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REVIEW ARTICLE

Neonicotinoids and their Metabolites in Human Biomonitoring: A Review

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Summary Neonicotinoids (NNDs) constitute a major class of insecticides with a broad and versatile spectrum of applications in agriculture. Hence, their residues are found in several environmental compartments and can be transferred *via* several pathways to numerous organisms. Despite their profound impact on honeybees and wild bees (impairment of memory, impact on immune system), their presence in humans is far less reported, possibly due to the low to moderate toxicological effects that they elicit. The aim of the present review is to emphasize on developments in the biomonitoring of NNDs. It focuses mainly on chromatographic analysis of NNDs and their metabolites in human biological fluids, discussing key features, such as sample preparation and analytical method validation. Nonetheless, case reports regarding intoxication incidents are presented, highlighting the significance of such cases especially in the developing world.

Additional Keywords: LC-MS, urine, Imidacloprid

Introduction

Insecticides are substances of chemical or biological origin that are used to control insects. Amongst the plethora of insecticides, neonicotinoids (NNDs) comprise a significant class of insecticides with numerous applications in agriculture. NNDs family includes, imidacloprid (IMI), thiamethoxam (THIAM), clothianidin (CLOTH), thiacloprid (THIAC), acetamiprid (ACET), dinotefuran (DINOT), nitenpyram (NITEN), nithiazine (NITH), imidaclothiz (IMCL), flonicamid (FLON), the fourth generation member sulfoxaflor (SULF) and cycloxaprid (CCLX). Exemplary compound of NNDs is IMI, whose sales in 2008 were estimated to ca. 5,450 tones in 2010 (Pollack, 2011) and its production was estimated at ca. 20,000 tones [see (Simon-Delso *et al*., 2015) and references therein]. The above data indicate the significance of NNDs for plant protection; nevertheless they imply their ubiquitous presence in the environment.

NNDs are systemic insecticides with chemical structures based on nicotine moiety (Figure 1). Consequently, their mode of action is similar to that of nicotine. Research studies have shown that NNDs bind in several and sometimes different domains in the insect nicotinic acetylcholine receptors [for the description of these receptors see the chapter by Jones and Sattelle, 2010] that results in differentiation of their bioactivity (Matsuda *et al*., 2005; Tomizawa *et al*., 2007a; Tomizawa *et al*., 2007b). Briefly, NNDs target the nicotinic receptors and provoke excitation of the nerve cells, causing trembling and shaking and eventually paralysis. The latter can lead to the death of the insects, depending on dose and exposure duration.

Active substances of NNDs have been alleged as one of the factors that lead to the development of the honeybee colony collapse disorder (CCD) syndrome (Vanengelsdorp *et al*., 2009). NNDs in particular seem to interplay in CCD (Lu *et al*., 2014), however further research is needed to elucidate CCD causality, since combined stress that

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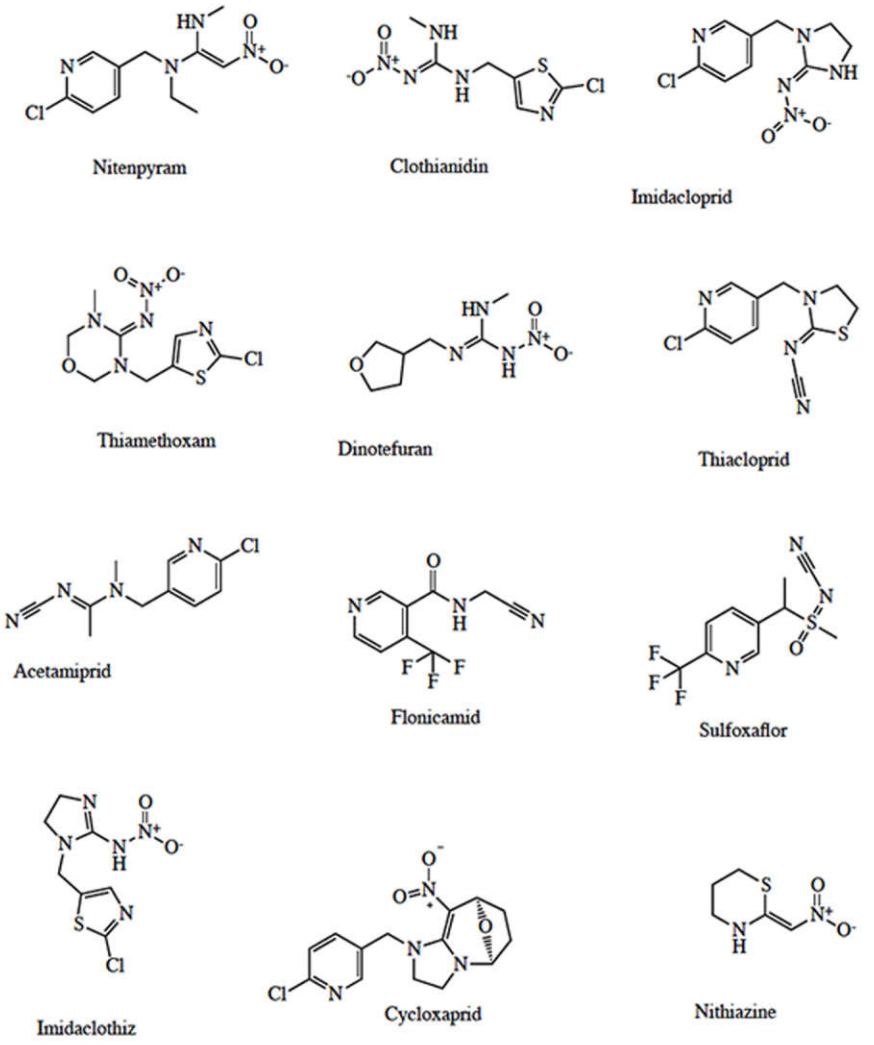

Figure 1. Chemical structures of Neonicotinoids

stems from parasites, pesticides and the poor flower diversity seems to be related to bee declines (Goulson *et al.,* 2015). Indicative symptoms provoked by sublethal doses of IMI in honeybees are the decrease in the hypopharyngeal gland size, and respiratory rhythm (Hatjina *et al*., 2013). Nonetheless, impairment of memory and brain metabolism has also been reported (Decourtye *et al*., 2003). In this context, NNDs' presence in human organisms is overshadowed by their impact especially on bees, and the relatively moderate to low toxicity that they exhibit with respect to other more hazardous for human health pesticides, such as organophosphates, carbamates and pyrethroids (Dawson *et al*., 2010).

Humans are exposed to numerous pollutants *via* their diet (Domingo *et al*., 2008; Lu *et al*., 2008; Marti-Cid *et al*., 2008b, a, c), the drinking water (Benotti *et al*., 2009), and the pollution of several environmental compart-

ments such as air (Dockery and Pope, 1994; Chen and Liao, 2006). Amongst the organic pollutants that impact the environment, pesticides possess a predominant role. Increasing number of works and modern applications are published in the domain of presence of pesticides in human biological fluids [see (Hernandez *et al*., 2005; Inoue *et al*., 2007; Jia *et al*., 2008)]. The latter are often encompassed in prospective cohort studies that try to elucidate diseases' causes and associate them with chemicals' exposure. Such studies have proved their efficacy to unveil important aspects of prenatal exposure (Bouchard *et al*., 2011; Engel *et al*., 2007).

Human biomonitoring is a leading field in bioanalysis, which covers all parts of the analysis of contaminants in biological fluids such as urine, blood, serum, saliva and body tissues. Amongst all matrices (invasive and non-invasive), blood and urine are the most frequently investigated. The collection of biomonitoring data regarding pesticides is of great interest since human exposure is portrayed, and association of concentration levels with potential toxicological effects is plausible.

Gagliardi and Pettigrove (2013) reported the improvement of aquatic ecosystem health after removal of intensive agriculture from an Australian region. Similarly, minimization of pesticides' use should be sought projecting in lowered residue levels found in human biological fluids and tissues and subsequently less impact on human health. Given this aspect, NNDs should be encompassed in monitoring schemes, and collection of pertinent works is of primary importance.

The present review summarizes all developments in the field of determination of NNDs and metabolites in human biological fluids. To our knowledge, all available works are included, and highlights of each one are discussed. In addition, case reports are presented that in some cases contain analytical approaches. In the same context, future prospects are provided with emphasis on the directions towards pertinent research endeavors should be focused.

Bioanalytical Methods

A biomonitoring study comprises a study population, data and biospecimen collection, sample preparation and purification, and finally chemical analysis. A fundamental prerequisite for a human biomonitoring study is to obtain information from the target population group regarding possible exposure to particular pollutants. By this way, the analytical methods become focused, and results more easily interpreted and related to possible health problems that might emerge. However, biological fluids are complex materials that contain macromolecules such as proteins, and other organic compounds that share common physicochemical parameters with the analytes of interest. Thus, the sample preparation step is also critical in providing pure samples, enriched in analytes considering that compounds of interest are usually found at low concentrations. Exceptions are the intoxication incidents in which levels are usually higher. One of the most common sample preparation techniques is liquid-liquid extraction (LLE) (Kataoka, 2003). It works through the extraction of analytes from the matrix using an organic solvent. Its traditional form has certain downsides that are observed in some occasions, such as the non-miscibility of solvents with the samples, and their difficulty in extracting polar and ionic compounds from aqueous media. Advances on LLE and several of its modified protocols have gained ground the last two decades and are extensively used in analysis of contaminants in various commodities (Bosch-Ojeda and Sanchez-Rojas, 2009; de Pinho *et al*., 2010). Another routine approach in the sample preparation is solid phase extraction (SPE). SPE has been broadly used in preparing the analysis of pesticides in biological fluids (Kataoka, 2003). It possesses certain advantages, such as high recovery, enrichment of analytes through pre-concentration, relatively short preparation time, and automation compatibility (Li, 2013; Li *et al*., 2013; Togola *et al*., 2014). Last but not least, protein precipitation is of the oldest ways of processing samples in bioanalysis. It

entails a denaturation stage that is accomplished by heating or the use of an organic solvent. After the solvent addition (usually acetonitrile (ACN) or methanol), the organic phase is separated from the protein by cyclomixing and centrifugation (Kole *et al*., 2011). Kole *et al*. (2011) have also reviewed recent advances in sample preparation in the bioanalysis domain including all available preparation steps prior to analysis.

A last but imperative parameter is the validation of the analytical methods. In the bioanalysis field, several validation guidelines and protocols (Bioanalytical-Validation; ICH, 2005) are adopted, which deal with particular validation parameters such as the limit of detection and quantification (LOD and LOQ), accuracy and precision. Matrix effects are also critical, considering the complexity of matrices, from which the analyst has to selectively extract the compounds of interest. A Belgian group reviewed successively these effects in bioanalytical methods, and proposed solutions to reduce or eliminate matrix interferences (Van Eeckhaut *et al*., 2009).

A critical feature that deserves attention is the *in vivo* metabolism of NNDs. It is acknowledged that the insertion of chemicals into humans' body is accompanied by several reactions that occur and usually breakdown the parent compounds to smaller molecules. Breakdown products should not be neglected, since it is well reported that some of these molecules exhibit significant toxicity in several organisms that can surpass those of parent molecules (Nauen *et al*., 2001; la Farre *et al*., 2008). Metabolism of NNDs is extensive, including several metabolic products produced by reactions such as reduction, demethylation, hydroxylation, and olefin formation. Hence, it is practically impossible to incorporate all metabolites in a targeted analytical method since many of them are not commercially available. In the group of NNDs, 6-chloronicotinic acid (6-CNA) is a common metabolite for IMI, NITEN, THIAC and ACET, considering that the latter share the chloropyridinyl moiety in their structure, and therefore it is widely included in analytical methods. CLOTH and THIAM contain the chlothiazole core and one of their key metabolites is 2-chloro-1,3-thiazole-5-carboxylic acid (2-CTCA). Finally, DINOT that contains the furanyl moiety is converted to 3-furoic acid (3-FA). Fundamental *in vivo* metabolites of NNDs are depicted in Figure 2.

Biomonitoring Studies

Of the first works published on NNDs and their metabolites was that of Uroz *et al*. (2001). The authors developed an analytical method for the monitoring of 6-CNA in human urine by gas chromatography tandem mass spectrometry (GC-MS/MS). Sample preparation consisted of acidification of urine and heating (for deconjugation to take place) and then the passage of the resulting mixture through an Amberlite XAD-4 cartridge. Amberlite XAD-4 resin, which is a polymeric sorbent with adsorbing potency for hydrophobic molecules, was selected after comparison with octadecyl carbon chain material (C18). Clean up was achieved with water of low pH and hexane. Then, 6-CNA was eluted with diethyl ether. Recoveries were optimum with acidic pH, although very low pH would decrease the resin's adsorption capacity. Though, 6-CNA is a molecule that is more compatible with liquid chromatography (LC), the authors choose gas chromatography. Hence, the dry sample was reconstituted in hexane and subjected to derivatization with a hexafluoroisopropanol (HFIP) using a carbodiimide as a coupling agent. After neutralization of the extract, the latter was injected into the GC-MS/MS system. Analysis time was short (6 min), monitoring the precursor ion of derivatized 6-CNA and several daughter ions. LOD of the method was determined to 16 pg/mL, which is the lowest reported in the literature for 6-CNA (LODs of NNDs and metabolites for selected works incorporated in this review are presented in Table 1). Finally, the application of the method to five urine samples of agricultural workers did not disclose the presence of 6-CNA.

Taira *et al*. (2011) reported 6-CNA pres-

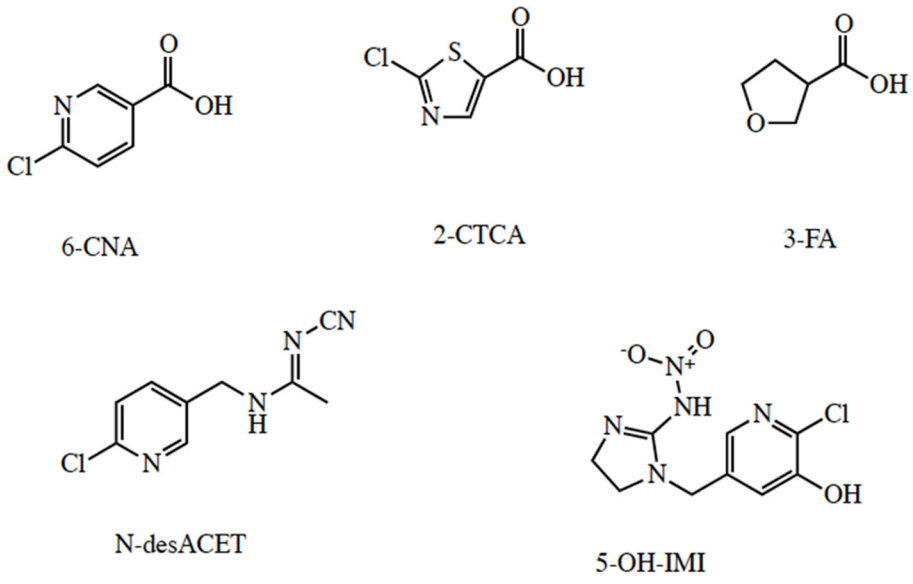

Figure 2. *In vivo* metabolites of Neonicotinoids.

Table 1. NNDs' and metabolites' LODs from selected works in human urine.

Analytes	LOD	Matrix	Detection	Reference
6-CNA	16 pg/mL	Urine	GC-MS/MS	(Uroz *et al.*, 2001)
6-CNA	400 ng/mL 2 ng/mL	Urine Urine	IC LC-MS (SIM)	(Taira *et al.*, 2011)
ACET N-desACET	0.068 ng/mL (LOQ) 0.55 ng/mL (LOQ)	Urine	LC-MS/MS	(Taira *et al.*, 2013)
6-CNA 2-CTCA 3-FA	0.1 ng/mL	Urine	GC-MS	(Nomura *et al.*, 2013)
ACET N-desACET IMI CLOTH DINOT FLON NITEN THIAC THIAM	0.2 ng/mL 0.2 ng/mL 0.2 ng/mL 0.5 ng/mL 0.2 ng/mL 1 ng/mL 1 ng/mL 0.1 ng/mL 0.2 ng/mL	Urine	LC-MS/MS	(Yamamuro *et al.*, 2014)

ence in the urine of six individuals that developed subacute nicotinic symptoms. Spot urine samples (spontaneous voided by the individuals) on the first visit and after were collected and subjected to ion chromatography (IC) analysis. IC was selected as a screen-

ing method since it exhibited acceptable recovery for 6-CNA. Confirmatory LC-MS was applied only to positive samples. Samples prior to chromatography were ultra-filtrated to remove proteins. LOD for the IC method was 0.4 mg/L_{urine}.

Regarding the LC-MS methodology, standard reversed phase separation was performed. Selected ion monitoring (SIM) was used to quantify 6-CNA, using the $[M+1]^+$ as quantitation ion. The LOD of the method was 2 μg/L_{urine}. Maximum 6-CNA concentrations ranged from 7.5 to 84.8 μg/L_{urine}. The origin of 6-CNA was attributed to the excessive intake of tea beverages and conventionally grown fruits. Nevertheless, the authors did not present an analytical study on NNDs' and metabolites' detection in food commodities, so as to strengthen the mentioned statement.

The same group two years later investigated NNDs metabolites in human urine of 3 patients suspected of subacute exposure to NNDs (Taira *et al*., 2013). This work came to fill gaps of the previous work, such as the non-inclusion of other metabolites of key members of NNDs family (showing selected metabolites of IMI, ACET, and CLOTH). To proceed to this study, a qualitative step was adapted aiming to unveil possible metabolic products. The latter was accomplished by liquid chromatography time-of-flight mass spectrometry (LC-TOF/MS). TOF/MS is known for its inherent advantages, which are: sensitivity, high mass range, and high-speed analysis. TOF/MS functioned using a database of nominal molecular weights of 57 known metabolites of the 3 NNDs of the study. For ACET, the dominant metabolite was *N*-desmethyl-acetamiprid (N-desACET) that until this work was reported only in rats' biological fluids. Subsequently, quantitation of TOF/MS identified compounds was performed with LC-MS/MS. Human urine samples were solid-phase extracted, and 10-folds concentrated. After loading urine samples in preconditioned cartridges, a wash step with H_2O was applied, and analytes were extracted with ACN. Acidic and basic extraction was also used utilizing 1.25 μL of formic acid and 10 μL of an ammonium hydroxide solution, respectively. Data analysis was based on data mining approach, which is a computerized procedure used to unveil patterns in large data sets. An almost 50% of nominated compounds were detected in positive controls by this screening methodology. Acidic SPE conditions exhibited the highest retention with only two undetectable substances. The qualitative TOF/MS analysis of human urine confirmed the presence of 8 metabolites, including N-desACET and 5-OH-IMI. The highlight of this work was the first report on N-desACET detection and quantitation in human urine at 3.2 ng/mL (one sample) that indicated human exposure to ACET. Taira has also reviewed the suspected health effects as a consequence of NNDs exposure in Japan, focusing on inhalation and oral exposure (Taira, 2014).

Nomura *et al.* (2013) published work on the detection and quantitation of NNDs' metabolites in human urine using GC-MS. Metabolites studied were 6-CNA, 2-CTCA and 3-FA. The method was validated after optimizing particular parameters. More specifically, a hydrolysis step was applied in order deconjugation to take place. Deconjugation was tested under acidic and basic conditions. It was found that addition of 50 μL of sulfuric acid was the optimum condition for deconjugation of the 3 NNDs. Then SPE was applied, by eluting compounds from preconditioned cartridge with methanol after column washing with 0.5 mL of 2% formic acid. SPE was performed on polymeric strong cation exchange column, also characterized by its non-polar retention mechanism. Methanol was chosen instead of ACN due to the observed minimization of interferences in the chromatogram. Division of the eluate was performed in order to proceed in separate analysis for 2-CTCA, and 3-FA and 6-CNA respectively. Derivatization of the analytes with trimethylsilyl group (a typical group used for such reactions) using BSTFA-TMCS proceeded smoothly for all target compounds. For the assessment of recovery, spiking was conducted at two different stages: initially at the beginning of

the extraction and then before derivatization. The calibration curve was constructed in the range of 0.6 to 10 μg/L, using pooled urine, exhibiting correlation coefficients for all analytes above 0.99. In the same context, within-run precision was determined at five concentration levels, with acceptable % relative standard deviation (RSD) values. Between-run precision was assessed similarly at 0.6 and 5 μg/L for five consecutive days, with RSD% below 13%. LOD and LOQ were calculated using the signal to noise (S/N) ratio of 3 and 10 in respect and were 0.1 and 0.3 μg/L, correspondingly. Application of the method to real samples unveiled a high frequency of detection for 3-FA, which is attributed to the frequent use of DINOT in Japan. Even though the presence of CLOTH is unambiguous in agricultural commodities in Japan, its metabolite 2-CTCA displayed low detection rate. The latter, as the authors state is unclear. Thus, it can be a challenge for future endeavors. Overall mean concentrations were 1.8 and 2.6 μg/L for 6-CNA and 3-FA, respectively. 2-CTCA was detected only in one farmer at 0.1 μg/L.

One year later, and as a compendium of their previous work, Ueyama *et al.* (2014) dealt with urinary NNDs metabolites. The main principal of this work was to focus solely on the urinary metabolites, overcoming overestimation of concentrations resulting from dietary intake. Consequently, they developed a straightforward method for simultaneous determination of urinary NNDs using LC-MS/MS. Sample preparation began with acidification of urine sample and addition of internal standard (IS). Then, the urine sample was incubated and applied to SPE. After conditioning, and a washing step, the majority of analytes were eluted with MeOH. NITEN and the IS were finally eluted with MeOH:ACN that contained 5% of ammonia (NH_3) solution. The use of NH_3 aided the elution of NITEN that exhibits high ionic binding to the SPE material. LODs varied from 0.01 to 0.12 μg/L. The concentration results showed that the Japanese population was exposed to NNDs. In particular, the detection frequencies were higher than 50% for all analytes, excepting NITEN. Overall, the authors pointed out two limitations. The first regarded the use of only one IS, and the second the difficulty to identify NNDs peak near LOD.

Yamamuro *et al.* (2014) developed a novel analytical method, for detecting NNDs in serum and urine. Until then most works on NNDs and metabolites were concentrated either in environmental or food samples that might contain NNDs or in biological fluids but with limited number of analytes. Therefore, this work came to fill this gap since it dealt with almost all NNDs. In addition, the authors included three ACET metabolites. The sample preparation step was simple. A low volume of sample was diluted with water and then purified, through a cartridge containing diatomaceous earth. This step although it seems as an SPE step, it works via LLE that occurs among the eluate (chloroform: isopropanol, 3:1), and a gel formed on the diatomaceous earth surface. Acceptable analytical performance was obtained only when elution was repeated with ten portions of low volumes (3 mL each) of the mentioned solvent mixture. The optimum mobile phase was a pH 3-buffered methanol, which provided substantial sensitivity, except FLON. Linearity of the calibration curve for each analyte was studied over a range of concentrations, starting from the LOQ up to 1 μg/mL. All correlation coefficient values were above 0.99, thus acceptable. Extensive validation of the method proved its efficacy and robustness. Sensitivity was substantial as depicted by the respective LOD values (serum 0.1-0.2 ng/mL, urine 0.1-1 ng/mL). It is foreseen that this approach can become a useful vehicle in forensic laboratories, which investigate human poisoning incidents with NNDs.

Jamin *et al.* (2014) published a cutting-edge work, in an untargeted profiling of pesticide metabolites in urine from pregnant women from a French epidemiological cohort. To carry out such profiling, the authors generated a pesticide metabolite list based on the likelihood of pesticide use in the study area. Analysis was accomplished

by liquid chromatography high-resolution mass spectrometry (LC-HRMS) using an Obritrap system. HRMS has already proved its effectiveness in the drug discovery domain (Ramanathan *et al*., 2011), in metabolomics studies (Xiao *et al*., 2012) and recently was reported in human exposure evaluation. This approach made effective the investigation of molecules on the basis of the theoretical mass of their quasi-molecular ions. Nineteen metabolites of IMI were screened. Nevertheless, no residues were detected.

Case Reports

Case reports usually refer to intentional (suicide attempts) ingestion of an amount of a pesticide formulation, a severe problem, especially in developing countries (Gunnell and Eddleston, 2003). Increased risk of suicide with exposure to pesticides has been reported particularly in intensive agricultural regions (Parron *et al*., 1996). The latter is becoming critical considering the risk projected to young people in these areas that have relative easy access to pesticides formulations (Kong and Zhang, 2010). Several case reports are published where NNDs are implicated. Typical symptoms-manifestations that develop in humans after such exposure to NNDs are disorientation, drowsiness, dizziness, cough, vomiting and abdominal pain. In the case of non-fatal incidents, after an initial treatment in the hospital (nasogastric lavage, instillation of activated charcoal), the patients are treated symptomatically and supportive, and finally discharged.

Wu *et al.* (2001) have presented a case report of acute poisoning with IMI formulation. More specifically a 64-year-old farmer was attempted to suicide using a bottle of insecticide containing IMI in *N*-methyl pyrrolidone (NMP), and a low percent of surfactant. The researchers concluded that it was rather difficult to determine whether the symptoms of drowsiness and dizziness, were provoked by IMI. However, the relative high concentration of the solvent (NMP) seemed to play a decisive role in intoxication. Proenca *et al.* (2005) published work on the fatal poisoning with IMI. To assess exposure in post-mortem samples the authors developed an LC-DAD-ESI/MS method that was capable to detect IMI and two of its metabolites (6-CNA and 5-OH-IMI). Samples of blood, urine and tissues were collected for toxicological analysis. Sample preparation was based on LLE with dichloromethane as organic solvent. Analysis was conducted by concomitant use of diode array (DAD) and mass spectrometer detector. IMI was detected in all post-mortem samples, but none of its two metabolites was detected. All specimens prior to targeted IMI and metabolites analysis were subjected to screening of other substances as well. None drug or pesticide was found in the samples. From analytical standpoint, the method was sensitive, exhibiting an LOD of 0.002 μg/mL$_{blood}$ and LOQ of 0.01 μg/mL$_{blood}$. Respective limits in urine were not reported. IMI levels varied from 0.29 μg/mL (in urine) to 2.05 μg/mL in blood. Conclusively, this study demonstrated how analytical methodologies could assist the resolving of cases that under routine examinations is difficult to understand the causative agents.

David *et al.* (2007) reported an incident regarding IMI poisoning. More specifically, a 22-year-old male with clinical toxicity was hospitalized after ingestion of 30 mL of IMI (17.8% concentration). Symptoms were as those above mentioned, and the patient was released on the 5th hospital day. Mohamed *et al.* (2009) published incidents from Sri Lanka, where IMI was involved after intentional self-poisoning (Mohamed *et al*., 2009). The latter was an outcome of a prospective observational cohort study of all poisoning presentations that was established during 2002 and lasted until 2007. More precisely, blood samples were taken, whenever possible so as to determine IMI levels (none metabolite were included). Plasma was isolated; SPE extracted, and the extract was subjected to LC-MS/MS analysis. Over this period, 68 patients were presented with a history of IMI exposure. Seven cases were occupational dermal exposure and not worrying, five involved co-ingestion of IMI with another

active substance and 56 were acute IMI self-poisoning. IMI residues were detected in 28 patients with a median admission plasma concentration of 10.6 ng/L.

Kumar *et al.* (2013) reported an accidental human poisoning with IMI, from rural India. The case report regarded a 60-year-old farmer that was exposed through inhalation. After hospitalization, the man was released. Data regarding IMI concentration in fluids was not provided, possibly due to the route of exposure. The same year, Lin *et al.* (2013) published a paper regarding a case report from Taiwan. The latter considered a suicide attempt by ingesting an IMI formulation. The authors, however, did not refer to IMI concentrations in biological fluids. An overview of cases reported until 2013 was also presented, including clinical details that are useful in incidents that end up to hospitalization and subsequent treatment of patients.

Fuke *et al.* (2014) reported the detection of IMI in biological fluids in a case of fatal intoxication. The authors developed an HPLC-DAD method monitoring IMI and 6-CNA. Specimens studied were whole blood, cerebrospinal fluid, humor, and urine. Sample preparation consisted of initial vortex mixing of a low volume of the liquid sample and concomitant extraction with ACN. Evaporation and two centrifugation steps provided the organic phase that was injected to the HPLC system. Prior to HPLC, a screening with GC-MS verified the presence of IMI, however, the poor chromatographic performance, favored HPLC analysis. Validation was performed after spiking drug-free blank blood. All validation characteristics were acceptable, with recoveries for IMI ranging from 86 to 105% for all fluids. 6-CNA was not detected in the samples analyzed. IMI in the femoral blood reached a maximum concentration of 105 μg/mL. Regarding cerebrospinal fluid its concentration was approximately half the one determined in the femoral blood. In this fatal case, since no other cause of death was evidenced, IMI intoxication was the cause of death. Same year Yeter and Aydn reported on the determination of ACET and one of its metabolites after fatal intoxications (Yeter and Aydin, 2014). Both biological fluids (postmortem blood and urine) and tissues were processed. Chromatographic analysis revealed ACET at 2.7 μg/mL in blood while its metabolite was not evidenced. None of the compounds was detected in urine samples.

Yeh *et al.* (2010) reported the acute multiple organ failure with IMI and alcohol ingestion. Specifically a 67-year-old man was transferred to the emergency in Taiwan hospital, after ingestion of an unknown amount of an insecticide containing IMI mixed with liquor. The incident led to arrhythmia and multiple organ failure within hours of intake. This incident argued the belief of the low mammalian toxicity of IMI and added a point to the increasing evidence that IMI can provoke kidney damage and other organ damages. Same year Iyyadurai *et al.* (2010) reported a fatal incident regarding IMI, after a suicidal attempt. The authors although stated (an often shortcoming also observed in other studies) that no data regarding serum IMI level were available.

Forrester (2014) provided an overall picture of NNDs exposure that occurred in Texas, USA, from 2000 to 2012. Of 1,142 exposures the 77% contained IMI and in less extent DINOT (17%). Both substances were detected along with other active substances as well. A seasonal trend favored mid-spring to mid-summer exposure reaching 50%. Almost all NNDs were detected including NITEN, ACET, THIAM, and CLOTH. The most common routes of exposure were ingestion, accounting for a 51%, dermal (44%) and ocular (11%).

Greek IMI biomonitoring

Our group the last decade is involved in biomonitoring studies in which pesticides are the target analytes (Kasiotis *et al.*, 2008; Kasiotis *et al.* 2011; Kasiotis *et al.*, 2012). In the frames of the ECOPEST project (ECOPEST) a biomonitoring study was conducted (blood and urine) that included 27 farmers. These farmers among several field applications, they applied seed treatment with IMI in cot-

ton crop fields. IMI and 6-CNA were incorporated in the developed analytical method that included a total of ten analytes (publication under preparation). The analyses showed the presence of IMI at levels ranging from 9.7-20.1 ng/mL$_{urine}$, and 6-CNA from 5.1 to 9.4 ng/mL$_{urine}$. Only one serum sample was positive with 6-CNA.

Conclusions

Human biomonitoring constitutes an indispensable tool in public health surveillance since it entails the prevalence of diverse pollutants in human organisms. It serves also as a valuable source of information in integrated health impact assessment. NNDs comprise a major pesticide category that deserves attention mostly due to their frequent use and broad spectrum of applications. Although, their human toxicity is moderate to low, monitoring schemes should encompass them. The latter are important in multiple exposure assessment that considers both cumulative toxicity and synergistic effects. Several reported analytical studies, verified the presence of NNDs and their metabolites. In this context, modern analytical tools, such as HRMS, is the trend that biomonitoring laboratories should pursue and encompass in their analytical schemes. Last but not least, the elucidation of undiscovered metabolic products should be a constant goal that is firmly connected to the diversity of pathways and reactions that organic substances undergo after their insertion in humans' body.

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ΑΡΘΡΟ ΑΝΑΣΚΟΠΗΣΗΣ

Ανασκόπηση της βιοπαρακολούθησης στον άνθρωπο, των νεονικοτινοειδών και των μεταβολιτών τους

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Περίληψη Τα νεονικοτινοειδή αποτελούν μια σημαντική κατηγορία εντομοκτόνων με ένα ευρύ φάσμα εφαρμογών στον τομέα της γεωργίας. Ως εκ τούτου, τα υπολείμματά τους βρίσκονται στο περιβάλλον και μπορούν να μεταφερθούν μέσω διαφόρων οδών σε πολλούς οργανισμούς. Παρά τις σοβαρές επιπτώσεις τους στις μέλισσες και τις άγριες μέλισσες (απώλεια μνήμης, επιπτώσεις στο ανοσοποιητικό σύστημα), η παρουσία τους στον άνθρωπο είναι λιγότερο περιγεγραμμένη, πιθανώς λόγω των χαμηλών έως μετρίων τοξικολογικών επιδράσεων που προκαλούν. Σκοπός της παρούσας ανασκόπησης είναι να δοθεί έμφαση στις εξελίξεις στη βιοπαρακολούθηση των νεονικοτινοειδών στον άνθρωπο. Επικεντρώνεται κυρίως στη χρωματογραφική ανάλυση των μητρικών μορίων και των μεταβολιτών τους σε ανθρώπινα βιολογικά υγρά, σχολιάζοντας βασικά χαρακτηριστικά των μεθόδων, όπως η προετοιμασία του δείγματος και η επικύρωση της αναλυτικής μεθόδου. Επιπλέον, παρουσιάζονται οι αναφορές σε περιστατικά δηλητηρίασης από νεονικοτινοειδή, τονίζοντας τη σημασία τους ιδίως στον αναπτυσσόμενο κόσμο.

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