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Original Research

Long-Term Investigation and Health Risk Assessment of Multi-class Fungicide Residues in Fruits

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Abstract

Fungicides are chemicals designed against fungi that are widely used in protection of fruits all over the world, for better quality and safety. These chemicals have been intensively used to protect Polish fruits for many years. The objective of this paper was to evaluate the fungicide residues monitored by chromatography and spectrophotometry in fruits in 2005-14 and health risk assessment. Fruits (974 samples) were analyzed for the presence of over 70 fungicides. Currants (68.5% of samples), apples (63.3%), and cherries (54.6%) were the fruits with residues found most frequently. Dithiocarbamates (27.4%) and captan (26.3%) were the most often detected. The highest concentrations were noted for boscalid and captan, ranging from 2.83 mg/kg (cherry) to 3.31 mg/kg (currant). The risk assessments involving hazards for human health were performed because of fungicide toxicity. The results of this investigation show how important it is to control fungicide residues in fruit, which require frequent chemical treatment for fungal diseases, to guarantee food quality and safety.

Keywords: fungicide residues, fruits, risk assessment, GC/ECD/NPD, HPLC/DAD/FLD, LC/MS/MS

Introduction

Fruits are unquestionably a rich source of vitamins, minerals, and dietary fiber, excellently assimilated by the human organism due to a "natural" manner of application. The demand for fruits systematically increases, and this phenomenon is surely impacted by increasingly greater interest in good nutrition and promotion of a healthy, balanced diet. Fruits are consumed in raw [1] or processed form, e.g., yogurts, kissels, jams, juices, marmalade, or ice cream [2].

According to 2014 data from the Agricultural Market Agency (ARR), Poland is an important fruit producer in Europe. Cherry, currant, raspberry, and apple production and export rank below Italy, Spain, and France, but above Greece and Germany. Poland is also a significant producer of strawberries, gooseberries, and chokeberries [3].

The durability period and nutritional values of fruits are determined by fungal diseases and chemical pollution caused by pesticides [4]. The most common fungal diseases attacking fruit include powdery mildew,

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Table 1. Analysed active substances and their substance groups.

Substance group	Active substances	Substance group	Active substances	Substance group	Active substances
Acylamino acid (Group 1)	benalaxyl[3,4]	Imidazole (Group 15)	prochloraz[3,4] imazalil[3,4]	Strobilurin (Group 29)	azoxystrobin[3,4] dimoxystrobin[3,4] kresoxim-methyl[3,4] pyraclostrobin[3,4] picoxystrobin[3,4] trifloxystrobin[3,4]
Amine (Group 2)	diphenylamine[3]	Morpholine (Group 16)	dimethomorph[3,4] fenpropimorph[3,4]		
Anilinopyrimidine (Group 3)	cyprodinil[3,4] mepanipyrim[3,4] pyrimethanil[3,4]	Oxazolidin (Group 17)	chlozolinate[3]		
Benzamide (Group 4)	zoxamide[3,4] fluopicolide[3,4]	Oxazole (Group 18)	famoxadone[3,4] vinclozolin[3,4]	Strobilurin (Group 30)	bensulfuron-methyl[4]
Benzimidazole (Group 5)	carbendazim[2,4] thiabendazole[3,4]	Quinoline (Group 19)	qinoxyfen[3,4]	Triazole (Group 31)	azaconazole[3,4] bitertanol[3,4] bromuconazole[3,4] cyproconazole[3,4] difenoconazole[3,4] diniconazole[3,4] epoxiconazole[3] fenbuconazole[3,4] flusilazole[3,4] flutriafol[3,4] fluquinconazole[3] hexaconazole[3,4] imibenconazole[3] metconazole[3,4] myclobutanil[3,4] paclobutrazol[3,4] penconazole[3,4] propiconazole[3,4] prothioconazole-desthio[3,4] tebuconazole[3,4] tetraconazole[3,4] triticonazole[3,4] triadimenol[3,4] triadimefon[3,4]
Benzophenone (Group 6)	metrafenone[3,4]	Oxathiin (Group 20)	flutolanil[3,4]		
Carbamate (Group 7)	bendiocarb[4] benfuracarb[4] dithiocarbamates*[1] iprovalicarb[3,4]	Pyrimidine (Group 21)	fenarimol[3,4]		
Carboxamide (Group 8)	boscalid[3,4]	Phthalimide (Group 22)	captafol[3,4] captan[3] folpet[3,4]		
Conazole (Group 9)	etaconazole[3,4]	Phenylamide (Group 23)	oxadixyl[3,4] metalaxyl[3,4]		
Chloronitrile (Group 10)	chlorothalonil[3]	Phenylpyrrole (Group 24)	fludioxonil[3,4]		
Chlorinated hydrocarbon (Group 11)	HCB[3]	Phenylurea (Group 25)	pencycuron[3,4]		
Chlorophenyl (Group 12)	dicloran[3] tolclofos-methyl[3,4] tecnazene[3] quintozene[3]	Pyrimidinol (Group 26)	bupirimate[3,4]		
Dicarboximide (Group 13)	iprodione[3,4] procymidone[3,4]	Phosphorothiolate (Group 27)	pyrazophos[3,4] isoprothiolane[3,4]		
Hydroxyanilide (Group 14)	fenhexamid[3,4]	Sulphamide (Group 28)	tolylfluanid[3,4] dichlofluanid[3,4]		

*determined as CS_2 residues [1] spectrophotometric, [2] HPLC/DAD/FLD, [3] GC/ECD/NPD, [4] LC/MS/MS

responsible for the destruction of grapes [5]. Powdery mildew is one of the most widespread and dangerous diseases, which can infect all volatile parts of a vine, including leaves, stems, inflorescence, and fruits [6]. Gray mold, caused by *Botrytis cinerea*, is the most destructive postharvest disease of grapes [7], apples [8], strawberries [9], and peaches [10]. The main harmful effect of gray mold is reduction of the yield and quality of the harvested fruit. In the period of vegetation and fruiting, apple and pear trees are very vulnerable to attacks of the pathogens *Venturia inaequalis* and *Venturia pirina*, causing apple scab and pear scab, respectively [11]. In turn, peach leaf curl, caused by the fungus called *Taphrina deformans*, infects leaves and shoots of peaches and nectarines [12].

To protect fruits against diverse fungal diseases, it is necessary to use plant protection products (p.p.p.) [13]. Fungicides are chemicals designed against fungi, widely used in plant protection all over the world [14], for better quality and safety. These chemicals are intensively used in protection of Polish fruits for many years. Currently, to protect fruit plants from fungal diseases, more than 80 fungicides are registered in Poland [15]. For example, azoxystrobin, kresoxim-methyl, trifloxystrobin, and pyraclostrobin (strobilurin fungicides) are used to treat grape powdery mildew [16]. Phenylpyrrole (e.g., fludioxonil), anilinopyrimidine (e.g., pyrimethanil) [17], and carboxamide (e.g., boscalid fungicides) are used to control gray mold.

Despite their many merits such as better yields and quality, pesticides are one of the most toxic, stable, and mobile substances in the environment. They may penetrate the tissues of fruits and appear in the pulp and juice of fruits

[18]. Widespread use of pesticides from various chemical groups, characterized by different mechanisms, may pose a hazard to human health and the natural environment due to their toxicity, high persistence, and bioaccumulation [19]. Pesticide residues in fruits, depending on significant properties of their active ingredients, may cause many adverse side effects, from allergies to chronic diseases and cancer [20].

The objective of this paper was to evaluate multi-class fungicide residues in Polish fruits in 2005-14 using spectrophotometric and chromatographic techniques (GC/ECD/NPD, HPLC/DAD/FLD, LC/MS/MS) and health risk assessment. Because of fungicide toxicity, the results of this investigation show how important it is to control these pesticides, in particular, in fruit requiring frequent chemical treatment for fungal diseases, to guaranty food quality and consumer safety.

Materials and Methods

Samples

In the framework of the official testing of residues of plant protection products conducted by the Ministry of Agriculture and Rural Development, in total 974 samples of fruits (nuts, berries, pome, and stone fruits) were analyzed at the Official Laboratory of Pesticide Residue in Bialystok, Poland (53°139'N latitude and 23°159'E longitude). These samples were collected from May 2005 to November 2014 by the regional inspectors of plant protection and seed according to a predetermined schedule. Raw fruit samples were subjected to the analytical procedures described below.

Chemicals and Reagents

All reagents were analytical grade. Fungicides (77 active substances) were obtained from Dr. Ehrenstorfer Laboratory (Germany). Standard stock solutions (purity for all standards >95%) of various concentrations were prepared in acetone and stored in dark glass bottles below 4ºC.

Pesticides (Table 1) belonging to fungicides from various groups in terms of chemical structure, e.g., anilinopyrimidine, benzimidazole, carboxamide, dicarboximide, phthalimide, strobilurin, sulphamide, triazole, and carbamate (31 groups), were analyzed.

Analytical Procedure

We used four methods for sample preparation as described in our earlier work [21-22]: two multi-residue methods (MRM) and two single residue methods (SRM) (Fig. 1).

Single Residue Method:

– Isolation and determination of dithiocarbamates (DTC) using spectrophotometry.

Dithiocarbamate residues were determined as a group (mancozeb, maneb, methiram, propineb, thiram, ziram) by a spectrophotometric method and expressed as carbon disulphide. Fifty grams of sample with 60 mL of hydrochloric acid (4 M) and tin (II) chloride (0.13 M) was heated for 45 minutes (temperature about 80ºC) to release carbon disulphide from dithiocarbamates to obtain pH between 11-12.

Carbon disulphide was separated and collected in a methanolic solution of potassium hydroxide, forming potassium xantogenate, which was next heated with zinc acetate to obtain zinc sulfide. Finally, the quantity of the formed complex (final volume 25 mL) was estimated and the absorbance at a wavelength of 662 nm on a spectrophotometer was measured (Helios Delta VIS and Nidet Evolution 220 LC).

– Isolation and determination of carbendazim residues using high-performance liquid chromatography (HPLC).

A representative sample of 20 g of 150 mL acetone was homogenized for 5 min. Then 2.5 g of celite was added and filtered through a Buchner funnel. The final filtrate was evaporated in a rotary evaporator, leaving about 20 mL. Then this solution was applied to a ChemElut cartridge containing diatomaceous earth. After 25 min of equilibration, the pesticides were eluted with dichloromethane. The organic solvent was evaporated to dryness using a rotary vacuum evaporator at 40°C and dissolved in a 2 mL volume of a mixture of acetonitrile/water (2:8, v/v). The extracts were analyzed with liquid chromatography (Waters Alliance 2695 chromatograph) with simultaneous use of a diode array detector (Waters 2996) at 285 nm and fluorescence detector (Waters 2475) (λ_{ex} = 285 nm, λ_{em} = 315 nm). The external standard method was used, by applying 100 µL of standard solution on the column (Supelcosil LC-18, 5 µm, 250 × 4.6 mm). The mobile phase was acetonitrile-phosphate buffer pH = 8, delivered at a flow rate of 0.8 ml/min, with a gradient composition consisting of 20% (v/v) acetonitrile for 2 min, a linear increase over 13 min to 50% acetonitrile, then an increase to 80% acetonitrile over 5 min, and finally a decrease at 20% acetonitrile over 5 min.

Multi-residue methods:

– Isolation and determination of fungicide residues using matrix solid phase disepersion (MSPD) and gas chromatography with a dual detection system (GC/ECD/NPD).

Two grams of homogenized sample was put in a mortar and manually blended with 4 g of solid support using a pestle to obtain a homogeneous mixture, and then quantitatively transferred with a spatula to a glass macro column packed with anhydrous sodium sulfate (5.0 g) and silica gel (2.5 g). Depending on the commodity, either silica gel (strawberries, raspberries, plums, grapes, peaches) or florisil (apples, currants, gooseberries, blueberries, sour cherries, sweet cherries, pears) was used. The analytes were eluted using 15 mL hexane/acetone (8:2, v/v) and 15 mL hexane/diethyl

Table 2. Optimised MRM transitions by direct ESI source infusion.

Pesticide	MRM transition, m/z	DP (V)	CE (V)	CXP (V)		Pesticide	MRM transition, m/z	DP (V)	CE (V)	CXP (V)
Azaconazole	300 > 159	86	37	10		Iprodione	330.1 > 244.9	61	21	14
	300 > 231	86	23	12			330.1 > 101	61	37	6
Azoxystrobin	404.1 > 371.9	61	19	20		Iprovalicarb	321.2 > 119.1	61	23	8
	404.1 > 344	61	33	18			321.2 > 203.2	61	12	10
Benalaxyl	326.1 > 148.1	76	27	8		Isoprothiolane	291 > 231	21	15	12
	326.1 > 208.2	76	21	12			291 > 188.9	21	29	10
Bendiocarb	224.11 > 167.1	36	13	10		Kresoxim-methyl	314.1 > 206	70	10	10
	224.11 > 109	36	23	8			314.1 > 116.1	70	19	8
Benfuracarb	411.2 > 195.1	66	28	10		Mepanipyrim	224 > 106	131	33	18
	411.2 > 89.9	66	21	10			224 > 66	131	59	10
Bensulfuron-methyl	411 > 149	101	27	8		Metalaxyl	280.1 > 220.1	41	19	12
	411 > 118.9	101	57	12			280.1 > 160.1	41	31	10
Bitertanol	338 > 269	50	15	16		Metconazole	320.1 > 70	56	63	8
	338 > 70	50	25	16			320.1 > 124.9	56	55	6
Boscalid	343 > 307	116	27	16		Metrafenone	409 > 209	61	19	12
	343 > 140	116	25	8			409 > 226.9	61	27	12
Bromuconazole	378 > 159	91	35	10		Myclobutanil	289 > 70	71	23	8
	378 > 70	91	61	8			289 > 125	71	45	8
Bupirimate	317.1 > 166.1	91	31	10		Oxadixyl	279.1 > 219.1	46	15	12
	317.1 > 108.1	91	33	8			279.1 > 133.1	46	29	8
Captafol	350 > 313.9	76	17	4		Paclobutrazol	294 > 70	66	51	10
	350 > 312	76	17	4			294 > 125.1	66	49	8
Carbendazim	192 > 160.1	71	27	10		Penconazole	284 > 70	56	21	8
	192 > 132.1	71	43	8			284 > 158.9	56	35	8
Cyproconazole	292 > 70	61	23	8		Pencycuron	329 > 124.9	70	29	6
	292 > 125	61	45	6			331 > 126.9	70	31	6
Cyprodinil	226.1 > 93	71	43	12		Picoxystrobin	368 > 205	36	13	12
	226.1 > 77	71	61	12			368 > 145	36	29	8
Dichlofluanid	350 > 223.9	60	21	12		Prochloraz	376 > 307.9	16	17	16
	350 > 123	60	39	6			376 > 70	16	29	10
Difenoconazole	406 > 251	96	35	14		Procymidone	284.1 >256	50	23	14
	406 > 188	96	59	10			284.1 > 67	50	50	8
Dimethomorph	388.1 > 301	36	29	16		Propiconazole	342 > 159	100	37	10
	388.1 > 165.1	36	41	10			342 > 69	100	23	8
Dimoxystrobin	327 > 205	61	15	12		Prothioconazol-desthio	312 > 69.9	96	61	8
	327 > 116.1	61	29	6			312 > 125	96	37	6
Diniconazole	326.1 > 70.1	25	63	8		Pyraclostrobin	388 > 194.1	41	17	12
	326.1 > 158.9	25	39	10			388 > 163.1	41	33	10

Continued

Etaconazole	328.1 > 159	61	37	10		Pyrazofos	374 > 222	81	29	7
	328.1 > 123	61	75	6			374 > 194	81	43	5
Famoxadon	392.1 > 331.1	50	11	18		Pyrimethanil	200.1 > 107	96	33	12
	392.1 > 238.1	50	23	14			200.1 > 82	96	32	9
Fenarimol	331.1 > 268	70	31	14		Qinoxyfen	308 > 196.9	46	43	10
	331.1 > 81	70	35	16			308 > 162	46	59	8
Fenbuconazole	337 > 125.1	96	35	8		Tebuconazol	308.1 > 70	41	57	8
	337 > 70	96	23	8			308.1 > 125.1	41	59	8
Fenhexamid	302.1 > 97.1	86	31	6		Tetraconazole	372 > 159	26	37	10
	302.1 > 55	86	57	8			372 > 70	26	73	10
Fenpropimorph	304.2 > 147.1	91	39	8		Thiabendazol	202 > 175	121	37	10
	304.2 > 117.1	91	77	8			202 > 131.1	121	45	8
Fludioxonil	266.1 > 229	65	17	12		Tolclofos-methyl	301 > 268.9	61	23	14
	266.1 > 157.9	65	45	8			301 > 174.9	61	35	10
Fluopicolid	383 > 172.9	91	31	10		Tolylfluanid	364 > 238	100	19	12
	383 > 108.9	91	89	6			364 > 137	100	39	8
Flusilazole	316.1 > 247	26	25	14		Triadimefon	294.1 > 197.1	51	21	12
	316.1 > 165.1	26	35	10			294.1 > 225	51	17	11
Flutolanil	324.1 > 262	16	25	14		Triadimenol	296 > 70	56	19	8
	324.1 > 242	16	35	14			296 > 227	56	15	8
Flutriafol	302 > 123	61	37	6		Trifloxystrobin	409.1 > 186	61	25	10
	302 > 109	61	37	5			409.1 > 206.1	61	19	12
Folpet	315 > 130	50	39	6		Triticonazole	318 > 70	71	49	8
	315 > 163	50	19	7			318 > 125	71	47	8
Hexaconazole	314.1 > 70	21	49	8		Vinclozolin	286 > 214	76	17	12
	314.1 > 159	21	37	10			286 > 174	76	45	10
Imazalil	297 > 159	81	31	10		Zoxamide	336.1 > 186.9	46	31	10
	297 > 201	81	23	10			336.1 > 159	46	55	10

DP–declustering potential; CE–collision energy; CXP–collision cell exit potential

ether/acetone (1:2:2, v/v/v). The extract was evaporated to dryness using a rotary vacuum evaporator at about 40ºC. Then the eluate was re-dissolved using 2 mL of hexane/acetone (9:1, v/v). The fruit samples by an Agilent (Waldbronn, Germany) model 7890A gas chromatograph equipped with EC and NP detectors were analyzed. A capillary column HP-5 (5 %-phenylmethylpolysiloxane) (30 m × 0.32 mm, 0.5 μm film thickness) and for confirmation of residues a mid-polarity column HP-35 ((35 %-Phenyl)-methylpolysiloxane (30 m × 0.32 mm, 0.5 μm film thickness) were used. The injector and detectors temperature were set at 210 and 300ºC, respectively. The oven temperature was programmed as follows: 120 to 190ºC at a rate of 16ºC min^{-1}, increased to 230ºC at 8ºC min^{-1}, and then to 285ºC at 18ºC min^{-1}, for 18 min. Helium (purity 5.0) was used as a carrier gas at a flow rate of 3.0 ml min^{-1}. Nitrogen (purity 5.0) as a makeup gas was used for EC, and NP detectors were set at 57 and 8 ml min^{-1}, respectively. The air (purity 5.0) and hydrogen (purity 5.0) (for NPD) gas flows were set at 60 and 3 mL min^{-1}, respectively. Two mL of the extracted sample solution was injected.

– Isolation and determination of fungicide residues using QuEChERS and liquid chromatography (LC-MS/MS).

Ten grams of homogenized fruit sample were weighted in a 50 mL polypropylene centrifuge tube. The sample was extracted with 10 mL of acetonitrile and shaken vigorously

for 1 min and vortexed for 1 min using a digital Vortex-Mixer (Velp Scientifica, Usmate, Italy). Next, 4 g $MgSO_4$, 1 g NaCl, 1 g trisodium citrate dehydrate, and 0.5 g disodium hydrogen citrate sesquehydrate were added. The tubes were immediately shaken for 1 min, vortexed in a Vortex-Mixer for 1 min, and then centrifuged for 5 min at 4,500 rpm. Acetonitrile extract was transferred into 15 mL tubes containing 150 mg anhydrous $MgSO_4$. Depending on the commodity, either 25 mg PSA (gooseberries, sweet cherries, pears, apples, plums, strawberries, grapes) or 25 mg PSA and 2.5 g GBC (raspberries, blueberries, blueberries) was used. The tubes were vortexed for 1 min and centrifuged at 4,500 rpm for 10 min. One ml of the final extract was filtered through a 0.2 m hydrophilic PTFE filter, transferred into the appropriately labeled autosampler vial, and subsequently analyzed via LC-MS/MS. An Eksigent Ultra LC-100 (Eksigent Technologies, Dublin, CA, USA) liquid chromatography system operated at a flow rate of 0.45 ml min^{-1} without split using a SunFire C_{18} 3.5 µm, 2.1 x 100 mm (Waters) analytical column, maintained at 50ºC during the experiments. The volume injected into the LC-MS/MS system was 10 µL. The binary mobile phase consisted of water with 0.5% formic acid and 5mM ammonium formate (phase A) and methanol with 0.5% formic acid and 5 mM ammonium formate (phase B). The initial composition of 95% A and 5% B (v/v) was held for 2.0 min., followed by linear ramping to 95% of B in 8 min. and was held for 7 min. After ramping, the mobile phase returned to the initial composition in 2 minutes. The total chromatographic run time was 25.0 min. System MS/MS 6,500 QTRAP (AB Sciex Instruments, Foster City, CA) was used for mass spectrometric analysis, equipped with an electrospray ionization source (ESI). The capillary voltage was maintained at 4,500V for positive ion mode and the temperature of the turbo heaters was set at 450ºC. For the nebulizer gas (GS1), auxiliary gas (GS2), and curtain gas (CUR), nitrogen was used at a pressure of 65, 45, and 35 psi, respectively. For the nebulizer and collision gases a nitrogen was used. Optimization of the compounds was performed by injecting individual standard solutions directly into the source (flow injection analysis methods, or FIA) (Table 2).

Validation of Methods

Validation of the analytical methods was carried out in accordance with European Commission (EC) guidelines. The validation studies were performed using pesticide-free fruit samples. Calibration standards were prepared in the fruit matrix solution to produce a final concentration of spiking concentrations:

- *For spectrophotometric*: 0.03 mg/kg, 0.1 mg/kg and 5.0 mg/kg;
- *For HPLC*: 0.02 mg/kg, 0.5 mg/kg, 2.0 mg/kg;
- *For GC*: range 0.002-0.05 mg/kg, range 0.05-0.5 mg/kg, and range 0.5-5.0 mg/kg;
- *For LC-MS/MS*: range 0.005-0.01 mg/kg, range 0.01-0.2 mg/kg, and range 0.2-1.0 mg/kg.

The accuracy and precision of the method were evaluated by performing recovery studies and expressed as relative standard deviation (RSD, %) and mean recovery, respectively. Repeatability was calculated for five days using five replicates for each level of three different concentration levels.

Quality Check

The laboratory participates in international proficiency tests organised by the Food Analysis Performance Assessment Scheme (FAPAS; Central Science Laboratory in York) and by the European Commission (University of Almeria) every year, and achieves correct results. All of the analyses were conducted using accredited methods by the Polish Centre of Accreditation (PCA) in compliance with PN-EN ISO/IEC 17 025.

Risk Assessment

The health risk estimation through a comparison of detected fungicide residues with the established Acceptable Daily Intake (ADI) or Acute Reference Dose (ARfD) was calculated. The level of residue concentration in a product was determined as the arithmetic mean of all the results obtained. In the case of results under the limit of detection (LOD) of analytical methods for intake calculations, LOD values were taken. The long- or short-term dietary consumer exposure to pesticide residues was estimated using an EFSA calculation model: Pesticide Residue Intake Model "PRIMo" revision 2. This model is based on national food consumption and unit weights implementing internationally agreed risk assessment methodologies to assess the exposure of consumers, accepting consumption at the level of the 97.5 percentile [23]. Additionally, long-term risk assessment comparing the pesticide toxicological data for groups: cluster E, adults and children, and the Polish general population by calculating the hazard quotient (HQ) was performed. Its value was estimated by dividing the international estimated daily intake with the relevant acceptable daily intake, which was considered to be safe levels of exposure over a lifetime.

Short-Term Risk

Short-term risk was estimated by comparing single intake of the highest detected residue of plant protection products (HR_P) full portion consumption data for the commodity unit (F) to a set volume ARfD (Acute Reference Dose). The estimated short-term intake (ESTI) was calculated according to the following formula [24]:

$$ESTI = \square (F \times HR\_P) / mean\_body\_weight$$

Long-Term Risk

The acceptable daily intake (ADI) is the estimated amount of a substance in food, expressed on a body weight basis, that can be ingested daily over a lifetime without appreciable chronic, long-term risk to any consumer. The

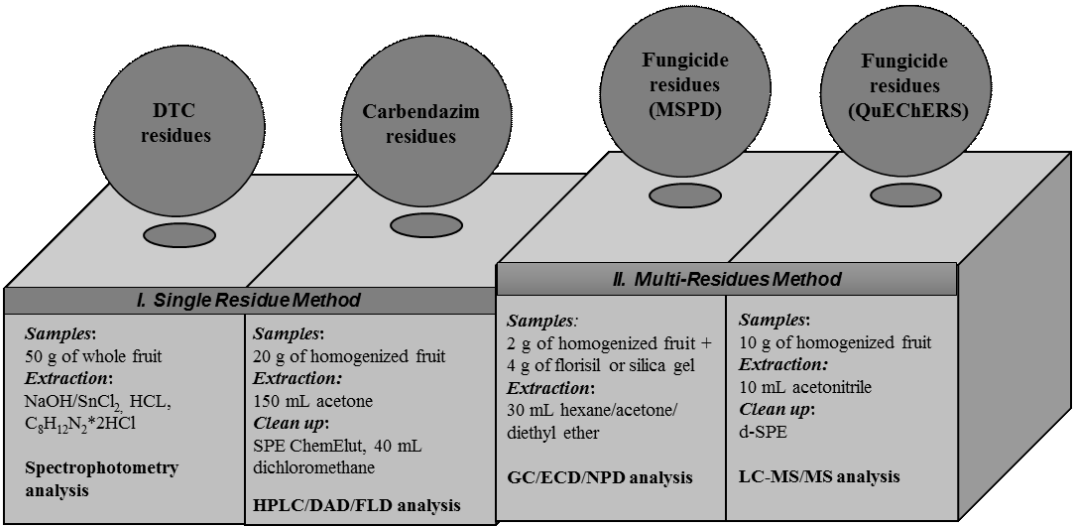


Fig. 1. Scheme of sample preparation procedures.

estimated daily intake (EDI) was calculated according to the following formula, where Fi is food-consumption data and RLi is residue level in the commodity:

$$EDI = \sum (Fi \times RLi) / mean\_body\_weight$$

The long-term risk was assessed by calculating the hazard quotient (HQ) by dividing the estimated daily intake by the relevant acceptable daily intake:

$$HQ = (EDI/ADI) \times 100\%$$

Results and Discussion

Analytical Methods and Validation Parameters

Four methods were used in sample preparation (Fig. 1). Procedure one of two multi-residue methods (MRM) for determination of 73 compounds using gas chromatography with selective detectors: electron capture (EC) and nitorgen phopsphorous (NP) is described [25]. Two single residue method (SRM) for determination of carbendazime using liquid chromatography and dithitocarbamates by spectrophotometric (described in our earlier published work [22, 26]) were used. LC/MS/MS for determining 66 compounds was performed and validation parameters are presented in Table 3. In 2014 the carbendazime was included in the MRM method and analysed by LC/MS/MS.

The preparation and analytical methods for fungicide residues in fruit sample were validated in terms of linearity and limits of detection respectively. The limit of quantification (LOQ) and the limit of detection (LOD) were calculated using signal-to-noise ratios (S/N) of 10:1 and 3:1 for the pesticide, respectively. The LOQs ranged from 0.02 to 0.05 mg/kg (*for spectrophotometric*), 0.01 to 0.04 mg/kg (*for HPLC*), 0.001 to 0.009 mg/kg (*for GC*), and 0.005 to 0.009 mg/kg (for *LC-MS/MS*). LODs for all pesticides analyzed were lower than the respective maximum residue levels (MRLs) established by the European regulation for fruits. Linearity was evaluated by the calculation of a five-point linear plot with three replicates, based on linear regression and correlation coefficient (R^2). All pesticides showed linearity in the concentration range 0.03-5.0 mg/kg (*for spectrophotometric*), 0.02-2.0 mg/kg (*for HPLC*), 0.003-5.0 mg/kg (*for GC*), and 0.002-1.00 mg/kg (*for LC-MS/MS*) with correlation coefficients higher than 0.99553. Mean recoveries for fruit samples spiked at three fortification levels ranged from 71.07 to 119.90% with exceptions of bupirimate, dimethomorph (40-70%) and triadimenol (121%), with RSDs of 0.9-9.4% for a period of five days. However, the range of 60-140% can be used in routine multiresidue analysis [27-28]. These results indicated that the validation parameters were good, and consequently that fungicides were satisfactorily determined using these methods.

These results suggest that the sample preparation procedures and various methods of instrumental analysis are suitable for the analysis of the wide-ranging group of fungicide residues in fruit samples.

Trends Over the Last 10 Years of Fungicide Residues in Fruits

A total of 974 fruit samples were analyzed in 2005-14. Fungicide residues in 52.0% of the samples were detected. There were pesticide residues below the MRLs in 50.2% samples, while 1.7% of tested samples exceeded MRLs. Pesticide residues were not detected in 48.0% of samples. The percentage of contaminated samples by fungicides in 2005 was 53.0%, in 2006 45.0%, in 2007 61.0%, in 2008 65.7%, in 2009 45.7%, in 2010 35.2%, in 2011 69.2%, in 2012 33.3%, in 2013 72.4%, and in 2014 56.1% (Fig. 2). The percentage of samples in which exceedances of the maximum residue levels (MRLs) of

Table 3. Validation results.

Pesticide	Apple			Black currant			Sour cherry		
	Recovery (mean) (%)	RSD (%)	U (%)	Recovery (mean) (%)	RSD (%)	U (%)	Recovery (mean) (%)	RSD (%)	U (%)
Azaconazole	93.8	7	17.2	86	7.0	19	101	7.8	16
Azoxystrobin	98.8	6	16.3	88	6.1	18	98	6.9	16
Benalaxyl	87.1	7	18.5	88	6.5	18	88	7.5	18
Bendiocarb	98.0	6	16.4	97	6.5	17	95	7.2	17
Benfuracarb	86.3	10	18.6	95	6.2	17	96	8.0	17
Bensulfuron-methyl	96.6	7	16.7	85	7.6	19	97	7.2	17
Bitertanol	83.0	9	19.4	75	6.6	22	83	9.4	19
Boscalid	95.2	8	16.9	86	6.9	19	95	8.1	17
Bromuconazole	85.7	7	18.8	84	7.3	19	85	8.2	19
Bupirimate	75.9	7	21.2	85	6.6	19	74	8.8	22
Captafol	99.6	7	16.2	73	6.7	22	101	7.8	16
Carbendazim	83.5	15	19.3	95	8.9	17	85	7.7	19
Cyproconazole	86.0	7	18.7	75	6.6	22	85	8.2	19
Cyprodinil	95.2	8	16.9	78	6.6	21	86	7.3	19
Dichlofluanid	83.1	8	19.4	83	10.6	19	82	11.2	20
Difenoconazole	86.2	10	18.7	73	6.7	22	92	7.4	18
Dimethomorph	75.9	7	21.2	85	6.6	19	74	8.8	22
Dimoxystrobin	85.8	7	18.8	85	6.3	19	85	7.9	19
Diniconazole	86.3	10	18.6	95	6.2	17	96	8.0	17
Etaconazole	93.8	7	17.2	81	6.6	20	98	6.9	16
Famoxadon	92.5	6	17.4	87	6.4	19	97	7.3	17
Fenarimol	76.6	8	21.0	96	6.5	17	86	8.2	19
Fenbuconazole	93.4	7	17.2	87	6.0	19	91	7.1	18
Fenhexamid	76.0	8	21.2	77	7.4	24	75	8.7	22
Fenpropimorph	75.5	6	21.3	82	6.1	20	77	8.5	21
Fludioxonil	86.2	10	18.7	95	8.9	17	95	8.5	17
Fluopicolid	77.3	7	20.8	86	6.8	19	83	9.4	19
Flusilazole	86.0	7	18.7	75	7.0	22	74	8.8	22
Flutolanil	95.2	8	16.9	94	6.9	17	95	8.5	17
Flutriafol	86.2	10	18.7	86	6.9	19	86	7.3	19
Folpet	84.9	8	19.0	92	5.7	17	94	7.6	17
Hexaconazole	93.8	7	17.2	86	7.0	19	101	7.8	16
Imazalil	76.6	8	21.0	96	6.5	17	86	8.2	19
Iprodione	74.0	8	21.7	95	6.2	17	92	7.4	18
Iprovalicarb	99.6	7	16.2	73	6.7	22	101	7.8	16
Isoprothiolane	86.0	7	18.7	75	6.6	22	86	7.2	19
Kresoxim-methyl	99.6	7	16.2	99	7.0	16	85	7.1	19
Mepanipyrim	97.0	10	16.6	95	8.9	17	78	8.5	21

Continued

Metalaxyl	98.8	6	16.3	88	6.1	18	98	6.9	16
Metconazole	77.3	7	20.8	75	7.0	22	95	7.8	17
Metrafenone	85.7	7	18.8	86	6.9	19	74	8.8	22
Myclobutanil	92.5	6	17.4	87	6.4	19	97	7.3	17
Oxadixyl	84.6	8	19.0	82	7.0	20	82	11.3	20
Paclobutrazol	86.9	7	18.5	76	7.6	21	86	7.4	19
Penconazole	93.4	7	17.2	87	6.0	19	91	7.1	18
Pencycuron	84.5	7	19.1	83	6.9	19	84	7.2	19
Picoxystrobin	100.6	7	16.0	85	6.9	19	104	7.2	15
Prochloraz	98.0	6	16.4	88	6.7	18	97	7.2	17
Procymidone	98.8	6	16.3	73	6.7	22	95	7.2	17
Propiconazole	97.0	7	16.6	96	6.5	17	85	7.1	19
Prothioconazol-desthio	86.0	7	18.7	75	7.0	22	74	8.8	22
Pyraclostrobin	76.4	11	21.1	99	7.0	16	94	9.1	17
Pyrazofos	87.4	7	18.4	84	7.3	19	85	7.9	19
Pyrimethanil	95.0	7	16.9	96	7.0	17	95	7.8	17
Qinoxyfen	76.1	8	21.1	88	6.7	18	88	7.5	18
Tebuconazol	99.6	7	16.2	99	7.0	16	85	7.1	19
Tetraconazole	86.3	10	18.6	95	6.2	17	96	8.0	17
Thiabendazol	95.0	7	16.9	81	6.6	20	95	7.3	17
Tolclofos-methyl	96.6	7	16.7	85	7.6	19	85	7.8	19
Tolylfluanid	86.3	10	18.6	95	6.2	17	96	8.0	17
Triadimefon	98.8	6	16.3	88	6.1	18	98	6.9	16
Triadimenol	93.8	7	17.2	86	7.0	19	101	7.8	16
Trifloxystrobin	83.5	15	19.3	94	6.9	17	95	9.9	17
Triticonazole	83.1	8	19.4	83	10.6	19	82	11.2	20
Vinclozolin	82.2	7	19.3	85	7.3	19	86	8.2	19
Zoxamide	83.0	9	19.4	95	6.2	17	95	8.1	17

U – uncertainty

fungicides were observed fell within the range of 0.8-2.6%, excluding 2007 and 2013, when it was 4.0% and 4.8%, respectively. No MRL exceedances occurred in 2012 and 2014.

According to Poland's Central Statistical Office (GUS), in 2005-14 mostly herbicides (55%) were used in chemical plant protection. The second important group of utilized preparations was fungicides (29.4%) [29]. Fig. 2 shows the trend of fungicide consumption in 2005-14 in Poland. The trend of fungicide consumption was characterized by small decreases and increases and was not strongly correlated with the prevailing weather conditions. Weather conditions play a crucial role in the development of fungal pathogens. On the development of fungal diseases, among others, air humidity, precipitation, and temperature have an impact. At high humidity and moderate air temperature, fruit bodies with ascospores matue quickly. Precipitation contributes to germination of spores and propagation of infections. In our study, the weather could influence the obtained results. For example, 2007 and 2013 resulted in a high percentage of samples with residues characterized by high precipitation and temperatures.

The Most Frequently Detected Fungicide Groups

In the research period under discussion, anilinopyrimidines, phthalimides, carbamates, carboxamides, and triazoles were the most frequently detected fungicide

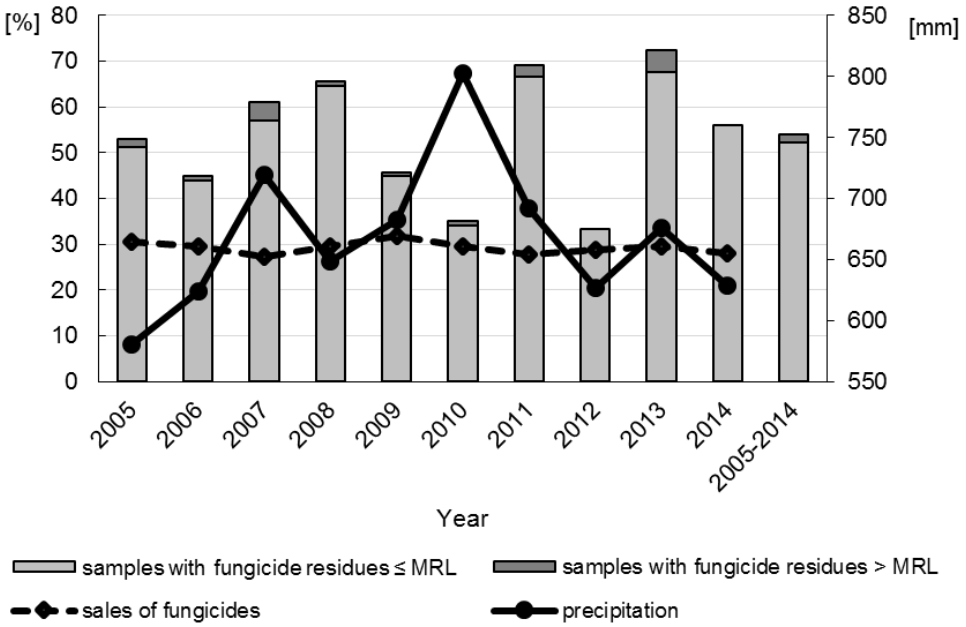


Fig. 2. Percentage of fruit samples contaminated by fungicides, sales of fungicides, and the average annual precipitation for Poland in 2005-14.

groups (Fig. 3, Table 1). The characteristics of the detected fungicide groups in this study are as follows.

Group 22, phthalimide fungicides: In 2005-07, as well as in 2009, the most frequently detected were phthalimides. Phthalimide fungicides such as captafol, captain, and folpet are multi-site inhibitors that affect energy production. The main mode of action of these substances consists in blocking of respiratory processes of pathogens by creating persistent compounds in the structure of enzymatic proteins. Phthalimide fungicides, blocking many enzymes acting at various stages of respiration, hinder the energetic processes of a fungus. Captan and folpet are carcinogenic substances.

Group 7, carbamate fungicides: In 2008 and in 2010-14 the most frequently detected fungicides were those from the carbamate group. Carbamate fungicides disrupt the formation of fungal cell walls by interfering with synthesis of phospholipids and fatty acids. They also affect mycelial growth, spore production, and germination.

Group 8, carboxamide fungicides: Beginning in 2009, substances from the carboxamide group were detected in the analyzed samples. Carboxamide fungicides inhibit mitochondrial complex II of fungal respiration, interrupting electron transport in the mitochondrial respiratory chain.

Group 30, triazole fungicides: Triazole fungicides were detected systematically at a similar level (below 10

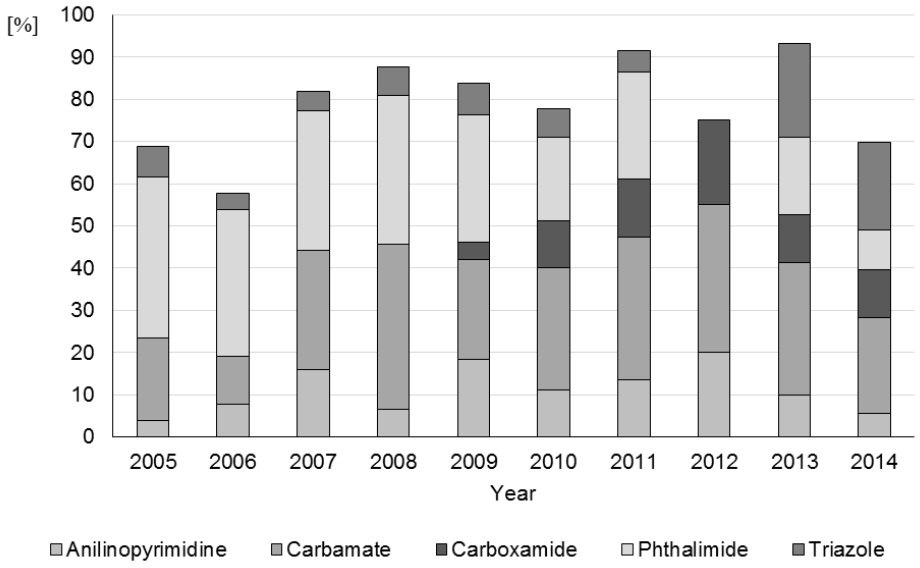
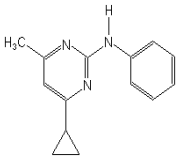
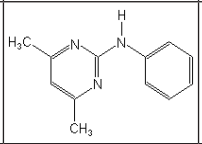
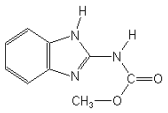
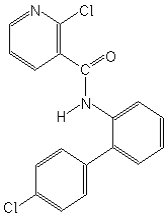
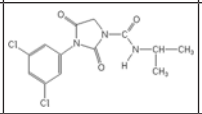
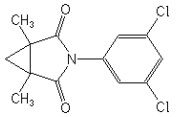
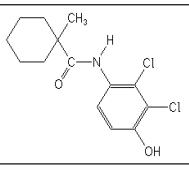
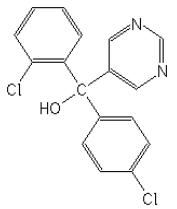
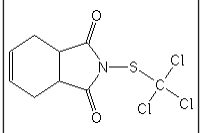
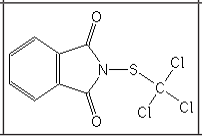


Fig. 3. The most frequently detected fungicide groups in 2005-14.

Table 4. Summary of fungicide residues detected in fruit samples in 2005-14.

Substance group	Active substance	Chemical structure	No. of samples with residues (%)	No. of samples > MRL (%)	Range of concentration (mg/kg)	Positive fruits (No. of detected, MRL)
Anilinopyrimidine *(Group 3)*	Cyprodinil		38 (3.9)	2 (0.2)	0.01-0.28	Blueberry (1, MRL=5), sweet cherry (1, MRL=1), apple (10, MRL=0.05–1), currant (2, MRL=5), strawberry (23, MRL=3–5), grape (1, MRL=5)
	Pyrimethanil		42 (4.3)	3 (0.3)	0.01-2.40	Apple (33, MRL=0.01–5), raspberry (5, MRL=10), strawberry (4, MRL=2–5)
Benzimidazole *(Group 5)*	Carbendazim		3 (0.3)	0 (0)	0.04-0.07	Apple (3, MRL=0.2)
Carbamate *(Group 7)*	Dithiocarbamates – DTC	-	212 (21.8)	0 (0)	0.05-2.74	Gooseberry (2, MRL=5), pear (14, MRL=5), apple (87, MRL=3–5), currant (62, MRL=5), plum (2, MRL=2), strawberry (24, MRL=2–10), sour cherry (21, MRL=1–2)
Carboxamide *(Group 8)*	Boscalid		42 (4.3)	0 (0)	0.01-3.31	Gooseberry (1, MRL=10), blueberry (1, MRL=10), sweet cherry (1, MRL=3), pear (4, MRL=2), apple (13, MRL=2), currant (8, MRL=10), strawberry (13, MRL=10), sour cherry (1, MRL=4)
Dicarboximide *(Group 13)*	Iprodione		14 (1.4)	0 (0)	0.01-0.62	Raspberry (2, MRL=10), strawberry (12, MRL=10–15)
	Procymidone		12 (1.2)	3 (0.3)	0.02-0.84	Raspberry (3, MRL=10), currant (2, MRL=0.02), strawberry (7, MRL=0.2–5)
Hydroxyanilide *(Group 14)*	Fenhexamid		16 (1.6)	0 (0)	0.03-0.71	Sweet cherry (1, MRL=5), raspberry (5, MRL=10), strawberry (10, MRL=5)
Pyrimidine *(Group 21)*	Fenarimol		16 (1.6)	0 (0)	0.01-0.08	Currant (1, MRL=1), sour cherry (15, MRL=1)
Phthalimide *(Group 22)*	Captan		204 (20.9)	0 (0)	0.01-2.83	Peach (2, MRL=3), sweet cherry (2, MRL=5), pear (11, MRL=4), apple (105, MRL=3), sour cherry (84, MRL=2–5)
	Folpet		11 (1.1)	0 (0)	0.02-2.70	Apple (4, MRL=3), strawberry (7, MRL=3)

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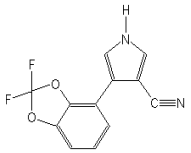
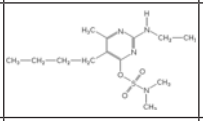
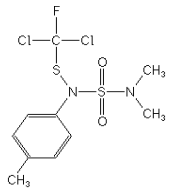
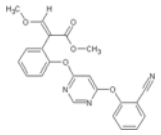
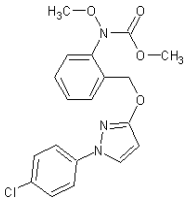
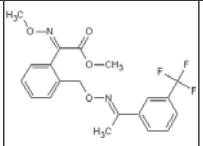
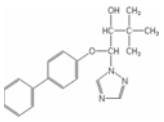
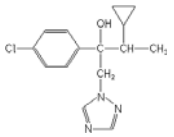
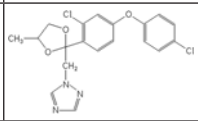
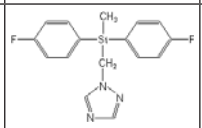
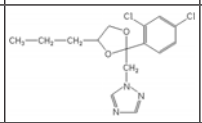
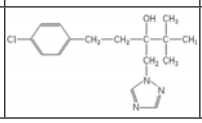
Phenylpyrrole *(Group 24)*	Fludioxonil		22 (2.3)	0 (0)	0.01-0.30	Sweet cherry (1, MTL=5), pear (1, MRL=5), apple (1, MRL=5), currant (1, MRL=3), strawberry (17, MRL=3), grape (1, MRL=5)
Pyrimidinol *(Group 26)*	Bupirimate		6 (0.6)	0 (0)	0.02-0.82	Gooseberry (2, MRL=5), currant (4, MRL=0.5–5)
Sulphamide *(Group 28)*	Tolylfluanid		34 (3.5)	1 (0.1)	0.02-1.80	Pear (1, MRL=5), apple (19, MRL=5), raspberry (4, MRL=5), currant (1, MRL=0.02), strawberry (9, MRL=2–5)
Strobilurin *(Group 29)*	Azoxystrobin		1 (0.1)	0 (0)	0.06	Currant (1, MRL=5)
	Pyraclostrobin		5 (0.5)	1 (0.1)	0.04-0.61	Gooseberry (1, MRL=3), pear (1, MRL=0.5), currant (3, MRL=3)
	Trifloxystrobin		22 (2.7)	0 (0)	0.01-0.16	Gooseberry (1, MRL=1), sweet cherry (1, MRL=1), pear (2, MRL=0.5), apple (11, MRL=0.5), currant (7, MRL=0.2–1)
Triazole *(Group 30)*	Bitertanol		2 (0.2)	0 (0)	0.10	Sour cherry (2, MRL=1)
	Cyproconazole		1 (0.1)	1 (0.1)	0.09	Gooseberry (1, MRL=0.05)
	Difenoconazole		27 (2.8)	2 (0.2)	0.02-0.43	Gooseberry (2, MRL=0.1), currant (24, MRL=0.2), sour cherry (1, MRL=0.3)
	Flusilazole		33 (3.4)	6 (0.6)	0.01-0.29	Pear (1, MRL=0.02), apple (7, MRL=0.02-0.2), currant (16, MRL=0.02–0.2), sour cherry (9, MRL=0.2)
	Propiconazole		1 (0.1)	1 (0.1)	0.14	Gooseberry (1, MRL=0.05)
	Tebuconazole		11 (1.1)	0 (0)	0.02-0.21	Apple (4, MRL=1), plum (6, MRL=0.5–1), sour cherry (1, MRL=5)

%) until 2011. In 2012, triazole residues were not detected. On the other hand, detection of substances from this group has increased over the last two years. Triazole fungicides are recognized as carcinogens and mutagens that can weaken the immune system (cancer-causing, reproductive disorders, endocrine disorders, and neurotoxic).

Group 3, anilinopyrimidine fungicides: In 2005-14, anilinopyrimidines were detected. The anilinopyrimidine fungicides registered on fruits include cyprodinil, pyrimethanil, and mepanipyrim. The main mode of action of these substances consists in blocking the synthesis of amino acids. This inhibits fungal growth both inside and outside the leaf.

The Most Frequently Detected Active Substances

The most commonly detected pesticides were: dithiocarbamates (212 samples), captan (204 samples), pyrimethanil and boscalid (42 samples), cyprodinil (38 samples), tolylfluanid (34 samples), flusilazole (33 samples), difenoconazole (27 samples), trifloxystrobin and fludioxonil (22 samples), fenhexamid and fenarimol (16 samples), iprodione (14 samples), procymidone (12 samples), tebuconazole and folpet (11 samples), bupirimate (six samples), pyraclostrobin (five samples), and carbendazim (three samples). Bitertanol in two samples and cyproconazole, propiconazole, and azoxystrobin only in one sample each were detected (Table 4).

Ditihocarbamates (carbamate, group 7) belonging to the most widespread active substances in the world, occurred the most frequently in apple and currant samples in this investigation. Overall, DTC fungicides found in this study were similar to those found in other studies from other countries (apples, pears, grapes) [30]. The level of DTC in samples ranged from 0.05 to 2.74 mg/kg. The levels were lower than the level of dithiocarbamates found in fruit and vegetable samples from southeastern Poland (3.24 mg/kg) [31]. Although dithiocarbamates were present in 212 samples (21.7% of all tested samples), any sample with levels above the MRL was noted.

Captan belonging to the phthalimide class (group 22), was identified in 204 samples of peaches, apples, pears, and sweet and sour cherries. Boscalid belonging to the class of carboxamide (group 8), was identified in 42 samples of strawberries, currants, gooseberries, apples, blueberries, pears, and sweet and sour cherries. The highest concentrations were noted for boscalid and captan, ranging from 2.83 mg/kg (for cherry samples) to 3.31 mg/kg (for currant samples). The highest concentration for boscalid was observed in grapes from Turkey (1.68 mg/kg) [32].

Some pesticide concentrations exceeded their MRLs, such as flusilazole (seven samples), pyrimethanil (three samples), procymidone (three samples), cyprodinil (two samples), difenoconazole (two samples), and cyproconazole, propiconazole, tolylfluanid (in one sample each). More details are presented in Table 4.

Results showed the detection of different triazole fungicides in fruit samples. Difenoconazole and flusilazole indicated the highest concentration of 0.43 mg/kg and 0.29 mg/kg in currant, tebuconazole with a concentration of 0.29 mg/kg in plum, bitertanol with a concentration of 0.10 mg/kg in sour cherry, propiconazole and cyproconazole with a concentration of 0.14 mg/kg and 0.09 mg/kg in gooseberry. Flusilazole is broad-spectrum fungicide used to control fungal diseases in cereals, fruits, vegetables, and nuts [25]. The level of flusilazole in samples ranged from 0.01 to 0.29 mg/kg, with six samples having levels above the MRL (0.20 mg/kg). This fungicide is moderately toxic to humans (class II) [33]. On the other hand, difenoconazole was identified in 27 samples of currants, gooseberries, and sour cherries. The level of difenoconazole in samples ranged from 0.02 to 0.43 mg/kg, with two samples having levels above the MRL (0.30 mg/kg). The World Health Organization [33] has classified difenocnazole as moderately toxic to humans (class II). The reported difenoconazole level was higher than its level in samples from Turkey (0.01-0.03 mg/kg) [32]. Accordingly, monitoring flusilazole and difenoconazole levels in fruits is important for human health.

During 10 years of testing, only 1.7% of fruit samples had residues exceeding MRL. In Brazil, 3% among the 160 vegetable samples analyzed in 2001-10 had residues exceeding MRL [34]. Zicarii et al. [35] reported that 3.2% among the 6,947 samples from Italy had residues above MRL. In turn, 8.4% of the 724 fruit and vegetable samples imported from South America to Denmark, Estonia, Finland, Norway, and Sweden had residues above the MRL [36].

Occurrence of Fungicide Residues in a Particular Group of Fruits

Berries and small fruits were the type of fruit where the residues of fungicides were the most frequently detected. Moreover, in the case of this type of fruit, among 320 analyzed samples, 12 (3.8%) showed values above MRLs. The most frequently identified was dithiocarbamates in currants. Results obtained by other authors [37-38] show that berries (e.g., currants) more frequently contain pesticide residues than other fruits. In turn, the most frequently detected fungicide in pome fruits and stone fruits was captain, which was found in cherry and apple samples.

The percentage of contaminated samples was high (above 50%) for three different fruits: currants (68.5%), apples (63.3%), and sour cherries (54.6%). Percentages of contaminated samples below 48% had strawberries (47.4%) and pears (47.7%). Currants and apples had fungicides detected in nine consecutive years, with the exception of the years 2011 and 2012.

Single and Multiple Fungicide Residues Detected in Samples

Of the 935 samples, 32.1% contained only one substance and 22% contained between two and five substances. The multi-residue samples are shown in Fig. 4.

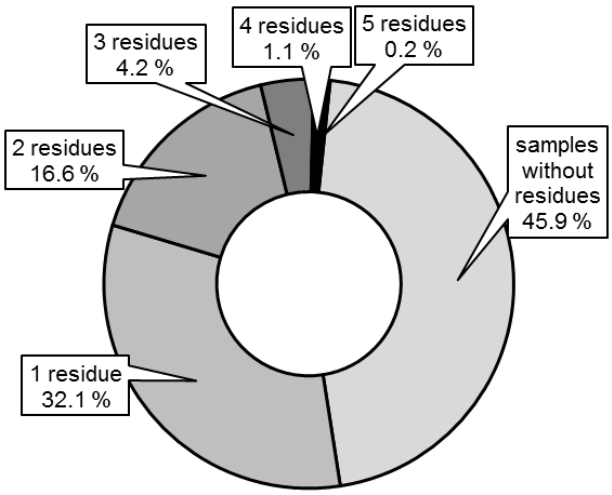


Fig. 4. Frequency of occurrence of samples without residues, with one residue, and with multi-residue in fruits.

Gooseberries had the highest number of samples with multiple residues (80.0% of positive gooseberry samples), followed by strawberries (50.0%), apples (45.9%), and currants (41.4%), which was in accordance with a study conducted by Sadło et al. [39-40]. Dithiocarbamates and captan were the substances most often found in the multi-residue samples.

Risk Assessment

Humans are acute and chronically exposed to pesticide residues in food. These compounds can be harmful to humans even at very small concentrations. In the case of safety evaluations of pesticide residue for food intake, ARfD and ADI are the standards for risk assessment and they are basing on the acute and chronic toxicity of pesticides for humans. Exposure estimation was conducted on the results of monitoring fungicide residue detected in fruit samples in order to determine the short- and long-term risks. Table 5a shows the results of the evaluation of the acute health risks of exposure of children and adults. Table 5b shows the long-term risk calculated additionally for Polish consumers and WHO cluster diet E (Austria, Belgium, Croatia, Czech Republic, Denmark, France, Germany, Hungary, Ireland, Luxemburg, Malta, Netherlands, Slovakia, Slovenia, Switzerland, United Kingdom, Northern Ireland, and Poland) related to fruit consumption containing fungicide residues. If the results of such analytes exceed 100%, there is a potential risk to consumers.

Short-Term Risk

In the case of residues exceeding MRL, informative notifications were sent under the Rapid Alert System for Food and Feed (RASFF). Short-term exposure was calculated for pesticides exceeding MRLs and is shown in Table 5a. In the case of fruit consumption from northeastern Poland, short-term exposure didn't exceed 25% for the group of toddlers and 5% for the adults. The highest values of short-term exposure for the children were connected with the presence of cyprodinil in apples (21.6% ARfD) and procymidone in currants (17.1% ARfD), and was 4.5% and 7.5% ARfD for the adults, respectively. The results for the short-term risk assessments should be considered good.

Long-Term Risk

The percent ratios of EDI to ADI of 23 pesticides were very low – 0.01% for azoxystrobine, fenhexamide, fenarimol, and fludioxonil (adults), and up to 30.27%

Table 5. Risk assessment in 2005-14.
a) Estimation of short-term (acute) dietary consumer exposure to pesticide residues.

Fruit	Active substance	HR (mg/kg)	MRL	Times exceeded MRL	ARfD* (mg/kg bw)	Adults		Children		Health risk
						Intake (mg/kg bw) $x10^{-4}$	% ARfD	Intake (mg/kg bw) $x10^{-4}$	% ARfD	
Apple	Cyprodinil	0.09	0.05	1.8	0.03	13.5	4.5	64.8	21.6	No
	Pyrimethanil	0.20	0.01	20	Not appl.	-	-	-	-	-
Currant	Difenoconazole	0.43	0.05	8.6	0.16	6.8	0.4	15.5	1.0	No
	Flusilazole	0.29	0.02	14.5	0.005	4.6	0.9	10.4	2.1	No
	Procymidone	0.57	0.02	28.5	0.012	9.0	7.5	20.5	17.1	No
Gooseberry	Cyproconazole	0.09	0.05	1.8	0.02	0.9	0.4	1.9	0.9	No
	Propiconazole	0.14	0.05	2.8	0.03	1.4	0.5	3.0	1.0	No
Strawberry	Tolylfluanid	0.49	0.02	24.5	0.25	12.9	0.5	24.0	1.0	No

HR – Highest residue, MRL – Maximum Residue Limits, ARfD – Acute Reference Dose, bw – body weight.
* ARfD values are derived from the pesticide database (EU Pesticides database) [41].

b) Risk estimates based on comparison of consumed groups of pesticides in the mean concentration with acceptable daily intake.

Substance group	Active substance	ADI (mg/kg b.w. d^{-1})	EDI (g/kg b.w. d^{-1}) $x10^{-3}$	HQ [%]					
				Polish general	UK adults (mean)	UK adults (97.5 percentile)	UK toddler (mean)	UK toddler (97.5 percentile)	WHO cluster E
Anilinopyrimidine (Group 3)	cyprodinil	0.03	7.631	0.16	0.07	0.96	0.16	2.48	0.15
	pyrimathanil	0.17	12.037	0.05	0.02	0.27	0.05	0.69	0.04
Benzimidazole (*Group 5*)	carbendazim	0.02	5.155	0.16	0.08	0.97	0.16	2.52	0.15
Carbamate (*Group 7*)	dithiocarbamate	0.05	90.717	0.26	0.53	6.83	1.16	17.72	1.05
Carboxamide (*Group 8*)	boscalid	0.04	16.551	0.13	0.12	1.56	0.26	4.04	0.24
Dicarboximide (*Group 13*)	iprodione	0.06	12.513	1.97	0.06	0.78	0.13	2.04	0.12
	procymidone	0.0028	8.676	1.16	0.91	11.66	1.98	30.27	1.79
Hydroxyanilide (*Group 14*)	fenhexamid	0.2	8.508	0.03	0.01	0.16	0.03	0.42	0.02
Pyrimidine (*Group 21)*	fenarimol	0.01	5.481	0.01	0.16	2.06	0.35	5.35	0.32
Phthalimide (*Group 22)*	captan	0.1	53.555	0.34	0.16	2.02	0.34	5.23	0.31
	folpet	0.1	13.595	0.09	0.04	0.51	0.09	1.33	0.08
Phenylpyrrole (*Group 24)*	fludioxonil	0.37	6.679	0.35	0.01	0.07	0.01	0.18	0.01
Pyrimidinol (*Group 26)*	bupirymate	0.05	5.995	0.08	0.04	0.45	0.08	1.17	0.07
Sulphamide (Group 28)	tolylfluanide	0.1	11.160	0.02	0.03	0.42	0.07	1.09	0.06
Strobilurin (*Group 29)*	azoxystrobin	0.2	5.059	0.13	0.01	0.10	0.02	0.25	0.01
	pyraclostrobin	0.03	6.171	0.04	0.06	0.77	0.13	2.01	0.12
	trifloxystrobin	0.1	5.674	0.07	0.02	0.21	0.04	0.55	0.03
Triazole (*Group 30)*	cyproconazole	0.02	5.091	0.16	0.07	0.96	0.16	2.49	0.15
	difenoconazole	0.01	7.134	0.45	0.21	2.68	0.46	6.97	0.41
	flusilazole	0.002	6.406	2.04	0.94	12.05	2.05	31.29	1.85
	propiconazole	0.04	5.144	0.08	0.04	0.48	0.08	1.26	0.07
	tebuconazole	0.03	5.904	0.13	0.06	0.74	0.13	1.92	0.11
	bitertanol	0.003	5.203	1.10	0.51	6.53	1.11	16.94	1.00

for procymidone and 31.29% for flusilazole for toddlers, calculated on the 97.5 percentile.

Conclusions

The sample preparation techniques such as MSPD and QuEChERS combined with spectrophotometric and chromatographic (GC/ECD/NPD, HPLC/DAD/FLD, and LC/MS/MS) methods were found to be suitable for the control of 77 fungicides in several fruits at concentrations lower than their established MRLs. The quantification with calibration curves made with spiked blank matrices was performed to completely remove any matrix effect. The methods were applied for 974 of fruit samples collected in Poland in the last 10 years (2005-14). Fungicide residues were found in 52.0% of the samples, with 50.2% samples containing pesticide residues below the maximum residue levels (MRLs), and 1.7% of tested samples exceeding MRLs. The percentage of contaminated samples was high (above 50%) for three different fruits: currants (68.5%), apples (63.3%), and sour cherries (54.6%). The present study shows that although fruits from northeastern Poland contained many fungicide residues, their consumption did not pose a danger to adult and child health.

The diversity of uses and the wide availability of fungicides causes their residues to reach the environment in an uncontrolled manner, and the human population is exposed to these substances. Therefore, monitoring pesticides in fruits is necessary in order to assess potential health risks and to improve the maximum residue limits (MRLs) for safe human consumption.

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