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Exposure of wild boars (*Sus scrofa* L) to neonicotinoid insecticides

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HIGHLIGHTS

- The presence of 7 neonicotinoids and its 5 metabolites in the meat tissue of wild boar was studied.
- Neonicotinoids and its metabolites were confirmed in 83% of tested samples.
- 48% of tested samples contained more than one neonicotinoid insecticide.
- The risk for consumers from neonicotinoids through the consumption of wild boar is low.

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ABSTRACT

The aim was to determine, for the first time, concentrations of 7 neonicotinoids (NEOs) and 5 metabolites in *Sus scrofa* from hunting areas in north-eastern Poland and assess the risk to consumers eating boar meat. 42 wild boar muscle samples were collected over a one-year period. The concentrations of 12 NEOs were determined by a fully validated LC-ESI-MS/MS protocol based on ultrasonic, freezing and cleanup EMR–lipid sample preparation. NEOs were present in over 83% of samples, 17% had no residue, and one pesticide was present in 36% of samples. Most often found were: clothianidin (35%), acetamiprid and imidacloprid (33%), thiacloprid (31%), thiamethoxam (9%), and the average concentrations were (ng g^{-1}): thiacloprid 6.2 > imidacloprid 5.7 > acetamiprid 4.6 > clothianidin 2.2 > thiacloprid 1.6 > thiamethoxam 1.0. Multi-residue samples were found, one with 7 and one with 5 NEOs. Two NEOs were present in 24%; 3 in 39% and 4 in 10% of samples. In the metabolic degradation of acetamiprid, imidacloprid and thiacloprid, it was observed that metabolites account for no more than 8.5% of the measured parent substance. Acetamiprid-n-desmethyl was noted most often (21%). Due to the detection of NEOs in a large proportion of samples, chronic and acute risk assessment were performed. The estimated chronic and acute risk for consumers from NEOs neonicotinoids through the consumption of wild boar was very low and amounted to respectively 0.02% of ADI and 0.86% of ARfD.

1. Introduction

Neonicotinoids (NEOs) are a group of systemic insecticides acting on the nicotinic acetylcholine receptors of the central nervous system of insects (Jeschke and Nauen 2008; Ghosal 2018) which have found wide application in agriculture and provide an alternative to organophosphates, carbamate and pyrethroid insecticides (Ahmed et al., 2014; Maienfisch et al., 2001; Cimino et al., 2017).

Acetamiprid (ACE), clothianidin (CLO), nitenpyram (NIT), imidacloprid (IMI), thiacloprid (THI), thiamethoxam (THX) (chlorinated) and dinotefuran (DIN) (non-halogenated) are in widespread usage in crop protection, used as *liquid* sprays, seed treatments (Douglas and Toker, 2015) or *as granules* in soil additives (Nataraja et al., 2016), and in veterinary pest control (Kundoo et al., 2018).

Neonicotinoids are highly effective, especially in controlling sucking insect pests (Ahmed et al., 2014). Some of these compounds are approved for use as seed treatments (CLO), some as foliar applications (ACE and THI) and some as both (IMI and THI) (Ding et al., 2018). However, the use of neonicotinoids is not restricted to the agricultural sector. In Poland, neonicotinoids based on ACE are approved for forest protection as foliar sprays and for agricultural

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aviation operations. In forested and city areas, NEOs are applied as tree soil drenches or injections (e.g., for control of emerald ash borer and hemlock wooly adelgid) (Roy et al., 2019). Douglas et al. (2015) reported that the widespread use of large-scale seed treatment resulted in a rapid increase in the use of neonicotinoids. In recent years, NEOs used as a seed coating have been a source of controversy (Sanchez–Bayo, 2014) because of suspected negative effects, primarily on bees - both domesticated and wild - and bumblebees (Whitehorn et al., 2012; Rundlof et al., 2015; Sanchez–Bayo, 2014; EFSA, 2016; Rundlof et al., 2015).

In 2013, the European Union introduced a moratorium on the use of three NEOs (IMI, CLO and THX), and in 2018, it further tightened the regulations prohibiting the use of these three pesticides due to the risk to pollinating insects (EC, 2018; EFSA 2019). In Poland, a temporary (180 days) permit was issued in 2018 and 2019, based on of art. 53 section 1 of Regulation (EC) No. 1107/2009 of the European Parliament (EC, 2018) and of the Council of October 21, 2009 concerning the placing of plant protection products (PPP) on the market and repealing Council Directive 79/117/EEC and Art. 7 section 1 of the Act of March 8, 2013 on PPP, for the use of rape seed treated based on CLO and THX (Cruiser and Modesto 480 FS).

Widespread agricultural use of systemic NEOs has resulted in their occurrence in various elements on environment (Chagnon et al., 2015; Hladik et al., 2018; Sluijs et al., 2015): soil (Bonmatin et al., 2015; Chagnon et al., 2015; Cycoń and Piotrowska-Seget, 2015), dust, wetlands (Main et al., 2014; Schepker et al., 2020), tap water (Wang et al., 2019), non-target plants and common foods (Chen et al., 2014; Hou et al., 2013). Neonicotinoids, as unintended contaminants, interact with and shape wetland aquatic insect communities (Cavallaro et al., 2019), and they influence crustacean species in commercial shrimp aquaculture (Anderson et al., 2015; Butcherine et al., 2019).

In addition to their documented, intended adverse effects on insects, NEOs adversely affect non-target organisms: terrestrial arthropods (Main et al., 2018) vertebrates (Gibbons et al., 2014), as well as vertebrates: rats (reduced sperm production, increased skeletal abnormalities, abortions, oxidative stress, thyroid lesions, neuro- and immunotoxicity) (Gawade et al., 2013; Bal et al., 2012), Nile tilapia (changes in the testicular tissue and gonads), salmon (Marlatt et al., 2019), and black-spotted pond frogs (destruction of DNA at very low level) (Gibbons et al., 2014) or rabbits (Burke et al., 2018). The risks of teratogenic effects of THI on chick embryos (Salvaggio et al., 2019), THI, CLO and THX on eared doves (Addy–Orduna et al., 2019) were investigated.

Toxicological characteristics of neonicotinoids are presented in Supplementary Material - Table S1.

Poisoning of wild animals by pesticides is less often described in the literature than in the case of domestic animals (Berny et al., 2007; Guitar et al., 2010; Bertero et al., 2020a). It mainly concerns cases of acute poisoning connected with death by pyrethroids, carbamates, organophosphates and organochlorines (Bertero et al., 2020b). Animals may play a fundamental role as bioindicators for environmental toxicants, even more so for the new group of neonicotinoid insecticides. Hladik et al. (2018) indicates the environmental risk associated with the use of NEOs.

To our knowledge, no information is available on potential effects of NEOs and their metabolites on wild boar. Likewise, there are very few scientific papers describing the occurrence of NEOs in non-target organisms. Some studies have reported NEO contamination in avian populations (Humann-Guilleminot et al., 2019), insectivorous birds (Hallman et al., 2014), eared doves (Addy-Orduna et al., 2019), house sparrows (Humann–Guilleminot et al., 2019), aquatic invertebrate (Schepker et al., 2020), shrimp (Butcherine et al., 2019) and bees (Tsvelkov et al., 2017; Siviter and Muth, 2020; Whitehorn 2012; Wang et al., 2020; Krupke et al., 2017).

In Europe, the wild boar is considered the most important game species on a national level, and hunting of wild boar is carried out year-round. Wild boars cause huge damage to agricultural crops, which level in recent decades has increased dramatically (Amici et al., 2012; Geisser and Reyer, 2004; Novosel et al., 2012; Orsoni et al., 2020; Pinna et al., 2007; Schley et al., 2008; Tack, 2018). The chemical impurities that the boar may have in its body are closely related to the chemical protection of crops it destroys, and thus, to its diet. Boar is an omnivorous species, however, the majority of its food are plants (90–99% of consumption) and a small amount of products of animal origin (1–10%), but this varies depending on the season (Schley and Roper, 2003). Maize is one of the most damaged annual crops, from sowing to harvest maturity (Geisser and Reyer, 2004; Roy et al., 2019), and for the boar, fresh maize is an important dietary component and amino acid source (Schley and Roper, 2003). Another source of neonicotinoid contamination is groundwater and ponds near production fields (Hladik et al., 2014; Schaafsma et al., 2015).

Curentlly, wild boar meat is seen as a natural and healthy food. However, next to the product's quality characteristics (Marchiori and Felicio, 2003; Ramazin et al., 2010), food safety parameters should be met (not only the absence of parasites).

A significant part of the research on wild animals describes the occurrence of a persistent group of organochlorines (Maršálek et al., 2013), as well as organophosphates insecticides, PCB and heavy metals in tissue samples (Mauro et al., 2017; Niewiadomska et al., 2013; Naccari et al., 2004; Pagliuca et al., 2005; Chiari et al., 2015; Tomza–Marciniak et al., 2014a, 2014b).

Since neonicotinoids are commonly used as crop protectants, it is expected that exposure of wild animals to these insecticides will have an effect on the food chain (Gibbons et al., 2014). Moreover, it is important to investigate the potential adverse effects of NEO exposure. The presence of NEO residues in wild boar may be the result of inhalation and dermal contact, consumption of plants or seeds on which neonicotinoids have been applied, consumption of insects which have been combated as a source of protein (Hallmann et al., 2014), and through contamination of soil and ground water.

Determination of insecticides in a meat matrix is a difficult task due to its nature, the presence of fat, interfering compounds coextracted with analytes, and very low insecticide concentration (Dimitrova e al., 2017; Ahmad et al., 2020). Pang et al. (2009) reported determination of 839 pesticides in animal muscles by gel permeation chromatography cleanup, GC-MS, and LC-MS/MS, Dimitrova et al. (2018) presented a procedure for determination of organochlorine insecticides in meat by gas chromatography with selective detector ECD, Ahmad and Khulod (2020) modified the QUECHERS protocol using GC/MS.

In the literature, there are no procedures presenting the methodology for the determination of NEOs and their metabolites in the meat of wild animals. Therefore, the first goal of our work was to develop a quick procedure dedicated for determination of 12 NEO compounds, including metabolites in the wild boar matrix, through modifications carried out at the stages of isolation, extraction and purification based on our previous methodological experiments in fatty bee matrices using LC-MS/MS (Kaczyński et al., 2017).

The attention paid to the toxicological characteristics of wild boar meat is definitely lower than in the case of breeding pigs, which are often subject to national monitoring (EFSA, 2020). Therefore, paying attention to the presence of NEOs and their metabolites in wild boar meat is extremely important. There is a need to estimate the presence of NEOs in wild boar tissues, determine their metabolic pathways and estimate the health risk of

consumers consuming wild boar meat. Here, we hypothesized that the diet of boar is likely linked to crops and the environment where neonicotinoids are used, and consuming boar meat can be a source of contamination and may cause health problems for the consumer of boar meat. Therefore, the next goal of this unique study is to evaluate environmental eco/toxicological risks by identifying neonicotinoids in the tissue of boar. The human health impact resulting from exposure to NEOs through consumption of wild boar products is not yet fully understood, and our data will be helpful for characterization of health risk.

We hope that this work will be the starting point for multidisciplinary research that should be carried out on neonicotinoid insecticides in the wild animals, the food chain, and with regard to the risk they pose to humans.

2. Material and methods

2.1. Analyzed neonicotinoids (NEO) and their metabolites

Analytical standards (purity > 95%) of the tested insecticides: acetamiprid (ACE), clothianidin (CLO), imidacloprid (IMI), nitenpyram (NIT), thiacloprid (THI), thiamethoxam (THX) and metabolites acetamiprid-n-desmethyl (ACE-D), dinotefuran (DIN), imidacloprid guanidine (IMI-G), imidacloprid olefin (IMI-O), imidacloprid urea (IMI–U), thiacloprid amide (THI-A) were obtained from Dr. Ehrenstorfer Laboratory (Augsburg, Germany) (Supplementary Material - Fig. S1). Triphenyl phosphate (TPP) as the internal standard (IS) was supplied from Sigma-Aldrich (Steinheim, Germany). The stock standard solutions (at 1000 μg mL^{-1}) were prepared by dissolving TPP in methanol. The working standard solutions of multiple compounds were prepared in concentrations of 0.1, 0.5, 1.0, 5.0, 10.0, 50.0 and 100.0 ng g^{-1} in methanol. All solutions were stored in dark glass bottles at 4 °C. Ultrapure water (LC grade 18 MΩ cm) used for analysis was obtained from a MilliQ water purification system (Millipore Ltd., Bedford, MA, USA). All organic solvents (acetonitrile, methanol, formic acid and ammonium formate) were of LC-MS grade and supplied by Sigma-Aldrich (St. Louis, MO, USA). QuEChERS kits with buffering citrate salts: magnesium sulfate ($MgSO_4$), sodium chloride (NaCl), trisodium citrate dihydrate ($Na_3C_6H_5O_7$*$2H_2O$), disodium hydrogen citrate sesquehydrate ($Na_2HC_6H_5O_7$*$1.5H_2O$)) and cleanup sorbent EMR-lipid were obtained from Agilent Technologies (Santa Clara, CA, USA). Hydrophilic polytetrafluoroethylene (PTFE) filters of 0.45 μm porosity were obtained from Sigma-Aldrich (St. Louis, MO, USA), 15 mL polypropylene tubes from Carl Roth, Karlsruhe, Germany, 2 mL Eppendorf microtubes from Eppendorf, Poland, and 2 mL glass vials from Agilent, USA.

2.2. Sample collection

Wild boar meat samples were obtained from Knyszyn Forest Landscape Park (Fig. 1), but we note that these animals were not specifically killed for the purposes of this work. These are protected areas in the Podlaskie Voivodeship of northeastern Poland with an area of 744.47 square kilometers, belonging to Natura 2000, a network of nature protection areas within the territory of the European Union.

Hunting was carried out according to a fixed Polish government schedule for the given month. The hunts were conducted in accordance with hunting law (PL Regulation, 2020) and hunting ethics. The study did not involve any killing beyond that carried out in the course of regular wildlife management (population control), which was conducted randomly (regardless of gender, age, weight). The wild animals were living and feeding in their natural habitats, located on the border of forest and agricultural areas, where the dominant crops were: maize, rape, wheat and potatoes. Wild boars (20 males and 22 females) were aged between 1 and 3 years, with weights from 25 to 70 kg. A total of 42 wild boars (*Sus scrofa* L) were collected during the period of May 2019–January 2020. Representative samples of shoulder muscle (about 200–250 g) were wrapped in polythene bags, labeled, placed in ice and sent to the laboratory. Characteristics of study subjects are presented in Supplementary Material - Table S2.

2.3. Sample preparation

Each meat sample was weighed and homogenized using a meat blender. Ten grams of the representative sample was transferred into a PTFE tube. Then, 50 μL of IS (TPP) at concentration 5 μg mL^{-1} were added, thoroughly mixed, and then left at room temperature (0.5 h).

Ten mL ACN (containing 1% formic acid) was added into the tube and vortexed for 1 min and put in the ultrasonic bath for 30 min (Fig. 2). The mixture containing: $MgSO_4$ (4 g), NaCl (1 g), $Na_3C_6H_5O_7$*$2H_2O$ (1 g), $Na_2HC_6H_5O_7$*$1.5H_2O$ (0.5 g) was added and thoroughly shaken for 0.5 h. This mixture was centrifuged for 5 min at 4500 rpm and frozen for 30 min at −70 °C. Cleanup of the sample was done by transferring 8 mL of the acetonitrile supernatant into a PTFE tube containing EMR-lipid sorbent, shaking for 60 s and centrifugation for 10 min at 4500 rpm. The eluate was 10-times concentrated and alalysed by LC-ESI-MS/MS according parameters described in Table 1.

2.4. Quality control and quality assurance

Procedural blanks of the meat matrix (wild boar meat, previously checked for free pesticides) were determined simultaneously for each set of the sample analysis by going through the same extraction and cleanup procedures.

2.5. Method validation

In order to ensure reliability of the results, the method was validated in accordance with SANTE/11813/2017 (SANTE, 2017) criteria to an extent adapted to the goals of the conducted research. The examined properties were: linearity, recovery, precision, limit of quantification (LOQ) and matrix effect (ME).

2.5.1. Recovery test

A recovery test was carried out in triplicate on samples fortified with the 12 NEO mix standard. The fortified samples as well as the blank were subjected to the same analytical procedures of extraction, cleanup and analysis. Residue levels for each pesticide were not corrected for the recovery values.

2.6. Risk assessment

In our research, the 'worst case scenario', i.e. large portion, assumed consumption data (0.1 kg per capita day^{-1}) as a theoretical value based on a survey of family hunters. In our survey of 100 Polish members of hunting families (data not published), we confirmed a maximum consumption of 0.1 kg per $capita^{-1}$ day^{-1}. The intake is then compared to the acceptable daily intake value (ADI) and acute references doses (ARfD) for each active substance.

Different NEOs act on the nAChR; thus the cumulative chronic risk assessment is the sum of exposure to individual neonicotinoids with similar molecular structures and the same mode of action (ACE inhibitors). The full procedure of risk assessment calculation has been described previously (Łozowicka et al., 2012). The following residue definition was proposed for risk assessment: as

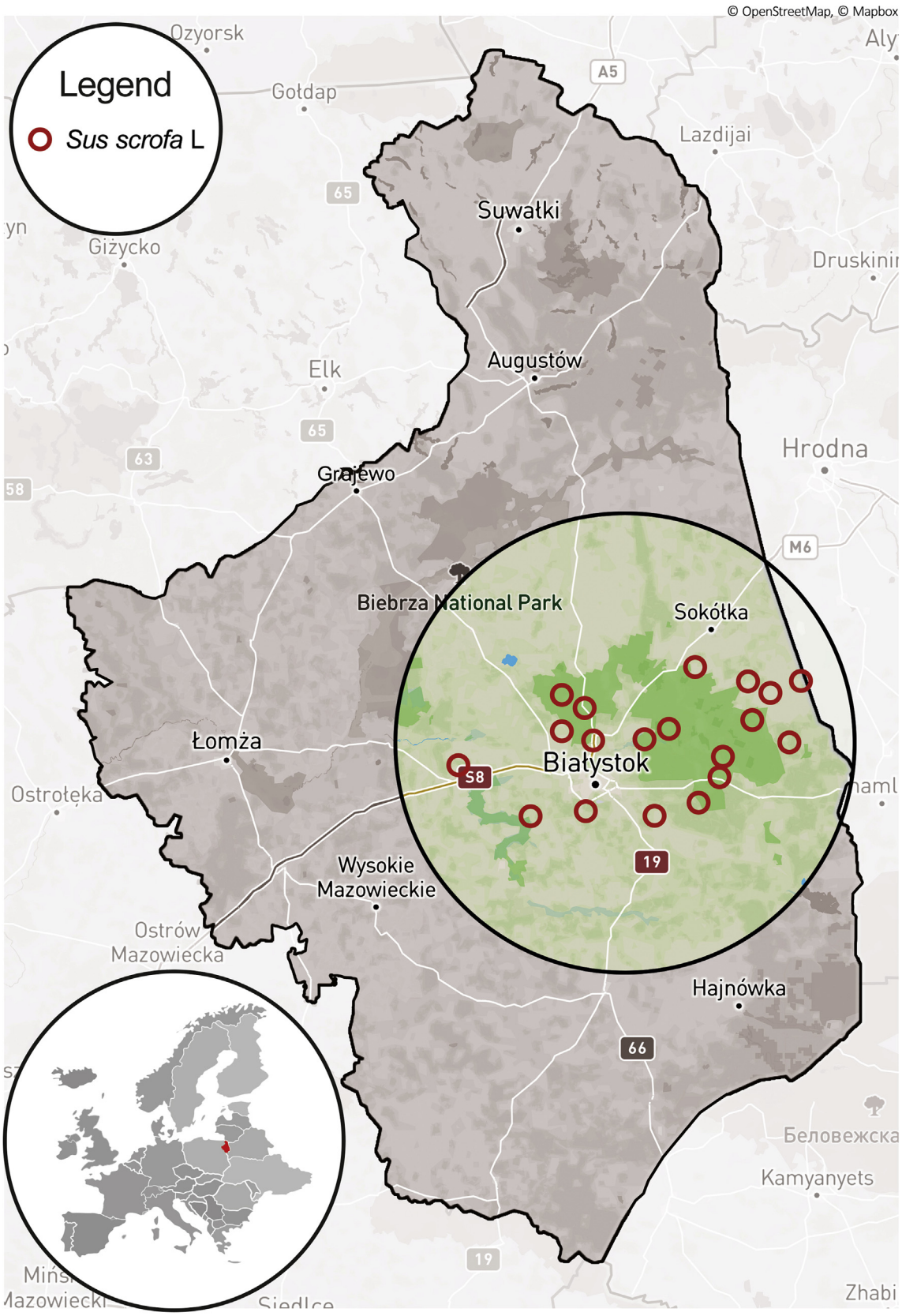

Fig. 1. Map of sampling.

Fig. 2. Wild meat sample preparation.

ACE the sum of ACE and acetamiprid-n-desmethyl (ACE-D), imidacloprid the sum of IMI, imidacloprid guanidine (IMI-G), imidacloprid olefin (IMI-O) and imidacloprid urea (IMI–U), thiacloprid the sum of THI and thiacloprid amide (THI-A).

3. Results

3.1. Analytical protocol for determination of NEOs

A fully validated protocol for analysis NEOs by LC-ESI-MS/MS based on modified QuEChERS methodology is presented. Sample cleanup based on solid-liquid extraction, and removal of the lipid fraction, is proposed. Eight experiments were carried out to prepare a sample of meat for testing, with and without ultrasonic treatment, with and without freezing, with and without purification at various time steps: P1) EMR-lipid with freezing, P2) EMR-lipid without freezing, P3) without cleanup and without freezing, P4) ultrasonic treatment 15 min without cleanup and without freezing, P5) ultrasonic treatment 30 min without cleanup and, without freezing, P6) ultrasonic treatment 60 min without cleanup and without freezing, P7) ultrasonic treatment 30 min EMR-lipid without freezing and P8) ultrasonic treatment 30 min EMR-lipid with freezing. Mean recoveries (n = 5) and relative standard deviation for the analyzed NEOs in wild boar meat tissue (at 10 ng g^{-1}) obtained with various extraction and cleanup procedures are presented in Table 2.

The lowest and highest mean recoveries and relative standard deviation were obtained for thiacloprid amide 38% – P1 and 107% P7. The use of EMR-lipid sorbent without freezing in the sample purification step (P1) resulted in unacceptable recoveries for all neonicotinoid metabolites, from 38% to 68%. The inclusion of the freezing stage in the procedure was unsuccessful, recovery for metabolites in the range of 43–61%, as in the third procedure without purification and without freezing, 40–61%. Ultrasonic isolation (Procedure 3–7) gave satisfactory recoveries, with the exception of 65% for imidacloprid olefin (P4). Matrix effects for the analyzed NEOs in wild boar meat tissue (at 10 ng g^{-1}) obtained with various extraction and cleanup procedures are presented in Supplementary Material - Table S3. The matrix effect was unacceptable in procedure (P4): ultrasonic treatment 15 min without cleanup and without freezing, (P5): ultrasonic treatment 30 min without cleanup and without freezing, (P6): ultrasonic treatment 60 min without cleanup and without freezing, −45%–42% (P4), −46% to 45% (P5), −49%–53% (P6). All compounds showed an acceptable recovery and minimal matrix effect in the case of (P7) ultrasonic treatment 30 min EMR-lipid without freezing and (P8) ultrasonic treatment 30 min EMR-lipid with freezing. The procedure of supporting the sample with ultrasonic treatment was used for the tests, and the use of EMR-lipid at the purification stage and 30 min prior to freezing.

Supplementary Material - Table S4 presents the values of recoveries (R), precision (RSD), uncertainties (U) for twelve pesticides. Recoveries for all compounds were acceptable between 71 and 115% for the four concentration levels 0.1 (ng g^{-1}), 1.0 (ng g^{-1}), 10.0 (ng g^{-1}) and 100.0 (ng g^{-1}). For example, for the lowest concentration level of 0.1 (ng g^{-1}), the recoveries were in the range: IMI-O 71% and DIN 115%. The RSDs for the four spiked levels were lower than 20% and were in the range: 1% IMI-O (at level 10.0 ng g^{-1}) and 15% ACE-D (at level 100.0 ng g^{-1}). LOQ were established at values 0.1 ng g^{-1}. Linearity was studied using matrix extracts of meat at seven concentration levels: 0.1, 0.5, 1.0, 5.0, 10.0, 50.0 and 100.0 ng g^{-1}, and deviation of the back-calculated concentration (DEV) was calculated as: % DEV=($C_{measured}$–C_{true})*100/C_{true}) (SANTE, 2017). Regression equations and DEV of analyzed pesticides are presented in Supplementary Material - Table S5. All of the tested insecticides showed acceptable values of DEV from −9 to 10%.

3.2. Occurrence of NEOs and their metabolites in wild boar samples

Studies on the occurrence of NEO and their metabolites were carried out in samples of the meat of wild animals, including 22 female wild boars aged: 6 boars - 3 years old, 10 boars - 2 years old and 6 boars - 1 year old, and 20 male boars aged: 7 boars - 3 years old, 11 boars - 2 years old and 2 boars - 1 year old. All animals were shot in the Knyszyn Forest with an area of 132,372.2 ha belonging to Natura 2000 Protected Areas and Special Protection Areas (SPAs) (Fig. 1).

Among the analyzed 7 neonicotinoids and 5 metabolites (Fig. 3), 5 compounds and 4 metabolites were determined (Supplementary material - Table S6). No dinotefuran, nitenpyram and metabolite imidacloprid olefins were detected. The most common were clothianidin (15 times), acetamiprid (14 times), imidacloprid (14 times) and thiacloprid (13 times). The ACE metabolite: ACE-D was present in 9 samples (0.2–12.2 ng g^{-1}). Moreover, of the three IMI metabolites tested, imidacloprid guanidine at a concentration of 5.4 ng g^{-1} and one sample of imidacloprid urea at a concentration of 1.5 ng g^{-1} were determined. In the case of the THI metabolite, thiacloprid amide was found in four samples within the concentration range of 0.1–0.4 ng g^{-1}.

Table 1
LC-MS/MS parameters for the neonicotinoids and metabolites.

	LC−MS/MS conditions							
Instrumetal sytem	Eksigent Ultra LC−100 (Eksigent Technologies, Dublin, CA, USA) liquid chromatography system coupled with AB Sciex 6500 QTRAP triple quadrupole mass spectrometer (AB Sciex Instruments, Foster City, CA)							
Chromatographic separation	Kinetex C-18 2.6 μm, 2.1 × 100 mm (Phenomenex, Torrance, USA); Mobile phase: (A) water with 0.5% formic acid and 2 mM ammonium formate and (B) methanol with 0.5% formic acid and 2 mM ammonium formate; Flow rate: 0.5 mL min^{-1}; Elution gradient: 0−1 min 1% B, increase to 8 min 95% B, hold 8−10 min, decries to 1% B at 12 min and hold at 1% to 15 min. Column temperature: 40 °C; Injection volume: 10 μL							
Detection	Positive Electrospray Ionization (ESI+) in multiple reaction monitoring mode Ion spray voltage: 5000 V; curtain gas (nitrogen): 30 psi; nebulizer gas (nitrogen): 60 psi; auxiliary gas (nitrogen): 50 psi; source temperature: 400 °C.							
Fragmentation parameters	Pesticide	Retention time (min)	Precursor ion (*m*/*z*)	Quantification Product ion (*m*/*z*), (CE/CXP (V))	Confirmation Product ion (*m*/*z*), (CE/CXP (V))	DP (V)	EP (V)	MRM Ratio (RSD) (%)
	Acetamiprid	4.71	223	125.9 (27/6)	99 (51/5)	80	10	6.6 (3.1)
	Acetamiprid-n-desmethyl	4.14	235	136 (44/10)	121 (38/15)	42	10	3.7 (4.3)
	Clothianidin	4.31	250	169 (19/10)	132 (21/6)	6	10	0.7 (3.7)
	Dinotefuran	2.94	203	157.1 (11/10)	113.1 (17/8)	36	10	0.6 (1.8)
	Imidacloprid	4.37	256	209.1 (21/12)	175.1 (27/10)	80	10	1.6 (1.7)
	Imidacloprid guanidine	3.42	212	127 (30/31)	177 (30/23)	60	10	1.5 (2.6)
	Imidacloprid olefin	3.93	254	236 (36/13)	171 (36/27)	48	10	2.4 (5.3)
	Imidacloprid urea	3.38	212	128 (30/27)	99 (30/25)	85	10	3.2 (4.7)
	Nitenpyram	3.31	271.1	126 (37/8)	237 (25/11)	61	10	0.2 (2.3)
	Thiacloprid	5.05	253	126 (29/6)	72.9 (81/8)	96	10	11.1 (3.3)
	Thiacloprid amide	4.34	236	132 (36/28)	94 (41/32)	75	10	4.3 (3.7)
	Thiamethoxam	3.73	292	211 (17/12)	181 (31/10)	61	10	2.1 (1.8)
Total run time	15 min							
Data collection	Analyst software version 1.6.2 (AB Sciex Instruments, Foster City, CA).							

Table 2
Mean recoveries (n = 5) and relative standard deviation (RSD, %) for the analyzed NEOs in wild boar meat tissue (at 10 ng g^{-1}) obtained with various extraction and cleanup procedures (P1−P8).

Procedure	P1	P2	P3	P4	P5	P6	P7	P8
Details	EMR-lipid with freezing	EMR-lipid without freezing	without cleanup and without freezing	ultrasound 15 min without cleanup and without freezing	ultrasound 30 min without cleanup and without freezing	ultrasound 60 min without cleanup and without freezing	ultrasound 30 min EMR-lipid without freezing	ultrasound 30 min EMR-lipid with freezing
Acetamiprid	74 (5)	76 (6)	80 (4)	85 (4)	95 (3)	94 (4)	90 (4)	91 (4)
Acetamiprid-n-desmethyl	52 (7)	54 (6)	48 (7)	75 (6)	93 (5)	96 (2)	89 (3)	86 (2)
Clothianidin	86 (6)	85 (4)	94 (3)	95 (6)	98 (2)	95 (4)	91 (5)	82 (6)
Dinotefuran	97 (4)	101 (5)	89 (4)	98 (6)	101 (4)	105 (3)	105 (2)	104 (5)
Imidacloprid	79 (5)	69 (6)	74 (5)	75 (4)	85 (6)	88 (6)	84 (7)	79 (4)
Imidacloprid guanidine	64 (3)	60 (4)	61 (6)	91 (7)	106 (5)	102 (6)	99 (4)	101 (3)
Imidacloprid olefin	51 (4)	55 (5)	57 (4)	65 (5)	83 (4)	88 (3)	84 (5)	72 (1)
Imidacloprid urea	53 (7)	58 (8)	52 (8)	82 (6)	98 (7)	93 (3)	92 (2)	100 (5)
Nitenpyram	68 (6)	65 (7)	61 (5)	88 (3)	93 (4)	96 (5)	88 (3)	101 (3)
Thiacloprid	76 (4)	80 (5)	81 (4)	92 (2)	105 (3)	98 (4)	100 (5)	103 (6)
Thiacloprid amide	38 (9)	43 (8)	40 (7)	81 (5)	109 (7)	102 (6)	103 (4)	107 (3)
Thiamethoxam	81 (3)	83 (5)	94 (5)	95 (4)	94 (2)	93 (4)	88 (6)	85 (5)

3.3. Multi-residue samples

Of the 42 samples of wild boar meat tested, seven did not contain any residue (17%) (Fig. 4). A total of 48% of the samples contained two or more NEO residues, up to seven. The highest percentage of samples were samples containing one compound (35%): thiacloprid (5 samples; 0.1–0.5 ng g^{-1}); clothianidin (n = 3; 1.3–12.2 ng g^{-1}); imidacloprid (n = 4; 0.1–0.5 ng g^{-1}); thiamethoxam (n = 2; 1.3–1.9 ng g^{-1}) and acetamiprid (1).

Nearly a quarter of the samples (24%) contained two compounds, in the combinations: CLO/THI (4.7; 0.5 ng g^{-1} and 1.3; 0.5 ng g^{-1}); THI/THI-A (2.4; 0.1 ng g^{-1}); CLO/IMI (0.4; 0.7 ng g^{-1} and 0.4; 0.2 ng g^{-1}); ACE/ACE-D (3.6, 0.3 ng g^{-1} and 13.5; 0.8 ng g^{-1}); ACE/IMI (0,1; 0,1 ng g^{-1}) and ACE/CLO (0,9; 0,2 ng g^{-1}). Three compounds were present in 10% of the samples: ACE/ACE-D/IMI (22.5; 1.3; 0.1 ng g^{-1}); ACE/CLO/IMI (1,3; 4,6; 0,1 ng g^{-1}); ACE/CLO/THX (0.1; 1.3; 0.2 ng g^{-1}); IMI/THI/THI amide (0.1; 3.0; 0.1 ng g^{-1}). Four active substances were noted in 10% of the

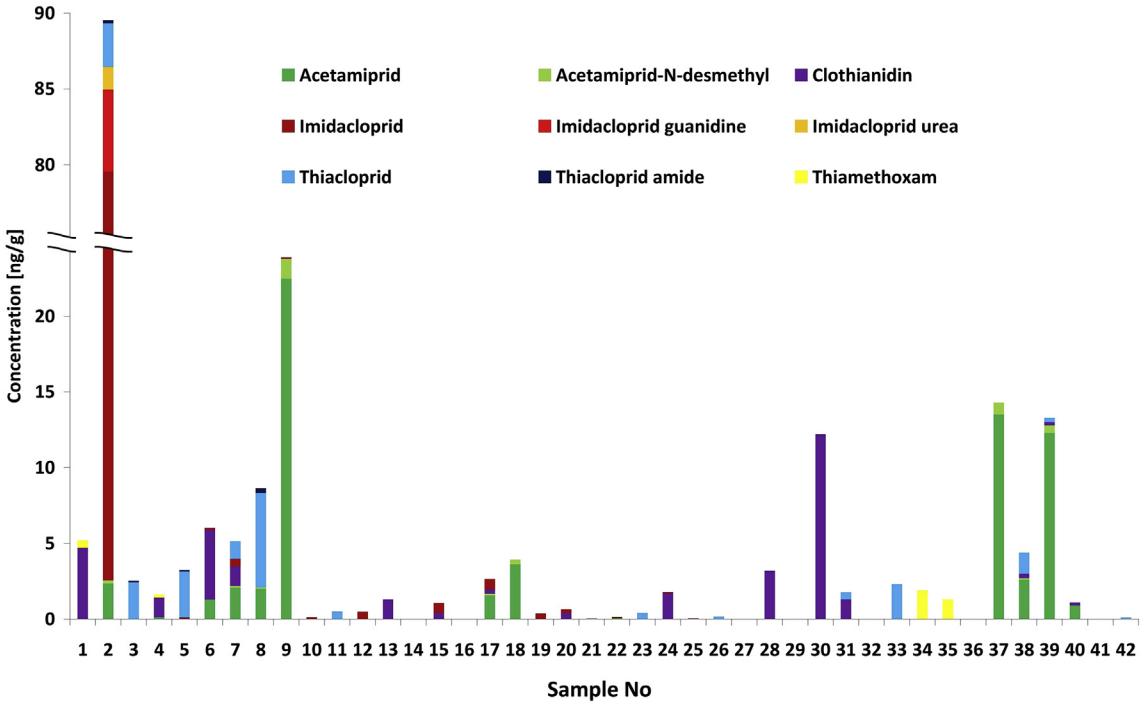

Fig. 3. Mean concentration of detected pesticides in individual samples of wild boars.

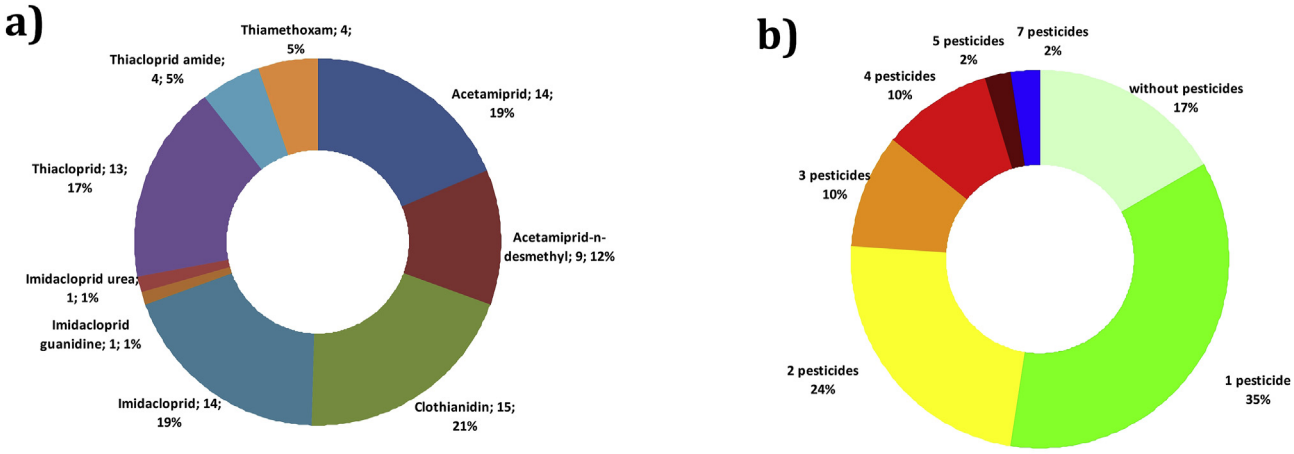

Fig. 4. Number of individual NEO pesticides detected in wild boar (a); pesticide free, one residue and multiresidues meat samples (b).

samples: ACE/ACE-D/THI (2.0; 0.1; 6.2; 0.3 ng g^{-1}); ACE/ACE-D/CLO/IMI (1.6; 0.1; 0.3; 0.7 ng g^{-1}); ACE/ACE-D/CLO/THX (2.6; 0.1; 0.3; 1.4 ng g^{-1} and 12.3; 0.5; 0.2; 0.3 ng g^{-1}). In 2% of the samples (one sample), the presence of five ACE/ACE-D/CLO/IMI/THI (2.1; 0.1; 1.3; 0.5; 1.2 ng g^{-1}) and seven compounds: ACE/ACE-D/IMI/IMI-G/IMI-U/THI/THI-A/THX (2.3; 0.2; 76, 9; 5.4; 1.5; 6.2; 0.3; 1.9 ng g^{-1}) was detected, respectively.

The concentrations of residues in samples with two residues ranged from 0.1 to 14.3 ng g^{-1}, with three residues 1.6–23.9 ng g^{-1}, with four residues 2.6–13.3 ng g^{-1}, the total concentration of residues in samples with five residues was 5.1 ng g^{-1} and with 7 residues 89.4 ng g^{-1}.

3.4. Risk assessment

The chronic exposure (long term) was estimated using a deterministic approach according to worst-case scenarios using a large portion (Łozowicka et al., 2012). The cumulative exposure, expressed as the sum of individual exposures to ACE, CLO, IMI, THI, THX, is very low (0.02% of ADI) (Table 3).

Short term consumer risk was estimated according 'worst case scenario' and was between 0.1 and 0.86% of ARfD (Supplementary material – Table S7). The highest ARfD was calculated for the sample with 7 neonicotinoids.

4. Discussion

The levels of occurrence of insecticides are very low, and interference from lipids is a huge problem from the perspective of obtaining the lowest possible limit of quantification in trace pesticide residue analysis in complex fatty biological matrices (Kaczynski et al., 2017; Ahmad and Khulod, 2020; Pang et al., 2009;

Table 3
Chronic of exposure to individual neonicotinoids.

	Acetamiprid	Clothianidin	Imidacloprid	Thiacloprid	Thiamethoxam
Body weigt (kg)	76	Consumption (average per day) (kg)			0.1
Acceptably Daily Intake (ADI) (mg kg^{-1} m.c)	0.025	0.097	0.06	0.01	0.026
Average residue (μg kg^{-1})	1.629	0.795	2.081	0.5261	0.093
Estimated Daily Intake (EDI) (ng)	0.00214	0.00105	0.00274	0.00069	0.00012
%ADI	0.0086	0.0011	0.0046	0.0069	0.0005
sum %ADI					**0.0217**

Song et al., 2018).

Lipids reduce the lifetime of the apparatus and reduce sensitivity due to suppression of analytes (Pagliuca et al., 2005). The necessity of lipid removal is well understood (Dankyi et al., 2015). There are many techniques for sample preparation and instrumental improvement of analyte recoveries (Song et al., 2018; Karthikeyan et al., 2019). Some of them are somewhat complex. Song et al. (2020a) described the cleanup process of porcine meat using multiwalled columns packed with two layers of carbon nanotubes, C18 and anhydrous magnesium sulfate ($MgSO_4$) as the top layer, while a mixture of florisil and $MgSO_4$ served as the bottom layer.

The metabolism of neonicotinoid insecticides takes place in six stages: 1) hydroxylation of the thiazolidine ring and subsequent glucuronidation 2) hydroxylation of the cyanamide moiety), 3) opening of the thiazolidine ring, 4) formation of an oxazole ring, 5) oxidation and subsequent methylation of the thiazolidine ring, and 6) oxidative cleavage of the methylene bridge, and involves oxidation (e.g., *N*-demethylation), reduction (e.g., conversion from *N*-nitro to imine), hydrolysis (e.g., conversion from cyano to amide), dehydrogenation, and *N*-acetylation (Ikenaka et al., 2018).

There are few works in the literature describing the determination of neonicotinoids and their metabolites. Ikenaka et al. (2018) optimized determination of 7 NEOs and 20 metabolites in green tea leaves, where the Presep RPP and ENVIcarb/PSA cartridges were connected in series, and analytes were eluted in dichloromethane/acetonitrile solution. Dankyi (2016) described simultaneous analysis of 5 NEOs in cocoa beans and shells based on the QuEChERS procedure, using the sorbent mixture of PSA/C18/graphitized carbon black during clean up and LC-MS/MS. In our study, based on our experience in pesticide determination (Kaczyński et al., 2017; Hrynko et al., 2021), ultrasonic-assisted extraction was used. Optimal recoveries vlues, with a low matrix effect, were obtained when prior freezing of the extracts and cleanup by EMR-lipid sorbent.

The LC-MS/MS analytical tool is extremely sensitive and selective in pesticide analysis (Karthikeyan et al., 2019), however, when working with difficult biological matrices, it has some limitations, especially when ESI ionization sources are used. Therefore, matrix effects were evaluated during method validation using a stable isotope-labeled internal standard. A positive ME was observed for: ACE, ACE-D, DIN, NIT and THX, max. up to 18%, and negative matrix effect for: CLO, IMI, IMI-G, IMI-O, IMI-U, THI, THI-A, within the range from −6% to −19%. These results show that the isolation, extraction, sorbent used and selected conditions applied in chromatographic analysis under controlled conditions of the internal standard are optimal for testing neonicotinoids and their metabolites in the meat matrix. The proposed method meets the validation criteria (SANTE, 2017) and enables testing of NEOs and metabolites at very low concentration levels (0.1 ng g^{-1}).

Over the past 10 years, the population of wild boars in Poland increased more than twice, and according to the data of the Polish Hunting Association (PL Regulation, 2020), it is currently about 300,000. Until recently, crops accounted for 25% of wild boars' diet, but now this number has risen to nearly 80%. They like practically everything: germinating corn, seeds, potatoes, young oats (Schley et al., 2003, 2008). Looking for worms in the ground, they can destroy an entire field in one night. This means that they are more often exposed to chemical substances used in agriculture. For example, the concentration of IMI in the wild boar (sample No.2) hunted May 10, was 83.8 ng g^{-1}. This means that they must have eaten treated corn grain or very small corn sprouts.

Nowadays, the treatment of seeds with NEOs is common, as they are relatively safe, easy to use and are less polluting to the environment. (Ding et al., 2018; Roy et al., 2019; Seagraves and Lundgren, 2012). NEO seed treatments have a long duration of activity against insects (Zhang et al., 2016; Nataraja et al., 2016; Ding et al., 2018). The effect of CLO and IMI seed treatments on natural enemies of winter wheat was described by Zhang et al. (2016).

The growing problem of the destruction of agricultural crops by the growing wild boar population means that they are subject to regular controlled hunting (Lombardini et al., 2017; Martin et al., 2017), and healthy hunted animals are intended for consumption. Thus, the consumption of wild boar meat and its products has also increased. Pesticides enter the human body through ingestion, inhalation or penetration via skin but the majority of persons become exposed via the intake of pesticide-contaminated food or water. The effects of pesticides on human health are highly variable. They may appear within days and are immediate in nature or they may take months or years to manifest and hence are called chronic or long-term effects. The cumulative toxicities of mixtures of selected NEOs (e.g. IMI, CLO, THI) are poorly understood (Maloney et al., 2017). Biomonitoring data provides the direct evidence that NEOs from any possible route of exposure can be absorbed by individuals and then excreted in urine. American, Chinese and Japanese (Song et al., 2020b; Ospina et al., 2019; Ikenaka et al., 2019) biomonitoring studies showed the presence of these increasingly popular compounds, replacing organophosphates and carbamates, in the urine of children over 3 years of age. In the American study (n = 3038), the most frequently detected were: 35% - ACE-D (0.20–34.7 ng mL^{-1}); 19.7% - 5-hydroxy imidacloprid (0.40–40.4 ng mL^{-1}); 7.7% - CLO (0.20–31.1 ng mL^{-1}); 4.3% - IMI (0.4–4.94 ng mL^{-1}) and <0.5% - ACE and THI. In the Japanese group (n = 46 children), in urine, THI concentrations were <0.13 ng mL^{-1} in approximately 30% of samples, and other NEOs: ACE-D, THX, DIN, and CLO, were 18.7, 1.92, 72.3, and 6.02 ng mL^{-1}, respectively.

In our research, the determined concentrations of NEOs are at a similar level to biomonitoring in urine, and we selected four groups of NEO in wild boar samples, depending on concentration range: I) 0.1–1.0 ng g^{-1}, 43 detections, where IMI was detected 13 times; II) 1.0–5.0 ng g^{-1}; 25 detections, where CLO was detected 8 times; III) 5.0–20.0 ng g^{-1}; 5 detections, where ACE was detected 2 times and IV) > 20 ng g^{-1}, 2 detections, ACE and IMI (single detections) (Table 4).

In our study, the most frequently detected were clothianidin (35% of samples) and acetamiprid (33%), also, IMI and THI were in 31% of samples, and THX in 10%. The arithmetic average of the concentrations was the highest for thiacloprid 6.2 ng g^{-1}, followed

Table 4
Concentration range and frequency of detected neonicotinoids and metabolites.

Concentration range	TOTAL	ACE	ACE-D	CLO	DIN	IMI	IMI-G	IMI-O	IMI-U	NIT	THI	THI-A	THX
	Number of detection												
0.1–1.0 ng g^{-1}	**43**	4	8	6		13					6	4	2
1.0–5.0 ng g^{-1}	**25**	7	1	8					1		6		2
5.0–20.0 ng g^{-1}	**5**	2		1			1				1		
>20 ng g^{-1}	**2**	1				1							

by: imidacloprid 5.7 ng g^{-1} > acetamiprid 4.6 ng g^{-1} > clothianidin 2.2 ng g^{-1} > thiacloprid 1.6 ng g^{-1} > thiamethoxam 1.0 ng g^{-1}, and the arithmetic averages of the concentrations of detected metabolites were, in order: thiacloprid amide 6.2 ng g^{-1} > imidacloprid guanidine 5.4 ng g^{-1} > imidacloprid urea 1.5 ng g^{-1} > acetamiprid-n-desmethyl 0.4 ng g^{-1}.

NEOs act as modulators of nicotinic acetylcholine for mammals and insects, and among the four detected metabolites in wild boar meat, ACE-D (0.1–0.8 ng g^{-1}) has nicotinic properties on nAChRs and is toxic to humans. The toxicological profile of these metabolites has not been fully investigated. In addition, the next metabolite detected in the highest concentration, THI-A (0.1–0.3 ng g^{-1}), does not have nicotinic properties, but it can hydrolyze to *N*-descyano-THI, which possesses very toxic activity toward mammals and nicotinic properties (oral LD_{50} 1.1 mg kg^{-1} bw for mice) (Tomizawa, 2004).

Clothianidin was the most frequently detected and possesses the status 'not approved' (EC, 2009), except in Poland, where a temporary (180 days) permit was issued in 2018 and 2019. The risk of bioaccumulation is low, and its acute toxicity to mammals is considered moderate. It is, however, a neurotoxicant. Long-term ingestion of clothianidin may result in reproductive and/or developmental effects (US EPA, 2005). Pecenka and Lundgren (2015) reported non-target effects of CLO on monarch butterflies and Tokumato et al. (2013) described the effects of exposure of clothianidin on the reproductive system of male quails.

Acetamiprid (example PPPs: Acetamiprid WP, Gazelle, Insyst, Beticol, Mospilan 20 SG and Antilop SG) is often used on the Polish crops of: rape, potatoes, field beans, lucerne, lupine, fruit trees and in forest, and ACE was detected in 33% of samples. The chemical reduction of the number of harmful forest insects is carried out using ground equipment (max. 2 treatments) and agricultural aviation equipment (max. 1 treatment) in the max. dose of 0.4 kg ha^{-1}. This means that acetamiprid gets into the body of the wild boar through food, contact and the respiratory tract. In toxicokinetic tests, ACE was absorbed rapidly after oral administration, and the highest concentration was observed in the adrenal glands, thyroid, liver and kidneys, without accumulation and main excretion in urine (Taira, 2014; EFSA, 2016). Metabolism studies were carried out on a group of farm animals (goats and poultry), which received ^{14}C-Ace in the dose of 10 mg kg^{-1} dry weight. It was estimated that most of the radioactive substance was excreted in urine and faeces, and the administered radioactivity was only recovered in organs, tissues, blood and milk or eggs (EFSA, 2016). Kenfack et al. (2018) showed the reproductive toxicity of ACE in male Guinea pigs.

In our studies, the parent compound acetamiprid and its metabolite were found in 9 samples, and ACE alone in 6 samples. The metabolite was not detected if ACE was at levels of 0.1–1.3 ng g^{-1}. In nine cases of ACE/ACE-D, the metabolite/parent substance ratio ranged from 3.8% to 8.5%. There was no relationship of higher ACE-D concentration as ACE concentration increased. In the sample where ACE was determined at 22.5 ng g^{-1} and ACE-D 1.3 ng g^{-1}, this ratio was 5.8%. In the sample where ACE/ACE-D concentration of 1.6/0.1 ng g^{-1} was recorded, the ratio was 6.4%, and in another sample ACE/ACE-D 2.3/0.2 ng g^{-1}, the ratio was 8.5%. The arithmetic mean concentration of ACE in these samples was 4.6 ng g^{-1} and of ACE-D 0.4 ng g^{-1}, and the average ratio was 3.8%. The highest concentration of total ACE + ACE-D was 23.8 ng g^{-1}. The metabolic profile was confirmed by feeding studies on cow and poultry, where ACE-D was recorded as the most abundant compound in all animal matrices (EFSA, 2016).

Thiacloprid possesses a mechanism of action similar to other NEOs and involves disruption of the insect's nervous system through stimulation of nicotinic acetylcholine receptors. The scientific research indicates that THI is harmful to bees, especially when its use is combined with other pressures on these insects (Wang et al., 2020). Thiacloprid has the status "not approved" (EC, 2009). According to the EU decision, THI may be in trade until August 3rd, 2020, while the final date of use is February 3rd, 2021. This means that, in Poland (2020) is the last year in which insecticides containing thiacloprid can be used on winter and spring rape and other crops: corn, potato, sugar beet and vegetables. In this study, the THI amide metabolite was found in four samples, single THI was reported in 11 samples. THI-A was not found in the samples where the THI concentration was lower than 2.3 ng g^{-1}. Where THI concentrations above 2.4 ng g^{-1} to 6.2 ng g^{-1} were recorded, the metabolite occurred in the 0.1–0.3 ng g^{-1} concentration range. The percentage ratio of metabolite to parent substance ranges from 3.3% to 7%, and is not correlated with THI concentration.

In mammals, IMI is hydrolyzed to 6-chloronicotinic acid, which is excreted or converted to guanidine (Tomlin, 1994). In plants, the major metabolites detected were imidacloprid-olefin and imidacloprid-guanidine (Tomlin, 2000). In our study, only one sample contained two metabolites, imidacloprid guanidine and imidacloprid urea, and no imidacloprid olefin was found. One of them, the metabolite IMI –guanidine, causes potential neurotoxity (Wang et al., 2018). The metabolites of imidacloprid guanidine with a concentration of 5.4 ng g^{-1} and imidacloprid urea with a concentration of 1.5 ng g^{-1} accounted for 7% and 1.95% of the IMI concentration value (76.9 ng g^{-1}), respectively. In 13 samples, IMI was at the low level 0.1–0.7 ng g^{-1}. Berheim et al. (2019) associated reduced activity of adult and fawn white-tailed deer with relatively high concentrations of IMI in spleens. The biological half-lives of CLO and IMI in soils were a few months and two to three years, respectively, as reported by Hopwood et al. (2012).

Thiamethoxam - an insecticide used to control a wide range of common pests (aphids; whiteflies; thrips; lacewings; leafhoppers; mealybugs; wireworms; ground beetles), according to EC Regulation 1107/2009 (repealing 91/414) (EC, 2009), possesses the status "not approved", except in Poland, where a temporary (180 days) permit was issued in 2018 and 2019. The discovery of thiamethoxam as a second-generation NEO was described by Maienfisch et al. (2001). Thiamethoxam is suspected to have carcinogenic activity (US EPA, 2003). Bredeson and Lundgren (2015) described the effect of a thiamethoxam seed treatment on pests and yield in cultivated sunflowers. Thiamethoxam was detected in four

samples, collected in pairs on May 11th and May 13th, and the concentration was 0.5 and 0.2 ng g^{-1}, with the additional presence of clothianidin. In contrast, a significantly higher concentration was recorded in the samples obtained on January 19th - 1.3 and 1.9 ng g^{-1}. It can be assumed that these compounds are derived from seed treatment of winter crops of rape and maize.

Acute and chronic effects of exposure to NEO pesticides on human health are discussed below. Recognizing potential risks of NEOs to mammals or humans, many human limit values for NEOs, such as acute/chronic reference dose (ARfD) and acceptable daily intakes (ADIs), have been established around the world (Supplementary Material - Table S1) (Wang et al., 2019).

Most of the current ADIs and ARfDs for NEOs were derived from the existing mammalian toxicity studies (Supplementary Material - Table S1) and adjusted by appropriate uncertainty factors (UFs) for conservatism. For example, the ARfD of ACE is 0.1 mg kg^{-1} bw day^{-1} as recommended by the U. S. Environmental Protection Agency (US EPA, 2002) based on rodent acute neurotoxicity (male LOAEL: 30 mg kg^{-1} bw day^{-1}) and chronic/oncogenicity studies (male and female LOAEL: 17.5 mg kg^{-1} bw day^{-1}), respectively. The European Union (EFSA, 2013; EC, 2017) refined ADI and ARfD for ACE to the even lower value of 0.025 mg kg^{-1} bw day^{-1}, when it applied a lower NOAEL of 2.5 mg kg^{-1} bw day^{-1} (developmental neurotoxicity). Health Canada (2010) recommended an ADI and ARfD for ACE at 0.008 mg kg^{-1} bw day^{-1} based on the NOAEL of 2.5 mg kg^{-1} bw day^{-1} and UF = 300. In this study, probabilistic hazard assessments using the ARfD approach were conducted for each neonicotinoid, and a geometric mean was used to calculate chronic exposure.

Additionally, we conducted a study of the correlation of the influence of age, weight and sex of wild boars, as well as the place of hunting, on the content of neonicotinoids and individual compounds (Merta et al., 2015) (Fig. 5). Our research shows that imidacloprid and its metabolites show the strongest correlation with the number of tested compounds and their total concentration. Clothianidin shows a weak correlation with the hunting circuit (0.23). The number of detected compounds is negatively correlated (−0.34) with gender. There was a strong and trivial correlation between the age and weight of animals (0.89) and the mean of these two factors with the hunting circumference (0.4). Very interesting was the low correlation between age and number of NEOs (0.08) and age and sum of NEOs (0.02). This may indicated of the lack of long-term accumulation of NEOs in wild boars meat and a sign of continuous consumption.

The “worst case” scenarios (large portion of consumption e.g. 100 g per capita-1 day-1), indicated that both acute and long term exposure is very low. The acute risk assessment was higher for multiresidue samples, but was generally low. The main way to exposure of neonicotinoids is consumption of plant origin food (Wu et al., 2020; Zhang et al., 2019; Ospina et al., 2019; Li et al., 2020;

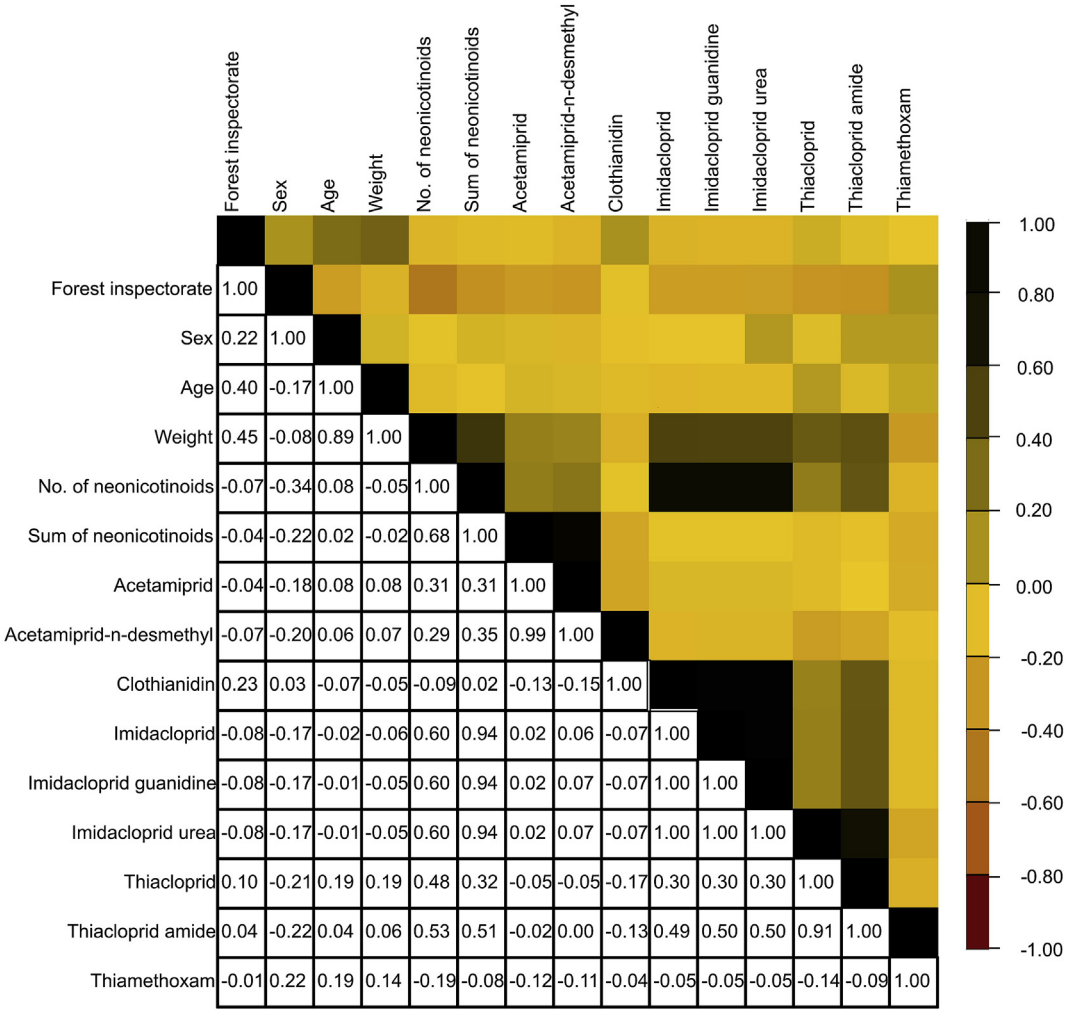

Fig. 5. Heatmap showing differentiation of neonicotinoid concentration depending on age, sex, weight, place and shooting date of wild boars.

Cimino et al., 2017). Often, as a potential sources of neonicotinoids are indicated drinking water (Mahai et al., 2021; Melin et al., 2020; Sultana et al., 2018) or air (Forero et al., 2017). Estimated neonicotinoid intake was usually within acceptable limits and did not exceed established reference doses. Unfortunately, studies on the health risks of consumers by neonicotinoids are still non-complex and fragmented. They usually relate to a specific type of food, country or region of the world, and the affected group of consumers or specific diets. The presented research on NEOs and their metabolites in wild boar meat is unique and should be deepened. These are concrete calculations for specific samples, but if we consider all routes of consumer exposure to NEOs, we need to be more critical toward these results.

5. Summary

The study was the first investigation to determine and characterize the spectrum of neonicotinoids pesticides present in the meat of game animals. For this purpose, the analytical procedure was modified during the sample preparation step by using 30 min ultrasonic techniques, supporting the extraction/isolation of NEO, freezing and purification step with the use of EMR-lipid sorbent and LC-MS/MS chromatographic analysis, and this made it possible to obtain satisfactory validation parameters and minimal matrix effect for all 12 tested neonicotinoids.

Despite the fact that the consumption of neonicotinoids in plant protection areas is increasing worldwide, there is relatively little information on their impact on human and animal health. There is also no data on potential exposure routes including consumption of the meat and meat products of wild boar, containing neonicotinoids and their metabolites. Due to the fact that more than 80% of wild boar meat samples contain NEOs, also in the form of multi-residue samples, within the range of 0.1–89.4 ng g^{-1}, the occurrence of NEOs raises some concern. The calculated hypothetic acute and chronic cumulative exposure is very low. We hope that the presented research on the presence of NEOs in wild boar meat will stimulate further scientific work on human health exposure and risk assessment. The research on the content of NEOs in the meat of hunted animals indicates the need to regularly monitor the quality of the obtained raw material as well as to exclude selected tissues of wild animals from consumption.

Credit author statement

Piotr Kaczyński, Conceptualization, Project administration, Methodology, Writing – original draft, Writing – review & editing. Bożena Łozowicka, Conceptualization, Investigation, Writing – original draft, Writing – review & editing. Maciej Perkowski, Resources, Data curation, Investigation Writing – original draft. Izabela Hrynko, Data curation, Formal analysis, Investigation. Wojciech Zoń;, Formal analysis, Visualization

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2021.130519.

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