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# Health risk for children and adults consuming apples with pesticide residue

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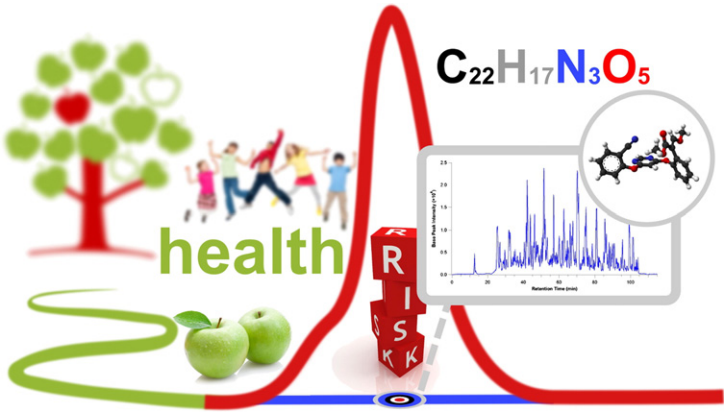
## HIGHLIGHTS

- Over 60% of apple samples contain pesticide residues.
- Thirty four pesticides were detected, the most frequently detected were fungicides.
- Insecticides were detected above MRL more often.
- Samples with multiple residues (up to 7) were noted.
- The estimated risk of acute exposure was highest for flusilazole and tebuconazole.

## GRAPHICAL ABSTRACT

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## ABSTRACT

The presence of pesticide residues in apples raises serious health concerns, especially when the fresh fruits are consumed by children, particularly vulnerable to the pesticide hazards. This study demonstrates the results from nine years of investigation (2005–2013) of 696 samples of Polish apples for 182 pesticides using gas and liquid chromatography and spectrophotometric techniques. Only 33.5% of the samples did not contain residues above the limit of detection. In 66.5% of the samples, 34 pesticides were detected, of which maximum residue level (MRL) was exceeded in 3%. Multiple residues were present in 35% of the samples with two to six pesticides, and one sample contained seven compounds. A study of the health risk for children, adults and the general population consuming apples with these pesticides was performed. The pesticide residue data have been combined with the consumption of apples in the 97.5 percentile and the mean diet. A deterministic model was used to assess the chronic and acute exposures that are based on the average and high concentrations of residues. Additionally, the "worst-case scenario" and "optimistic case scenario" were used to assess the chronic risk. In certain cases, the total dietary pesticide intake calculated from the residue levels observed in apples exceeds the toxicological criteria. Children were the group most exposed to the pesticides, and the greatest short-term hazard stemmed from flusilazole at 624%, dimethoate at 312%, tebuconazole at 173%, and chlorpyrifos methyl and captan with 104% Acute Reference Dose (ARfD) each. In the cumulative chronic exposure, among the 17 groups of compounds studied, organophosphate insecticides constituted 99% acceptable daily intake (ADI). The results indicate that the occurrence of pesticide residues in apples could not be considered a serious public

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health problem. Nevertheless, an investigation into continuous monitoring and tighter regulation of pesticide residues is recommended.



## 1. Introduction

The health benefits associated with the regular consumption of fruit have been reported extensively over the last several decades. Epidemiological studies have shown that the consumption of apples has been associated with health benefits (Eberhardt et al., 2000). Apples are rich in flavonoids, polyphenols, vitamins, and minerals and contain many useful phytochemicals. The procyanidins epicatechin and catechin have strong antioxidant activity and have been found to inhibit low-density lipoprotein oxidation in vitro (Aprikian et al., 2001; da Silva Porto et al., 2003; Hyson et al., 2000;) and increase high-density lipoproteins, lowering the risk of type 2 diabetes (Cooper et al., 2012). Scientific evidence indicates that a diet rich in apples can decrease the risk of chronic diseases (Boyer and Liu, 2004; Davis et al., 2006; Yoon and Liu, 2007) and can induce weight loss in middle-aged overweight women (de Oliveira et al., 2003). Apples are one of the very few individual foods with the capacity to reduce cancer risk (Arts et al., 2001; Feskanich et al., 2000; Le Marchand et al., 2000).

It was found that women ingesting apples had a 13–22% decrease in cardiovascular disease risk (CDR) (Sesso et al., 2003) and that a reduced risk of death from CDR existed for men (Hertog et al., 1993). Another study (He and Liu, 2008) indicated that flavonoid-rich apples are one of three foods (along with red wine and pears) that decrease the risk of mortality for both coronary heart and CDR among post-menopausal women. Whole apples were found to protect against asthma as well as against bronchial hyperreactivity due to their anti-inflammatory and antioxidant properties (Shaheen et al., 2001; Woods et al., 2003). Apples contain high levels of antioxidants that reduce the risk of many neurodegenerative diseases, such as Alzheimer's and Parkinson's, counteract the ageing process, and may help to maintain brain performance (Rogers et al., 2004; Wu et al., 2004).

Prevention is a more effective strategy than treatment of chronic diseases. Fruits that contain significant amounts of bioactive components may provide desirable health benefits beyond basic nutrition and play an important role in the prevention of chronic diseases. However, apples must not contain toxic substances above defined safe limits.

Pesticides are commonly used in apple production (Sauphanor et al., 2009; Simon et al., 2011; Rawn et al., 2008; Pennel, 2006; Eurostat, 2002) to control phytophages or pests that may damage crops during production, storage or transport (Ticha et al., 2008). Pesticides allow growers to increase the amount of usable apples from each tree at the time of harvest. Pesticides may also improve the quality and shelf-life of certain foods. Pesticides have been linked to a wide range of human health hazards, ranging from short-term impacts such as headaches and nausea to chronic impacts such as cancer, reproductive harm, and endocrine disruption (Baldi et al., 2001; Benbrook, 1996; Rivas et al., 2007). Chronic health effects may occur years after even minimal exposure to pesticides in the environment, or in food and water, and pesticides can cause many types of cancer in humans (Alexander et al., 2012; Rusiecki et al., 2006). Several of the most prevalent forms include leukaemia, non-Hodgkin's lymphoma, and brain, bone, breast, ovarian, prostate, testicular and liver cancers (Cantor et al., 1992). A study reported that children who live in homes where their parents use pesticides are twice as likely to develop brain cancer as those that live in residences in which no pesticides are used (Bradman et al., 2007; CDC, 2002). There is also mounting evidence that exposure to pesticides disrupts the endocrine system (Mnif et al., 2011), the reproductive system, and embryonic development. Endocrine disruption can cause infertility and a variety of birth defects and developmental defects in offspring, including hormonal imbalances and incomplete sexual development, impaired brain development, behavioural disorders, and many others (Alavanja et al., 2004).

Children are particularly susceptible to the hazards associated with pesticide use (Bradman et al., 2007; Dalvie et al., 2014). They also represent a specific sub-population among the consumer population. The toxicity of pesticides in infants and children may differ quantitatively and qualitatively from that in adults. There is now considerable scientific evidence that the human brain is not fully formed until the age of 12, and childhood exposure to some of the most common pesticides may greatly impact the development of the central nervous system. Children have more skin surface for their size than adults, absorb proportionally greater amounts of many substances through their lungs and intestinal tracts, and take in more air, food and water per body weight than adults (Garry et al., 2002). The immune system, nervous system, and detoxifying mechanisms of children have not developed completely, leaving them less capable of resisting the introduction of toxic pesticides into their systems (CDC, 2002). Researchers have found that pesticide exposure can induce a poisoning response linked to asthma (Hoppin et al., 2008). The combination of likely increased exposure to pesticides and lack of bodily development for combating the toxic effects of pesticides means that children are suffering disproportionately from their impacts. Considering the multitude of risks associated with pesticide intake by infants, the European Union set a strict restriction for pesticides in infant food (EC, 2009).

The goal of this study was to assess the presence of pesticide residues in apples produced and consumed in Poland and those exported to countries of the European Union and Russian Federation, as well as to evaluate the health effect of detected residues on various consumer age groups with the utilisation of cluster diet models.

## 2. Material and methods

### 2.1. Samples

The 696 samples of apples (Table 1) from the north-eastern and central Poland were collected over a nine-year period (Fig. 1) (from 2005 to 2013) during official inspections from producers supplying apples to the domestic and European markets as well as from exporters to the Russian Federation.

### 2.2. Chemicals and reagents

All reagents used were of residue analysis grade. Acetone, acetonitrile, dichloromethan, diethyl ether and n-hexane for pesticide residue analysis were provided by J.T. Baker (Deventer, Holland), along with Florisil (60–100 mesh) and silica gel activated for 8 h at 600 °C. Anhydrous sodium sulphate and celite were purchased from Fluka (Seelze-Hannover, Germany). ChemElut cartridge containing diatomaceous earth was obtained from J.T. Baker (USA).

**Table 1**
Number of tested samples.

| Year | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 | 2012 | 2013 | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| Number of samples | 32 | 22 | 61 | 46 | 65 | 303 | 100 | 24 | 43 | 696 |

**Fig. 1.** Locations of sampling sites in Poland.

### 2.3. Pesticide standards

Pesticides (182 active substances) were obtained from Dr. Ehrenstorfer Laboratory (Germany). Standard stock solutions of various concentrations were prepared in acetone and stored at 4 °C (purity > 95%). Standard working solutions were prepared by dissolving appropriate amounts of stock solutions in n-hexane/acetone (9:1, v/v) mixture.

### 2.4. Compound groups for consideration

Analysed pesticides (Table 2) were selected based on the frequency of their application in apple orchards and in storage, the persistence of pesticides in the environment (e.g. DDT), their toxicological profile (e.g. acetylcholinesterase (AChE) inhibitor, sodium channel modulator), and their mechanism of action. The studied compounds belong to fungicides, herbicides, and insecticides from various groups in terms of chemical structure, e.g. carboxamide, anilinopyrimidine, benzimidazole, dicarboximide, dithiocarbamate, phenylpyrrole, phthalimide, quinnie, strobilurin, sulphamide, triazole, neonicotinoid, carbamate, organophosphate, pyrethroid, sulfite ester and unclassified (17 groups).

### 2.5. Analytical methods

The apples were analysed by three different analytical methods, covering up to 182 pesticides, including isomers and metabolites. A multiresidue method (MRM) was used for the determination of 180 pesticide residues by gas chromatography (GC) with selective detectors and two single-residue methods (SRM); the determination of dithiocarbamates using spectrophotometry and the determination of carbendazim residues using high performance liquid chromatography (HPLC) were performed. Samples were analysed at the Laboratory of Pesticide Residues in Białystok. These methods were validated and accredited in accordance with PN-EN ISO/IEC 17025 (ISO, 2005) by the Polish Center of Accreditation, PCA. Details of the aforementioned analytical methods were as previously described (Łozowicka and Kaczyński, 2009a; Łozowicka et al., 2011).

#### 2.5.1. MRM — determination of 180 pesticide residues using gas chromatography (GC)

A homogenised sample of 2 g of apples was placed into a mortar with 4 g of the solid support Florisil and was blended manually. The homogeneous mixture was transferred into a glass macro-column packed with anhydrous sodium sulphate (5.0 g) and silica gel (2.5 g). The analytes were eluted using 15 ml hexane/acetone (8:2, v/v) and 15 ml hexane/diethyl ether/acetone (1:2:2, v/v/v). The extract was dried using a rotary vacuum evaporator at a temperature of approximately 40 °C. Then, the eluate was re-dissolved using 2 ml of hexane/acetone (9:1, v/v). The final solution was transferred into a GC vessel and placed onto the rack of the autosampler. GC analysis was performed with a model 7890A Agilent gas chromatograph (Waldbronn, Germany) equipped with an automatic split/splitless injector and two selective detectors: electron capture (ECD) and nitrogen–phosphorous (NPD).

**Table 2**
Analysed active substance of pesticides.

| | |
|---|---|
| Insecticides and acaricides (92) | Acetamiprid (0.01); acrinathrin (0.02); aldrin (0.005); alpha-cypermethrin (0.03); alpha-HCH (0.005); azinphos-ethyl (0.02); azinphos-methyl (0.02); beta-cyfluthrin (0.02); beta-HCH (0.01); bifenthrin (0.01); bromopropylate (0.01); bromophos-ethyl (0.02); bromophos-methyl (0.01); buprofezin (0.02); cadusafos (0.01); carbaryl (0.05); carbofuran (0.02); chlorfenvinphos (0.01); chlorpyrifos (0.01); chlorpyrifos methyl (0.01); cyfluthrin (0.01); cypermethrin (0.03); deltamethrin (0.05); diazinon (0.01); dichlorvos (0.001); dicofol (0.02); diflubenzuron (0.01); dieldrin (0.005); dimethoate (0.01); endosulphan sum of alpha-endosulphan, beta-endosulphan, endosulphan-sulphate (0.03); endrin (0.005); esfenvalerate (0.02); ethion (0.01); ethoprophos (0.02); fenazaquin (0.02); fenthion (0.01); fenitrothion (0.01); fenoxycarb (0.01); fenpropathrin (0.01); fenpyroximate (0.01); fenthion (0.01); fenvalerate (0.02); fipronil (0.005); formothion (0.01); gamma-HCH (lindane) (0.005); HCB (0.005); heptachlor (0.005); heptachlor-epoxide (0.005); heptenophos (0.01); hexythiazox (0.05); indoxacarb (0.02); isofenphos (0.01); isofenphos-methyl (0.01); lambda-cyhalothrin (0.01); malathion (0.02); mecarbam (0.02); methacrifos (0.05); mevinphos (0.01); methidathion (0.01); methoxychlor (DMDT) (0.01); DDT sum of o,p′-DDT, p,p′-DDD, p,p′-DDE, p,p′-DDT (0.001); parathion-ethyl (0.01); parathion-methyl (0.01); permethrin (0.04); phenthoate (0.01); phosalone (0.01); phosmet (0.01); phoxim (0.01); pirimicarb (0.02); pirimiphos (0.01); pirimiphos-methyl (0.01); profenofos (0.01); propoxur (0.01); pyridaben (0.02); pyriproxyfen (0.05); quinalphos (0.01); tebufenpyrad (0.01); teflubenzuron (0.03); terbufos (0.01); tetrachlorvinphos (0.01); tetradifon (0.01); thiacloprid (0.04); thiamethoxam (0.01); triazophos (0.01); omethoate (0.01); zeta-cypermethrin (0.02) |
| Fungicides (66) | Azaconazole (0.01); azoxystrobin (0.02); benalaxyl (0.03); bitertanol (0.013); boscalid (0.01); bromuconazole (0.01); bupirimate (0.005); captan (0.01); carbendazim (0.02); chlorothalonil (0.01); cyazofamid (0.01); cyproconazole (0.01); cyprodinil (0.01); dichlofluanid (0.01); dicloran (0.01); difenoconazole (0.05); dimethomorph (0.05); dimoxystrobin (0.01); diniconazole (0.01); diphenylamine (0.01); dithiocarbamates[a] (0.05); epoxiconazole (0.01); famoxadon (0.01); fenarimol (0.01); fenbuconazole (0.01); fenchlorphos (0.01); fenhexamid (0.01); fenpropimorph (0.02); fludioxonil (0.01); fluquinconazole (0.01); flusilazole (0.01); flutriafol (0.01); folpet (0.01); hexaconazole (0.01); imazalil (0.01); imibenconazole (0.01); iprodione (0.02); kresoxim-methyl (0.01); mefenoxam (0.01); mepanipyrim (0.01); metalaxyl (0.01); metconazole (0.01); myclobutanil (0.01); oxadixyl (0.03); paclobutrazol (0.02); penconazole (0.02); pencycuron (0.03); picoxystrobin (0.01); prochloraz (0.01); procymidone (0.01); propiconazole (0.01); pyrazophos (0.01); pyrimethanil (0.01); quinoxyfen (0.01); quintozene (0.01); tebuconazole (0.01); tecnazene (0.02); tetraconazole (0.01); tolclofos-methyl (0.01); tolylfluanid (0.02); triadimefon (0.02); triadimenol (0.05); trifloxystrobin (0.01); qvinclozolin (0.01); zoxamide (0.02) |
| Herbicides and growth regulators (24) | Acetochlor (0.02); atrazine (0.01); bromacil (0.01); chlorpropham (0.01); cyanazine (0.01); cyprazine (0.01); diflufenican (DFF) (0.01); flurochloridone (0.01); lenacil (0.02); metazachlor (0.01); metholachlor (0.02); metribuzin (0.02); napropamide (0.02); nitrofen (0.01); oxyfluorfen (0.01); pendimethalin (0.02); prometryn (0.01); propachlor (0.02); propaquizafop (0.03); propazine (0.01); propham (0.02); propyzamide (0.02); simazine (0.01); trifluralin (0.01) |

[a] Values in parentheses indicate the LOQ.

A fused-silica capillary 5%-phenyl-methylpolysiloxane column of 30 m × 0.32 mm and a film thickness 0.50 μm supplied by Agilent (Waldbronn, Germany) was used. The injection port and detector (ECD and NPD) temperatures were 210 and 300 °C, respectively, with helium as the carrier gas at a flow rate of 3 ml/min. Nitrogen was used as a make-up gas at a flow rate of 57 ml/min (ECD) and 8 ml/min (NPD) in addition to hydrogen at 3.0 ml/min and air at 60 ml/min (NPD). The furnace parameters were as follows: the initial temperature of 120 °C was increased to 190 °C at 16 °C/min, then to 230 °C at 8 °C/min, and finally to 285 °C at 18 °C/min and was maintained for 10 min (ECD and NPD). The volume of the final injected sample extract was 2 μl.

*2.5.2. SRM — determination of carbendazim residues using high performance liquid chromatography (HPLC)*

A representative sample of 20 g was homogenised with 150 ml acetone for 5 min. Then, 2.5 g of celite was added to the extract and filtered. The final filtrate was evaporated, and 20 ml of 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 and dissolved in 2 ml of an acetonitrile/water mixture (2:8, v/v). The final solution was placed into an HPLC vessel and placed onto the rack of the autosampler. HPLC analysis was performed using a model 2695 Waters Alliance (Milford, MA, USA) liquid chromatograph with a photodiode (Waters 2996) set to 285 nm and fluorescent detectors (Waters 2475) ($\lambda_{ex}$ = 285 nm, $\lambda_{em}$ = 315 nm). The external-standard method was used by applying 100 μl of the standard solution onto the column (Supelcosil LC-18, 5 μm, 250 mm × 4.6 mm). The mobile phase was acetonitrile/phosphate buffer with 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.

*2.5.3. SRM — determination of dithiocarbamates using spectrophotometry*

Dithiocarbamate residues were determined by a modified colorimetric method (Łozowicka and Kaczyński, 2009b). This method allows the determination of dithiocarbamate fungicides as a group (mancozeb, maneb, methiram, propineb, thiram, and ziram), expressed as carbon disulphide. A sample of 50 g was heated for 45 min (80 °C) with 60 ml of hydrochloric acid and tin(II) chloride to release carbon disulphide from dithiocarbamates in an alkaline pH. The dithiocarbamates decomposed with the emission of carbon disulphide. $CS_2$ was separated and collected in a methanolic solution of potassium hydroxide in which $CS_2$ formed potassium xanthogenate, which was then heated with zinc acetate to obtain zinc sulphide. In an acidic medium, this compound released hydrogen sulphide, which formed methylene blue in a reaction with N,N-dimethyl-1,4-phenylenediammonium dichloride in the presence of Fe(III) ions. Finally, the quantity formed was estimated by measuring the absorbance at 662 nm on a spectrophotometer (Helios Delta VIS). The absorbance was converted into the concentration, and the results were expressed in mg $CS_2$/kg.

*2.5.4. Validation of methods*

Validation of the analytical methods was carried out in accordance with European Commission (EC) guidelines (EC, 2009, 2013). The validation studies were performed using pesticide-free fruit samples, which were previously analysed. Calibration standards were prepared in a matrix solution to produce a final concentration of three spiking concentrations: for GC: 1st range 0.001 to 0.05 mg/kg, 2nd range 0.05–0.5 mg/kg and 3rd range 0.25–3.0 mg/kg, for HPLC: 1st range 0.01 to 0.1 mg/kg, 2nd range 0.05–0.5 mg/kg and 3rd range 0.25–3.0 mg/kg, for spectrophotometric: 1st range 0.05–0.1 mg/kg; 2nd range 0.1–1.0 mg/kg and 3rd range 1.0–5.0 mg/kg.

The accuracy and precision of the method were evaluated by performing recovery studies and are 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. Sensitivity was evaluated by determining the limit of detection (LOD) and the limit of quantification (LOQ) of the assay. The LOD and LOQ were calculated using the signal-to-noise ratio (S/N) criteria in all cases (LOD = 3 S/N, LOQ = 10 S/N).

Using matrix solid phase dispersion 180 pesticides were extracted and analysed by gas chromatography with dual detection system: electron capture and nitrogen–phosphorus, carbendazim by liquid chromatography and ditiocarabamates by spectroscopic techniques. Linearity was evaluated by the calculation of a five-point linear plot with three replicates, based on linear regression and squared correlation coefficient ($R^2$). All pesticides showed linearity in the concentration range of (GC) 0.001–3.0 mg/kg, (HPLC) 0.01–3.0 mg/kg and spectrophotometric 0.5–5.0 mg/kg with correlation coefficients higher than 0.994. The matrix effect on the detectors response for the studied pesticides and matrices was evaluated in the present work. To determine if there is a different response between matrix-matched standards and standards in solvent, matrix-matched standards were used. In this study recovery experiments for 182 pesticides at three spiking levels between 0.001 and 3.0 mg/kg for a period of five days were performed. Mean recoveries for apples spiked at three fortification levels ranged from 71.07 to 119.90% with the exception of captan, dicloran, phosmet, propazine (56–70%) and bifenthrin, dichlofluanid, tecnazene (120–134%) with RSDs of 0.9–9.4% for a period of five days were performed. Each pesticide was fortified at its LOQ level, at the MRL or at 10 times the LOQ level and at a third intermediate level. However, a range of 60–140% may be used in routine multiresidue analysis (EC, 2009, 2013). The accuracy and precision of the method via recovery experiments with fortified samples were tested. Precision method expressed as the repeatability (ten replicates) of the recovery determinations at the studied spiked levels and RSDs for all compounds have been defined (>20%). These results indicate that the recoveries and accuracy of pesticides were good. Consequently, the pesticides were satisfactorily determined using these methods.

The LOD values of individual pesticides were calculated based on the noise level in the chromatograms at S/N of 3:1. The LOQs of the proposed method were calculated by considering a value 10 times that of the background noise. For most compounds the values obtained were lower than their respective MRLs. The LOQs ranged from 0.001 to 0.004 mg/kg. All pesticide analysed LODs were lower than the respective maximum residue levels (MRLs) established by the European regulation for apples. Validation parameters are presented in the Supplementary material (Table S1).

*2.5.5. Quality check*

The laboratory successfully participated every year in the proficiency testing schemes organised and run by the European Commission (University of Almeria) and by the Food Analysis Performance Assessment Scheme (FAPAS; Central Science Laboratory in York). Results of participation in proficiency testing are presented in the Supplementary material (Table S2). All of the analyses were conducted with the use of three methods accredited by the Polish Centre of Accreditation (PCA) in compliance with PN-EN ISO/IEC 17 025.

*2.6. Risk assessment*

*2.6.1. Food-consumption data and different population groups*

The EFSA PRIMo model (EFSA, 2008) was used for deterministic exposure calculations. The model is based on the EFSA food-consumption database, which includes national food-consumption data from several member states. At present, the model includes consumption data for adult consumers from 12 member states and data for children from 7 member states. Furthermore, the model includes data from the 11 WHO European regional diet and the 4 WHO cluster diets, B, D, E and F. The consumption data used for the exposure calculations for six

consumer groups are shown in Tables 4–6. Dietary exposure assessments should cover the general population as well as critical groups (e.g., infants and children) that are vulnerable or are expected to have significantly different exposures than adults. There is a lack of complete studies for Polish consumers because these studies only account for the consumption of the general population (consisting of people from birth to death) and the average consumption (50th percentile) (Szponar et al., 2003).

With respect to the various population groups, special attention is necessary for infants and 1–2-year-old children, who are considered to be the most exposed. This is because infants and young children present the highest food-consumption levels per kilogramme of body weight. For example, UK toddlers consume 14.9 g/kg/body weight/day of apples and German girls consume 12.1 g/kg b.w./day, whereas adults consume approximately five times less, 2.7 g/kg b.w./day. In most cases, the exposure assessed in this population group is consequently higher than that estimated for all other age groups, and this guides the risk-assessment process.

In this working example, we decided to calculate the acute and cumulative dietary exposures of potential consumers of Polish apples using deterministic modelling based on the consumption data of consumers from Poland, the United Kingdom and Germany, selected from the EFSA PRIMo model and two WHO cluster diets.

1) Polish general population (above three years, mean diet, consumption 2.043 g/kg b.w./day) (Szponar et al., 2003)
2) German girls (2–4 years old, diet at the 97.5th percentile, consumption 12.0681 g/kg b.w./day)
3) UK adults (50+ years old, mean, consumption 0.4105 g/kg b.w./day)
4) UK adults (50+ years old, diet at the 97.5th percentile, consumption 2.6816 g/kg b.w./day)
5) UK toddlers (infants less than 1.6 years old, mean diet, consumption 1.7055 g/kg b.w./day)
6) UK toddlers (infants less than 1.6 years old, diet at 97.5th percentile, consumption 14.8689 g/kg b.w./day)
7) WHO Cluster D (Albania, Armenia Azerbaijan, Belarus, Bosnia and Herzegovina, Bulgaria, Georgia, Iran, Kazakhstan, Kyrgyzstan, Moldova, Republic of Montenegro, Romania, Russian Federation, Serbia, Tajikistan, Macedonia, Turkmenistan, Ukraine, Uzbekistan, diet at 97.5th percentile; consumption 0.665 g/kg b.w./day) (Sy et al., 2013)
8) WHO Cluster E (Austria, Belgium, Croatia, Czech Republic, Denmark, France, Germany, Hungary, Ireland, Luxemburg, Malta, Netherlands, Poland, Slovakia, Slovenia, Switzerland, United Kingdom of Great Britain and Northern Ireland, diet at 97.5th percentile; consumption 0.8467 g/kg b.w./day)

*2.6.2. Short-term or acute exposure assessment*

The dietary exposure to pesticides has been calculated in order to assess the acute (short-term) consumer health risk for the six populations (Table 4). The following input values are required to calculate the actual acute exposure:

- High residue concentrations obtained from analysis of apples during 2005–2013 to which the consumer was exposed
- Food consumption, taken from the EFSA PRIMo (EFSA, 2008).

The estimated short-term intake (ESTI) was calculated according to the following formula (Renwick, 2002):

$$\mathrm{ESTI} = \sum \frac{\mathrm{F} \times \mathrm{HR.P}}{\mathrm{mean\_body\_weight}}$$

**Table 3**
Levels of detected pesticide residues in apples and quantification limits (LOQ).

| Active substance | LOQ [mg/kg] | Number of samples | | | | Concentration range [mg/kg] | |
|---|---|---|---|---|---|---|---|
| | | without residues | with residues (total) | with residues < MRL | with residues > MRL | min | max |
| Boscalid | 0.01 | 549 | 106 | 106 | 0 | 0.010 | 0.32 |
| Captan | 0.02 | 493 | 162 | 162 | 0 | 0.020 | 3.00 |
| Carbendazim | 0.02 | 621 | 34 | 34 | 0 | 0.020 | 0.19 |
| Chlorothalonil | 0.01 | 654 | 1 | 1 | 0 | 0.020 | 0.02 |
| Cyprodinil | 0.01 | 614 | 41 | 40 | 2 | 0.010 | 0.34 |
| Difenoconazole | 0.02 | 654 | 1 | 1 | 0 | 0.020 | 0.02 |
| Dithiocarbamate | 0.05 | 563 | 92 | 92 | 0 | 0.050 | 1.87 |
| Fludioxonil | 0.01 | 651 | 4 | 4 | 0 | 0.020 | 0.07 |
| Flusilazole | 0.01 | 637 | 18 | 15 | 3 | 0.010 | 0.30 |
| Folpet | 0.02 | 647 | 8 | 8 | 0 | 0.020 | 0.53 |
| Iprodione | 0.02 | 654 | 1 | 1 | 0 | 0.070 | 0.07 |
| Myclobutanil | 0.02 | 654 | 1 | 1 | 0 | 0.500 | 0.50 |
| Procymidone | 0.02 | 654 | 1 | 1 | 0 | 0.020 | 0.02 |
| Propiconazole | 0.03 | 654 | 1 | 1 | 0 | 0.030 | 0.15 |
| Pyraclostrobin | 0.04 | 648 | 7 | 7 | 0 | 0.040 | 0.21 |
| Pyrimathanil | 0.01 | 569 | 86 | 84 | 2 | 0.010 | 0.48 |
| Tebuconazole | 0.005 | 650 | 5 | 5 | 0 | 0.006 | 0.50 |
| Tetraconazole | 0.005 | 653 | 2 | 2 | 0 | 0.010 | 0.02 |
| Tolylfluanid | 0.02 | 635 | 20 | 20 | 0 | 0.020 | 0.29 |
| Trifloxystrobin | 0.01 | 623 | 32 | 32 | 0 | 0.010 | 0.10 |
| Acetamipirid | 0.01 | 600 | 55 | 55 | 0 | 0.010 | 0.09 |
| Bifenthrin | 0.01 | 653 | 2 | 2 | 0 | 0.010 | 0.02 |
| Chlorpyrifos ethyl | 0.005 | 602 | 53 | 53 | 0 | 0.005 | 0.16 |
| Chlorpyrifos methyl | 0.005 | 644 | 11 | 10 | 1 | 0.008 | 1.00 |
| Cypermethrin | 0.02 | 641 | 14 | 14 | 0 | 0.020 | 0.05 |
| Diazinon | 0.01 | 643 | 12 | 6 | 6 | 0.010 | 0.05 |
| Dimethoate | 0.01 | 645 | 10 | 4 | 6 | 0.010 | 0.30 |
| Esfenvalerate | 0.02 | 653 | 2 | 2 | 0 | 0.020 | 0.03 |
| Fenazaquin | 0.02 | 632 | 23 | 22 | 1 | 0.020 | 0.12 |
| Fenitrothion | 0.01 | 653 | 2 | 0 | 2 | 0.010 | 0.02 |
| Lambda-cyhalothrin | 0.02 | 653 | 2 | 2 | 0 | 0.020 | 0.02 |
| Phosalone | 0.01 | 652 | 3 | 1 | 2 | 0.010 | 0.25 |
| Pirimicarb | 0.01 | 594 | 61 | 61 | 0 | 0.010 | 0.12 |
| Propargite | 0.1 | 636 | 19 | 19 | 0 | 0.100 | 0.65 |

where: ESTI — estimated short-term intake, F — full portion consumption data for the commodity unit, HR.P — the highest residue level.

An estimate of pesticide intake in the diet was compared to the ARfD. The acute hazard index was calculated as follows:

$$aHI = \frac{ESTI}{ARfD}$$

where: ARfDs — Acute Reference Doses.

Acute Reference Doses (ARfDs) are defined as "an estimate of the amount of a substance in food or drinking water, normally expressed on a body weight basis, that can be ingested in a period of 24 h or less without appreciable health risks to the consumer on the basis of all known facts at the time of the evaluation" (WHO/FAO, 2002).

*2.6.3. Chronic or long-term exposure assessment*

The dietary exposure to pesticides has been calculated in order to assess the chronic (long-term) consumer health risk for the six populations (Tables 5 and 6). The following input values are required to calculate the actual chronic exposure:

- Mean residue concentrations obtained from analysis of apples during 2005–2013 to which the consumer was exposed (two scenarios)
- Food consumption, taken from the EFSA PRIMo (EFSA, 2008).

Exposure was calculated according to the approach of estimated daily intake (EDI) given in the "Guidelines for predicting dietary intakes of pesticides residue". The exposure to pesticide residues in apples is calculated, relative to the mean body weight, as the average residue concentration (RLi) in the apples, multiplied by the consumption (Fi) of apples: in the chronic exposure risk assessment, the estimated total dietary exposure was compared to the toxicological reference value, acceptable daily intake (ADI).

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 ADI is set on the basis of all known facts at the time of evaluation, taking into account sensitive groups within the population (e.g. children and the unborn).

The ADIs used for calculation of the HQs for individual pesticides are those accepted in the EU (EFSA) or by JMPR, when available.

The estimated daily intake (EDI) of pesticide residues was calculated as follows:

$$EDI = \sum \frac{F_i \times RL_i}{mean\_body\_weight}$$

where: EDI — estimated daily intake, Fi — food-consumption data, RLi — residue level in the commodity.

The long-term risk assessment of intakes compared to pesticide toxicological data was performed by calculating the hazard quotient (HQ) by dividing the estimated daily intake by the relevant acceptable daily intake:

$$HQ = \frac{EDI}{ADI} \cdot 100\%$$

where: ADI — acceptable daily intake.

The hazard index (HI) for a given diet is calculated by summing the hazard quotients (HQs) for each pesticide (p) in the diet:

$$cHI = \sum HQ$$

HI will be used for the sum of HQs from the pesticides that the consumer is exposed to. If the HI exceeds 100%, the mixture has exceeded the maximum acceptable level and thus, there might be a risk (Reffstrup et al., 2010).

*2.6.4. "Worst-case" and "optimistic scenario" of chronic risk*

In this study, the health risk of chronic exposure to consumers was assessed in two scenarios.

*Scenario 1* is considered to be a rather unrealistic "worst" case scenario, involving calculation of the mean by assuming that the samples without detectable residues (<LOQ) contain residue concentrations at the numerical level of the LOQ.

*Scenario 2* ("optimistic scenario") involved calculation of mean residue concentrations by replacing the reported LOQ values with a zero.

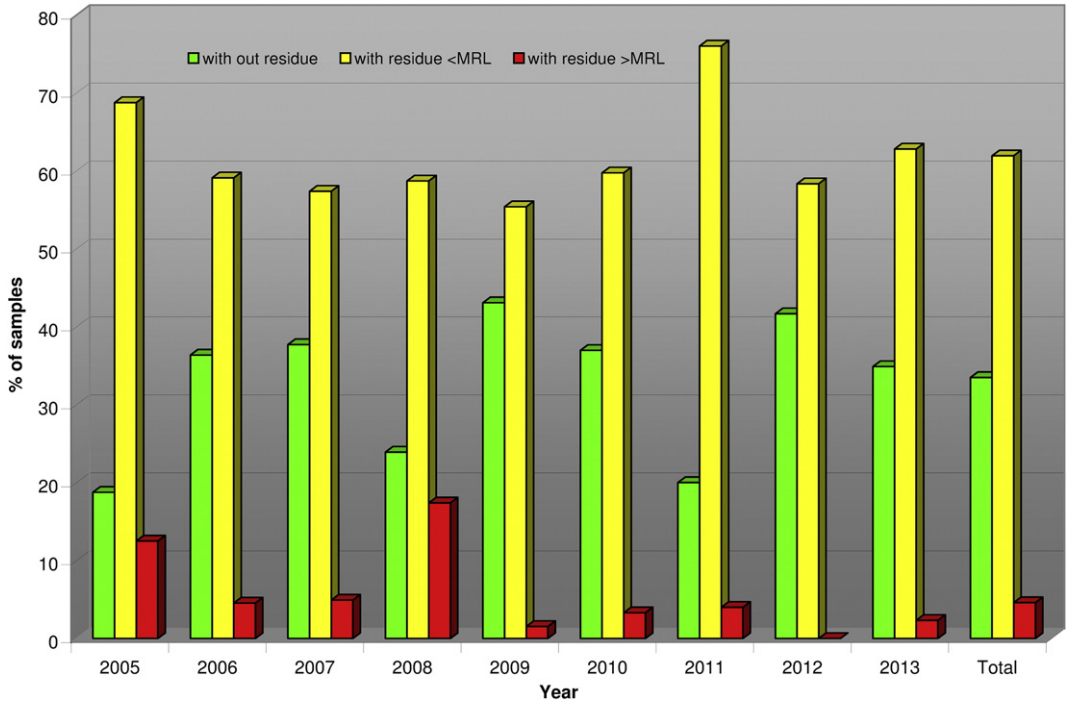


**Fig. 2.** The % of apple samples collected during 2005–2013: without residue, with residue below the MRL, and at the MRL or above the MRL.

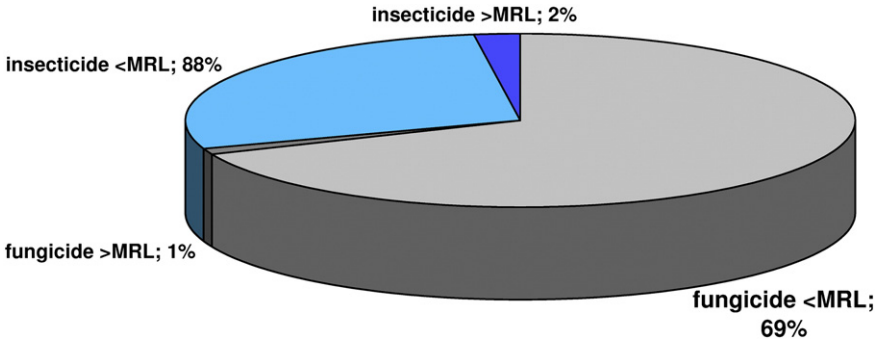


**Fig. 3.** Detections of insecticides and fungicides in apple samples.

This scenario implies that samples with non-detectable residues are completely free of the pertinent pesticide.

*2.6.5. Estimation of the cumulative exposure and risk assessment*

A cumulative risk assessment, which evaluates exposures based on a common mechanism of toxicity, was conducted to evaluate the risk from apples resulting from seventeen pesticide groups. The most numerous groups were: organophosphates (chlorpyrifos etyl, chlorpyrifos methyl, diazinon, dimethoate, fenitrothion, phosalone), triazoles (difenoconazole, flusilazole, mychlobutanil, propiconazole, tebuconazole, tetraconazole) and pyrethroids (bifenthrin, cypermethrin, esfenvalerate, lambda-cyhalothrin), anilinopyrimidines (cyprodinil, pyrimethanil) and strobilurins (pyraclostrobin, trifloxystrobin). The calculated cumulative residue is a simple arithmetic addition of residues of similar groups of chemicals that have different toxicities (potency).

## 3. Results and discussion

*3.1. Pesticide residues found in apples*

This study shows evidence of the presence of pesticide residues in apples (Table 3), including multiple residues in a single sample. A comparison of the detected levels with human health standards would therefore be important.

The percentage of apples analysed in the 2010 EU-coordinated control programme (EFSA, 2013) exceeding the MRL was (1.3%), and the percentage of samples with measurable pesticide residues below or at the MRL accounted for 65.2%. Compared to the results of the 2007 EU-coordinated control programme, using 2010 results, the percentages of samples free of detectable residues were lower (36% in 2007 and 32% in 2010), and 94 unique pesticides were found. The most frequently found active substances were dithiocarbamates (21.4% of samples analysed for this pesticide), captan/folpet (sum) (19.3%), diphenylamine (14.6%), and chlorpyrifos (13.24). MRL exceedances were detected for 15 active substances in 27 samples. For dicofol (sum), the median of the four residue levels (above the LOQ) was higher than 300% of the MRL; the origin of the samples exceeding the dicofol MRL was not reported. It should be noted that dicofol is no longer allowed in Europe.

Apples produced in Denmark (Petersen et al., 2012) in 2004–2011 had residues of 12 different pesticides in 46% of the samples, and 2% contained residues above MRLs. According to Danish monitoring, foreign apples had residues of 54 different pesticides in 80% of the samples and 3% contained residues above the MRL, including Polish apples with 78% residues, of which 2% were above the MRL.

Apples produced in India had residues of chlorpyrifos, endosulphan, dicofol, cypermethrin, fenvalerate, propargite, carbendazim, carbosulphan, thiamethoxam and mancozeb (Singh et al., 2009). Higher levels of pesticide residues were found in apple fruits from Bulgaria produced in the conventional way, with fenitrothion and chlorpyrifos residues

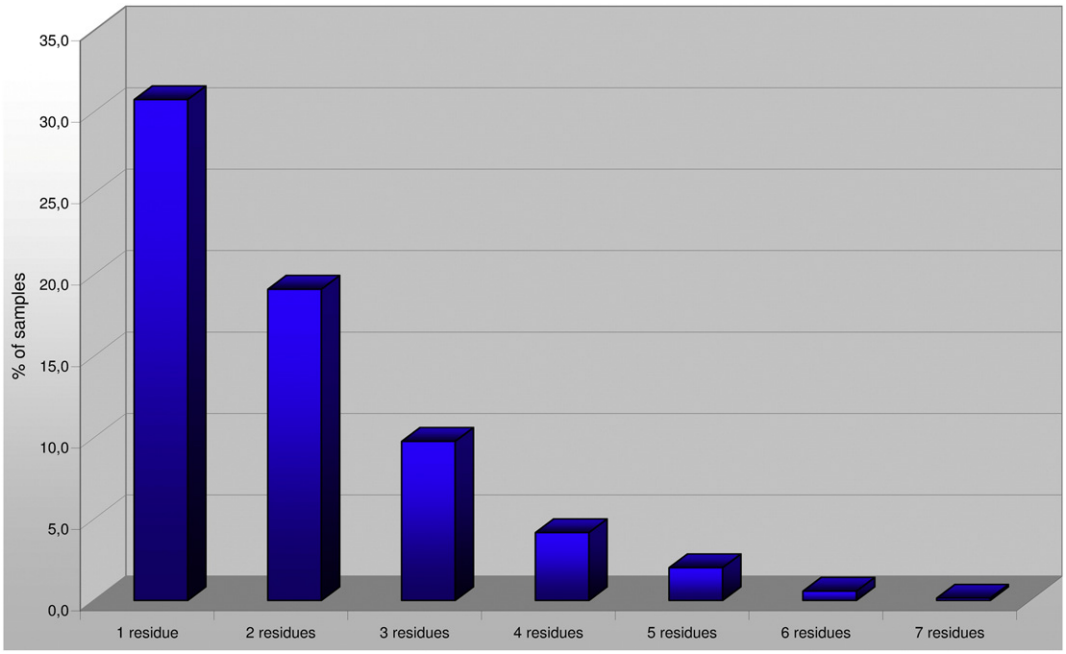


**Fig. 4.** Detected multiple residues samples.

detected frequently (Mladenova and Shtereva, 2009). Pesticide residues were also detected in 59.5% of the samples of fresh apples from the Czech Republic. The maximum residue levels (European Union MRLs) were exceeded only in 1.4% of samples, but residues were detected in 33% of the samples. Organophosphorus insecticides and fungicides representing phtalimides, sulphamides and dicarboximides were the most frequently found residues (Stepán et al., 2005).

In 2007, apples from another part of Poland included 5.6% of samples exceeding the national maximum residue level. The most frequently detected fungicides were dithiocarbamate, captan, pyrimethanil, and the insecticides chlorpiryfos, diazinon and pirimicarb (Machowska et al., 2008).

In our study, Fig. 2 shows the frequencies of samples with and without residue detections in the individual years of the study and the average frequencies for samples of apples produced in the northeastern and central part of Poland. As previously described, the residue data included a total of 659 samples. Pesticides were found in approximately 66.6% of the samples, and 3% contained residues above the MRL. In many cases, no detectable amount of pesticides was found, but this does not necessarily mean that the content is zero.

The lowest percentage of samples with residues was 56% in 2009, and the highest (81%) occurred in 2005. The highest percentage of samples with residues exceeding the maximum residue levels was noted in 2008 (17.4% of samples), the year in which Polish regulations were harmonised with EU regulations (Fig. 2). In 2012, no noncompliant cases were stated. Study of the distribution of samples with and without residues does not make it possible to determine an unequivocal trend line.

In total, 34 different pesticides, belonging to 18 different chemical groups, were found (see Table 3). The most prevalent pesticides were captan (146 determinations, 22.2%), with its highest concentration at 3.0 mg/kg and an arithmetic mean of 0.19 mg/kg, which is a non-systemic fungicide used to control fungal diseases in a wide range of fruit and other crops. Captan residue levels in other fruits and vegetables have also been measured. Boscalid was identified in 15.5% of the samples (102 determinations), which is a systemic fungicide used to control fungal diseases in a wide range of fruit and other crops; dithiocarbamates (84 determinations, 12.7%), which are a group of non-systemic fungicides used in a wide range of fruits and vegetables; pirymethanil (82 determinations, 12.4%); pirimicarb (61, 9.3%), which is a systemic insecticide used against various pests in a wide range of crops; chlorpyrifos ethyl (49, 7.4%), which is a non-systemic insecticide used to control various pests in fruit and other crops; acetamipryd (48, 7.3%); cyprodinil (39, 5.9%); carbendazim (34, 5.2%), which is a systemic fungicide; and trifloxystrobin (30, 4.6%).

The compounds detected in apples belonged to two groups: fungicides and insecticides (Fig. 3). Nearly twice as many fungicides were detected (69% of detections) as insecticides (28% detection). The situation is reversed in terms of the maximum residue level violations, with twice as many violations being noted for insecticides (2%) as for fungicides.

### 3.1.1. Multiple residues

Multiple residues in one sample can result from the application of different types of pesticides used to protect the apples against different pests or diseases, e.g., insecticides, fungicides and herbicides. Pesticide formulations often contain multiple active ingredients, which have different modes of action, which is often recommended by national authorities in integrated strategies to minimise the development of pest resistance to pesticides. In addition to the agricultural practices

**Table 4**
Risk estimates based on comparison of consumed pesticides in the highest concentration with Acute Reference Doses.

| Active substance | ARfD [mg/kg b.w./d] | ESTI [g/kg b.w./d] | aHI [%] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Polish general | German girls (97.5 percentile) | UK adults (mean) | UK adults (97.5 percentile) | UK toddler (mean) | UK toddler (97.5 percentile) | WHO cluster D | WHO cluster E |
| Acetamipirid | 0.1 | 0.09 | 1.29 | 7.60 | 0.26 | 1.69 | 1.07 | 9.37 | 0.42 | 0.53 |
| Bifenthrin | 0.03 | 0.02 | 0.95 | 5.63 | 0.19 | 1.25 | 0.80 | 6.94 | 0.31 | 0.40 |
| Boscalid | Not appl. | 0.32 | – | – | – | – | – | – | – | – |
| Captan | 0.3 | 3.00 | 14.30 | 84.48 | 2.87 | 18.77 | 11.94 | 104.08 | 4.66 | 5.93 |
| Carbendazim | 0.02 | 0.19 | 13.59 | 80.25 | 2.73 | 17.83 | 11.34 | 98.88 | 4.42 | 5.63 |
| Chlorothalonil | 0.6 | 0.02 | 0.05 | 0.28 | 0.01 | 0.06 | 0.04 | 0.35 | 0.02 | 0.02 |
| Chlorpyrifos ethyl | 0.1 | 0.16 | 2.33 | 13.77 | 0.47 | 3.06 | 1.95 | 16.97 | 0.76 | 0.97 |
| Chlorpyrifos methyl | 0.1 | 1.00 | 14.30 | 84.48 | 2.87 | 18.77 | 11.94 | 104.08 | 4.66 | 5.93 |
| Cypermethrin | 0.2 | 0.05 | 0.36 | 2.11 | 0.07 | 0.47 | 0.30 | 2.60 | 0.12 | 0.15 |
| Cyprodinil | Not appl. | 0.34 | – | – | – | – | – | – | – | – |
| Diazinon | 0.025 | 0.05 | 2.86 | 16.90 | 0.57 | 3.75 | 2.39 | 20.82 | 0.93 | 1.19 |
| Difrnoconazole | 0.16 | 0.02 | 0.18 | 1.06 | 0.04 | 0.23 | 0.15 | 1.30 | 0.06 | 0.07 |
| Dimethoate | 0.01 | 0.30 | 42.90 | 253.43 | 8.62 | 56.31 | 35.82 | 312.25 | 13.97 | 17.78 |
| Dithiocarbamate | 0.6 | 1.87 | 4.46 | 26.33 | 0.90 | 5.85 | 3.72 | 32.44 | 1.45 | 1.85 |
| Esfenvalerate | 0.05 | 0.03 | 0.86 | 5.07 | 0.17 | 1.13 | 0.72 | 6.24 | 0.28 | 0.36 |
| Fenazaquin | 0.1 | 0.12 | 1.72 | 10.14 | 0.34 | 2.25 | 1.43 | 12.49 | 0.56 | 0.71 |
| Fenitrothion | 0.013 | 0.02 | 2.20 | 13.00 | 0.44 | 2.89 | 1.84 | 16.01 | 0.72 | 0.91 |
| Fludioxonil | Not appl. | 0.07 | – | – | – | – | – | – | – | – |
| Flusilazole | 0.005 | 0.30 | 85.81 | 506.86 | 17.24 | 112.63 | 71.63 | 624.49 | 27.93 | 35.56 |
| Folpet | 0.2 | 0.53 | 3.79 | 22.39 | 0.76 | 4.97 | 3.16 | 27.58 | 1.23 | 1.57 |
| Iprodione | Not appl. | 0.07 | – | – | – | – | – | – | – | – |
| Lambda-cyhalothrin | 0.0075 | 0.02 | 3.81 | 22.53 | 0.77 | 5.01 | 3.18 | 27.76 | 1.24 | 1.58 |
| Myclobutanil | 0.31 | 0.50 | 2.31 | 13.63 | 0.46 | 3.03 | 1.93 | 16.79 | 0.75 | 0.96 |
| Phosalone | 0.1 | 0.25 | 3.58 | 21.12 | 0.72 | 4.69 | 2.98 | 26.02 | 1.16 | 1.48 |
| Pirimicarb | 0.1 | 0.12 | 1.72 | 10.14 | 0.34 | 2.25 | 1.43 | 12.49 | 0.56 | 0.71 |
| Procymidone | 0.012 | 0.02 | 2.38 | 14.08 | 0.48 | 3.13 | 1.99 | 17.35 | 0.78 | 0.99 |
| Propargite | Not appl. | 0.65 | – | – | – | – | – | – | – | – |
| Propiconazole | 0.3 | 0.15 | 0.72 | 4.22 | 0.14 | 0.94 | 0.60 | 5.20 | 0.23 | 0.30 |
| Pyraclostrobin | 0.03 | 0.21 | 10.01 | 59.13 | 2.01 | 13.14 | 8.36 | 72.86 | 3.26 | 4.15 |
| Pyrimathanil | Not appl. | 0.48 | – | – | – | – | – | – | – | – |
| Tebuconazole | 0.03 | 0.50 | 23.84 | 140.79 | 4.79 | 31.29 | 19.90 | 173.47 | 7.76 | 9.88 |
| Tetraconazole | 0.05 | 0.02 | 0.57 | 3.38 | 0.11 | 0.75 | 0.48 | 4.16 | 0.19 | 0.24 |
| Tolylfluanid | 0.25 | 0.29 | 1.66 | 9.80 | 0.33 | 2.18 | 1.38 | 12.07 | 0.54 | 0.69 |
| Trifloxystrobin | Not appl. | 0.10 | – | – | – | – | – | – | – | – |

mentioned above, other possible reasons for the occurrence of multiple residues are the mixing of lots that were treated with different pesticides, either during sampling or over the course of the sorting of the commodities (e.g., sorting for quality classes); residues resulting from soil uptake in cases in which pesticides have high persistence in the soil; residues resulting from spray drift from neighbouring plots or cross-contamination in the processing of the crops (e.g., by washing practices) (Coronado et al., 2011); or contamination during handling, packing and storage.

Apple samples containing one, two and multiple residues were noted (Fig. 4). Ten pesticides were found most frequently in combination with one or more other residues. A single residue was detected in 30.8% of the samples, and two in 19.4%. The most frequent combinations of two pesticides measured in the same sample were captan/boscalid (47 samples), cyprodinil/fludioxonil (190 samples) and boscalid/pyraclostrobin (149 samples). The concentrations of the two detected compounds fell within the range of 0.05 to 4.1 mg/kg, whereas 9.6% of the apples contained three residues in concentrations ranging from 0.07 to 0.24 mg/kg, and most often, it was the combination of dithiocarbamates/captan/pirimicarb. In 4.17% of the samples, four residues were found in the highest average concentration: pyrimethanil/diazinon/fenitrothion/cypermethrin (total concentration of 0.75 mg/kg), pirimicarb/trifloxystrobin/captan/acetamiprid (total concentration of 0.62 mg/kg), and the combination of boscalid/captan/carbendazim/dithiocarbamate (total concentration of 0.65 mg/kg). Five compounds were found in 2% of the samples in the highest mean concentration of 0.34 mg/kg. In 0.6% of the samples, six compounds were detected, and the following compounds were found in the highest concentration of 0.87 mg/kg: chlorpyrifos ethyl/dithiocarbamates/cyprodinil/esfenvalerate/flusilazole/pirymethanil. Seven compounds: pirymethanil/captan/fozalon/fenazaquin/dithiocarbamate/cyprodinil/cypermethrin were detected in one sample with a concentration of 1.02 mg/kg.

Apples are susceptible to storage diseases caused by approximately 20 species of fungi, with some of them occurring ubiquitously, and others more rarely or sporadically. The most frequently occurring fungi include fungi from the *Pezicula* genus, *Botrytis cinerea*, *Penicillium expansum* and *Monilinia fructigena* (Neri et al., 2009). The harmfulness of these diseases is significant because they cause the total destruction of apples (Weber, 2009; Weiss et al., 2006). Certain fungi (from the genera *Penicillium*, *Alternaria*, *Fusarium*) produce mycotoxins while developing on apples that are harmful to human health. For this reason, orchard farmers implement intensive fungicidal protection. For example, an apple orchard-protection programme conducted in 2009 (conventional apples from a Polish official inspection) encompassed 33 treatment procedures with twenty different plant-protection products: mainly fungicides (30 applications), herbicides (2) and insecticide (1) to give a total application level of 38.6 kg of pure active substances (all pesticides) per hectare. The performance of the treatment procedures at the appropriate times and doses guarantees that possible

**Table 5**
Risk estimates based on comparison of consumed groups of pesticides in the mean concentration with acceptable daily intake (scenario 1).

| Active substance | ADI [mg/kg b.w./d] | EDI [g/kg b.w./d] | HQ [%] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Polish general | German girls (97.5 percentile) | UK adults (mean) | UK adults (97.5 percentile) | UK toddler (mean) | UK toddler (97.5 percentile) | WHO cluster D | WHO cluster E |
| Boscalid | 0.04 | 0.02037 | 0.10 | 0.61 | 0.02 | 0.14 | 0.09 | 0.76 | 0.03 | 0.04 |
| Cyprodinil | 0.03 | 0.01317 | 0.09 | 0.53 | 0.02 | 0.12 | 0.07 | 0.65 | 0.03 | 0.04 |
| Pyrimathanil | 0.17 | 0.01720 | 0.02 | 0.12 | 0.00 | 0.03 | 0.02 | 0.15 | 0.01 | 0.01 |
| | | **cHI** | **0.11** | **0.65** | **0.02** | **0.14** | **0.09** | **0.80** | **0.04** | **0.05** |
| Carbendazim | 0.02 | 0.02127 | 0.22 | 1.28 | 0.04 | 0.29 | 0.18 | 1.58 | 0.07 | 0.09 |
| Iprodione | 0.06 | 0.02008 | 0.07 | 0.40 | 0.01 | 0.09 | 0.06 | 0.50 | 0.02 | 0.03 |
| Procymidone | 0.0028 | 0.02000 | 1.46 | 8.62 | 0.29 | 1.92 | 1.22 | 10.62 | 0.47 | 0.60 |
| | | **cHI** | **1.53** | **9.02** | **0.31** | **2.01** | **1.28** | **11.12** | **0.50** | **0.63** |
| Dithiocarbamate | 0.05 | 0.06835 | 0.28 | 1.65 | 0.06 | 0.37 | 0.23 | 2.03 | 0.09 | 0.12 |
| Fludioxonil | 0.37 | 0.01014 | 0.01 | 0.03 | 0.00 | 0.01 | 0.00 | 0.04 | 0.00 | 0.00 |
| Captan | 0.1 | 0.04182 | 0.09 | 0.50 | 0.02 | 0.11 | 0.07 | 0.62 | 0.03 | 0.04 |
| Folpet | 0.1 | 0.02093 | 0.04 | 0.25 | 0.01 | 0.06 | 0.04 | 0.31 | 0.01 | 0.02 |
| | | **cHI** | **0.13** | **0.76** | **0.03** | **0.17** | **0.11** | **0.93** | **0.04** | **0.05** |
| Chlorothalonil | 0.015 | 0.01002 | 0.14 | 0.81 | 0.03 | 0.18 | 0.11 | 0.99 | 0.04 | 0.06 |
| Pyraclostrobin | 0.03 | 0.04051 | 0.28 | 1.63 | 0.06 | 0.36 | 0.23 | 2.01 | 0.09 | 0.11 |
| Trifloxystrobin | 0.1 | 0.01041 | 0.02 | 0.13 | 0.00 | 0.03 | 0.02 | 0.15 | 0.01 | 0.01 |
| | | **cHI** | **0.30** | **1.76** | **0.06** | **0.39** | **0.25** | **2.16** | **0.10** | **0.12** |
| Tolylfluanid | 0.1 | 0.02216 | 0.05 | 0.27 | 0.01 | 0.06 | 0.04 | 0.33 | 0.01 | 0.02 |
| Difenoconazole | 0.01 | 0.02000 | 0.41 | 2.41 | 0.08 | 0.54 | 0.34 | 2.97 | 0.13 | 0.17 |
| Flusilazole | 0.002 | 0.01077 | 1.10 | 6.50 | 0.22 | 1.44 | 0.92 | 8.00 | 0.36 | 0.46 |
| Myclobutanil | 0.025 | 0.02074 | 0.17 | 1.00 | 0.03 | 0.22 | 0.14 | 1.23 | 0.06 | 0.07 |
| Propiconazole | 0.04 | 0.03018 | 0.15 | 0.91 | 0.03 | 0.20 | 0.13 | 1.12 | 0.05 | 0.06 |
| Tebuconazole | 0.03 | 0.00581 | 0.04 | 0.23 | 0.01 | 0.05 | 0.03 | 0.29 | 0.01 | 0.02 |
| Tetraconazole | 0.004 | 0.00503 | 0.26 | 1.52 | 0.05 | 0.34 | 0.21 | 1.87 | 0.08 | 0.11 |
| | | **cHI** | **2.13** | **12.57** | **0.43** | **2.79** | **1.78** | **15.49** | **0.69** | **0.88** |
| Acetamipirid | 0.07 | 0.01072 | 0.03 | 0.18 | 0.01 | 0.04 | 0.03 | 0.23 | 0.01 | 0.01 |
| Pirimicarb | 0.035 | 0.01191 | 0.07 | 0.41 | 0.01 | 0.09 | 0.06 | 0.51 | 0.02 | 0.03 |
| Chlorpyrifos ethyl | 0.01 | 0.00670 | 0.14 | 0.81 | 0.03 | 0.18 | 0.11 | 1.00 | 0.04 | 0.06 |
| Chlorpyrifos methyl | 0.01 | 0.00717 | 0.15 | 0.87 | 0.03 | 0.19 | 0.12 | 1.07 | 0.05 | 0.06 |
| Diazinon | 0.0002 | 0.01020 | 10.42 | 61.54 | 2.09 | 13.67 | 8.70 | 75.82 | 3.39 | 4.32 |
| Dimethoate | 0.001 | 0.01123 | 2.29 | 13.55 | 0.46 | 3.01 | 1.91 | 16.69 | 0.75 | 0.95 |
| Fenitrothion | 0.005 | 0.01002 | 0.41 | 2.42 | 0.08 | 0.54 | 0.34 | 2.98 | 0.13 | 0.17 |
| Phosalone | 0.01 | 0.01054 | 0.22 | 1.27 | 0.04 | 0.28 | 0.18 | 1.57 | 0.07 | 0.09 |
| | | **cHI** | **13.62** | **80.45** | **2.74** | **17.88** | **11.37** | **99.12** | **4.43** | **5.64** |
| Bifenthrin | 0.015 | 0.01002 | 0.14 | 0.81 | 0.03 | 0.18 | 0.11 | 0.99 | 0.04 | 0.06 |
| Cypermethrin | 0.05 | 0.02015 | 0.08 | 0.49 | 0.02 | 0.11 | 0.07 | 0.60 | 0.03 | 0.03 |
| Esfenvalerate | 0.02 | 0.02002 | 0.20 | 1.21 | 0.04 | 0.27 | 0.17 | 1.49 | 0.07 | 0.08 |
| Lambda-cyhalothrin | 0.005 | 0.02000 | 0.82 | 4.83 | 0.16 | 1.07 | 0.68 | 5.95 | 0.27 | 0.34 |
| | | **cHI** | **1.24** | **7.33** | **0.25** | **1.63** | **1.04** | **9.03** | **0.40** | **0.51** |
| Propargite | 0.01 | 0.10469 | 2.14 | 12.63 | 0.43 | 2.81 | 1.79 | 15.57 | 0.70 | 0.89 |
| Fenazaquin | 0.005 | 0.02041 | 0.83 | 4.93 | 0.17 | 1.09 | 0.70 | 6.07 | 0.27 | 0.35 |

Bold — cumulative Hazard Index for compounds of the same mode of action.

residues are at safe levels. In an apple sample originating from this orchard, only captan in a concentration of 0.01 mg/kg and pirymethanil in a concentration of 0.02 mg/kg were detected.

According to the current EU legislation, the presence of multiple residues in one sample is not a reason for considering a sample as not compliant with the MRL legislation, as long as the individual residues do not exceed their respective MRLs. Legal actions must be imposed by the member states in cases in which one or more MRLs are exceeded (EFSA, 2013).

### 3.1.2. Samples above MRL

As a result, this analysis shows that, in most cases in which multiple residues are found on apples, the measured residues are present in concentrations below the MRLs. Residues in concentrations above MRLs occurred in 3% of the individual samples (Fig. 2). However, even if the individual MRLs for pesticides are not exceeded, a food item may be of concern if the occurrence of the individual substances causes the same toxicological effect in humans. The maximum residue limit (MRL) was exceeded in 24 samples, including diazinon (six samples: 0.02; 0.02; 0.02; 0.03; 0.5 mg/kg, MRL = 0.01 mg/kg), dimethoate (six samples: 0.04; 0.05; 0.1; 0.1; 0.2; 0.3 mg/kg, MRL = 0.02 mg/kg, flusilazole (three samples: 0.03; 0.1; 0.3 mg/kg, MRL = 0.01 mg/kg), cyprodinil (two samples: 0.08; 0.1 mg/kg, MRL = 0.05 mg/kg); pirymethanil (two samples: 0.3; 0.48 mg/kg, MRL = 0.01 mg/kg), phosalone (two samples: 0.03; 0.25 mg/kg, MRL = 0.01 mg/kg), fenitrothion (two samples, 0.02; 0.02 mg/kg, MRL = 0.01 mg/kg) and fenazaquin (one sample, 0.12 mg/kg, MRL = 0.01 mg/kg). Compounds not recommended for use as protection in apple orchards, including tolyfluanide or phosalone, were present in four samples.

### 3.2. Exposure calculations

The long-term (nine years) monitoring had the goal of obtaining a representative picture of the chemical levels present in Polish apples. The results from the analytical programme have been used to calculate the exposure for the six populations by multiplying a mean or high of the residues by a mean and high percentile of consumption. In this paper, assessments of exposure to a mixture of pesticides were performed using the hazard index (HI). According to the methodologies currently used in consumer-risk assessment, the exposure assessment was calculated separately for each pesticide. However, because consumers may be exposed to more than one pesticide, either within one meal or over a longer period of consumption of different foods, it is important to assess whether the combined exposure to different pesticides actually present in the food being eaten poses a risk to consumer health.

There is no internationally agreed upon method for the risk assessment of the cumulative exposure to multiple residues of pesticides (Boobisa et al., 2008). Ideally, the long-term exposure assessment should be calculated by means of probabilistic modelling, using the

**Table 6**
Risk estimates based on comparison of consumed groups of pesticides in the mean concentration with acceptable daily intake (scenario 2).

| Active substance | ADI [mg/kg b.w./d] | EDI [g/kg b.w./d] | HQ [%] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Polish general | German girls (97.5 percentile) | UK adults (mean) | UK adults (97.5 percentile) | UK toddler (mean) | UK toddler (97.5 percentile) | WHO cluster D | WHO cluster E |
| Boscalid | 0.04 | 0.01182 | 0.06 | 0.36 | 0.01 | 0.08 | 0.05 | 0.44 | 0.02 | 0.03 |
| Cyprodinil | 0.03 | 0.00383 | 0.03 | 0.15 | 0.01 | 0.03 | 0.02 | 0.19 | 0.01 | 0.01 |
| Pyrimathanil | 0.17 | 0.00907 | 0.01 | 0.06 | 0.00 | 0.01 | 0.01 | 0.08 | 0.00 | 0.00 |
| | | **cHI** | **0.04** | **0.22** | **0.01** | **0.05** | **0.03** | **0.27** | **0.01** | **0.02** |
| Carbendazim | 0.02 | 0.00216 | 0.02 | 0.13 | 0.00 | 0.03 | 0.02 | 0.16 | 0.01 | 0.01 |
| Iprodione | 0.06 | 0.00010 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Procymidone | 0.0028 | 0.00003 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 |
| | | **cHI** | **0.00** | **0.01** | **0.00** | **0.00** | **0.00** | **0.02** | **0.00** | **0.00** |
| Dithiocarbamate | 0.05 | 0.02543 | 0.10 | 0.61 | 0.02 | 0.14 | 0.09 | 0.76 | 0.03 | 0.04 |
| Fludioxonil | 0.37 | 0.02543 | 0.01 | 0.08 | 0.00 | 0.02 | 0.01 | 0.10 | 0.00 | 0.01 |
| Captan | 0.1 | 0.04573 | 0.09 | 0.55 | 0.02 | 0.12 | 0.08 | 0.68 | 0.03 | 0.04 |
| Folpet | 0.1 | 0.00110 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 |
| | | **cHI** | **0.10** | **0.57** | **0.02** | **0.13** | **0.08** | **0.70** | **0.03** | **0.04** |
| Chlorothalonil | 0.015 | 0.00003 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Pyraclostrobin | 0.03 | 0.00087 | 0.01 | 0.04 | 0.00 | 0.01 | 0.00 | 0.04 | 0.00 | 0.00 |
| Trifloxystrobin | 0.1 | 0.00086 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 |
| | | **cHI** | **0.01** | **0.05** | **0.00** | **0.01** | **0.01** | **0.06** | **0.00** | **0.00** |
| Tolylfluanide | 0.1 | 0.00259 | 0.01 | 0.03 | 0.00 | 0.01 | 0.00 | 0.04 | 0.00 | 0.00 |
| Difenoconazole | 0.01 | 0.00003 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Flusilazole | 0.002 | 0.00007 | 0.01 | 0.04 | 0.00 | 0.01 | 0.01 | 0.05 | 0.00 | 0.00 |
| Myclobutanil | 0.025 | 0.00072 | 0.01 | 0.03 | 0.00 | 0.01 | 0.00 | 0.04 | 0.00 | 0.00 |
| Propiconazole | 0.04 | 0.00021 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 |
| Tebuconazole | 0.03 | 0.00097 | 0.01 | 0.04 | 0.00 | 0.01 | 0.01 | 0.05 | 0.00 | 0.00 |
| Tetraconazole | 0.004 | 0.00004 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 |
| | | **cHI** | **0.02** | **0.14** | **0.00** | **0.03** | **0.02** | **0.17** | **0.01** | **0.01** |
| Acetamipirid | 0.07 | 0.00155 | 0.00 | 0.03 | 0.00 | 0.01 | 0.00 | 0.03 | 0.00 | 0.00 |
| Pirimicarb | 0.035 | 0.00269 | 0.02 | 0.09 | 0.00 | 0.02 | 0.01 | 0.11 | 0.01 | 0.01 |
| Chlorpyrifos ethyl | 0.01 | 0.00232 | 0.05 | 0.28 | 0.01 | 0.06 | 0.04 | 0.35 | 0.02 | 0.02 |
| Chlorpyrifos methyl | 0.01 | 0.00229 | 0.05 | 0.28 | 0.01 | 0.06 | 0.04 | 0.34 | 0.02 | 0.02 |
| Diazinon | 0.0002 | 0.00036 | 0.37 | 2.16 | 0.07 | 0.48 | 0.31 | 2.66 | 0.12 | 0.15 |
| Dimethoate | 0.001 | 0.00129 | 0.26 | 1.56 | 0.05 | 0.35 | 0.22 | 1.92 | 0.09 | 0.11 |
| Fenitrothion | 0.005 | 0.00129 | 0.05 | 0.31 | 0.01 | 0.07 | 0.04 | 0.38 | 0.02 | 0.02 |
| Phosalone | 0.01 | 0.00054 | 0.01 | 0.07 | 0.00 | 0.01 | 0.01 | 0.08 | 0.00 | 0.00 |
| | | **cHI** | **0.79** | **4.65** | **0.16** | **1.03** | **0.66** | **5.73** | **0.26** | **0.33** |
| Bifenthrin | 0.015 | 0.00004 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Cypermethrin | 0.05 | 0.00069 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 |
| Esfenvalerate | 0.02 | 0.00007 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 |
| Lambda-cyhalothrin | 0.005 | 0.00007 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 |
| | | **cHI** | **0.01** | **0.04** | **0.00** | **0.01** | **0.01** | **0.05** | **0.00** | **0.00** |
| Propargite | 0.01 | 0.00711 | 0.15 | 0.86 | 0.03 | 0.19 | 0.12 | 1.06 | 0.05 | 0.06 |
| Fenazaquin | 0.005 | 0.00003 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 |

Bold — cumulative Hazard Index for compounds of the same mode of action.

distributions of the individual food consumptions reported by the respondents of food surveys and the distribution of the measured residue concentrations identified in monitoring programmes.

Because a methodology for probabilistic calculations is not yet available, in this paper, we decided to perform indicative chronic, cumulative risk assessments based on a conservative ("point estimates", in essence: multiplying a residue value by a single consumption value) "worst-case scenario", which is likely to overestimate the real consumer exposure, and an "optimistic scenario". The main advantage of a deterministic method is that it is relatively easy to perform and does not require sophisticated software.

The HI (sum of hazard quotients (HQ) for individual pesticides) method assumes that the effects following cumulative exposure can be predicted by the mathematical model of dose-addition, and it is designed for the risk assessment of substances that have the same effect or a common mode of action, e.g., organophosphate pesticides or the triazole group. Because the HI method assumes the same type of adverse effect for all of the detected pesticides, it is a relatively conservative (precautionary) approach to cumulative risk assessment.

Tables 4, 5 and 6 show the intake and average exposure in μg/kg b.w/day and in μg/day/person for children, adults and the general population.

*3.2.1. Pesticides contributing the most to chronic exposure*

The risk assessment for single detected pesticides in apples collected in 2005–2013 was performed by estimation of the hazard quotient (HQ), which was calculated by dividing the exposure by the acceptable daily intake (ADI) for individual pesticides.

The calculations reflect the "worst-case scenario", assuming that apples have been treated with all 34 pesticides included in the provisional assessment group and contained residues of each of the pesticides at least at the level of quantification.

Table 7 shows details for each pesticide and groups with the same mechanism (EFSA, 2009). Because the HQ method assumes the same type of adverse effect for all of the detected pesticides, it is a relatively conservative approach for cumulative risk assessment. The HQs for the individual pesticides ranged from 0.01% to 75.8%, with most of the HQs being below 100%, indicating no risk of adverse effects following exposure to individual pesticides. Diazinon contributed the most, but it did not exceed 76% of the acceptable daily intake for the most critical population of toddlers (UK toddler). Among the detected pesticides, diazinon has the lowest acceptable daily intake value ADI = 0.0002 mg/kg. For cluster diets D and E, diazinon constitutes 3.4 and 4.4% of the ADI, respectively. Residual traces of diazinon introduce neurotoxins into the body, which damage and poison the nervous system. Their toxicologically relevant effect is that they inhibit acetylcholine esterase activity (Feigenbrugel et al., 2005; White et al., 2003). Diazinon was approved until December 2007, and it is assumed that crops legally treated with diazinon in 2007 were still on the EC market in 2008. Out of the 1423 apple samples collected in 2008 from national control programmes, 18 samples contained quantifiable diazinon residues above the reporting level. The MRL was exceeded in 13 samples; of these samples, six were of European origin. It is expected that the diazinon residue levels will decrease in 2009 following restrictions for its use. Nevertheless, it is recommended to continue monitoring diazinon residues in food commodities. Member states are recommended to check for possible misuses at the national level on domestic products and check if LOQ MRLs for imported products are exceeded.

According to the unrealistic "worst-case scenario", the overall exposure resulting from residues of all detected pesticides ranged from 5.0% (UK adults, mean) to 181.93% UK toddlers (97.5 percentile) of the toxicological threshold for long-term exposure. The HI of 147.7% for children (2–4 years old) and 181.9% for toddlers is considered to indicate a risk of adverse effects following cumulative exposure to all of the detected pesticides. Diazinon, dimethoate, procymidone, flusilazole and lambda cyhalotrin were identified as the main contributing pesticides. All other pesticides resulted in an exposure below 1% of the toxicological reference value. In the "optimistic scenario", (Table 6), in which the limits of detection of the 34 detected compounds were not accounted for, only their average concentration, the greatest exposure was to diazinon, at approx. 3% ADI for UK toddlers. Such an approach indicates that none of the detected compounds or their groups present risk, and the cumulative risk for all compounds is equal to approx. 10% ADI.

*3.2.2. Groups of pesticides contributing the most to exposure*

A cumulative risk assessment begins with the identification of a group of chemicals, called a common mechanism group (CMG), that induces a common toxic effect by a common mechanism of toxicity. Pesticides are determined to have a "common mechanism of toxicity" if they act the same way in the body: that is, the same toxic effect occurs in the same organ or tissue by essentially the same sequence of major biochemical events. We distinguished five groups: organophosphates (OPs), N-methyl carbamates, triazoles, pyrethrins, and pyrethroids. Organophosphates (OPs) are a group of closely related pesticides used in agriculture and at nonagricultural sites that affect the functioning of the nervous system.

According to the unrealistic "worst-case scenario" (Table 5), the overall exposure resulting from residues of organophosphate, triazole and pyrethroid pesticides ranged from 4.4%, 0.7%, and 0.4% (WHO cluster diet D) to 99.1%, 15.5%, and 9% (UK toddlers 97.5th percentile) of the toxicological threshold for long-term exposure.

*3.2.3. Pesticides exhibiting the highest acute exposure*

To calculate the acute exposure from pesticide residues, the international estimated short-term intake (IESTI) method was used. This method requires data on the consumption of large portions (usually the 97.5th percentile from single-day consumption data among consumers), along with typical unit weights of the edible part of commodities and the body weights of the population associated with the food-consumption data. The baseline assumption is that a consumer may eat a large portion (high-level consumer at the top end of the distribution curve among consumers only) of a food that may contain residue levels higher than that of the composite sample, which was derived from supervised field trials.

Table 4 shows the HI for the general population, children and adults. The results show that children and UK toddlers have the highest exposure per kg b.w., followed by adults. The reason for the highest exposure for children is that they consume more per kg bodyweight compared to adults. The greatest consumption was observed for the UK toddler group (97.5th percentile), 14.8689 μg/kg b.w./day, and German girls (97.5th percentile), 12.0681 μg/kg b.w./day. Because the exposure per kg b.w. is highest for children, the HI is also highest for children. It can be seen in Table 4 that the HI for children is many times higher than for UK adults (97.5th percentile). In cases where MRLs are exceeded, an apple sample containing flusilazole at a concentration of 0.3 mg/kg constituted a real threat to children's health (aHI = 624.9% for UK toddlers and 507% for German girls). In the case of UK adults with consumption at the 97.5th percentile, the risk cannot be dismissed. Calculations accounting for average diet and population show that the general risk is less than 100%.

Flusilazole, a triazole fungicide, has an effect on reproductive development and is possibly a carcinogen. Flusilazole is an ergosterol biosynthesis inhibitor, which has a broad spectrum of activity against diseases caused by fungi and almost any class of pathogens, with the exception of *Peronosporales*. The fungicide has been applied worldwide to several cereals, as well as vegetables, fruits, and nuts. The data obtained by di Renzo et al. (2013) after in vitro and in vivo exposure suggest that the basal low level of one azole residue could shift the dose–response curve of the "moving" fungicide, supporting the additive effects of co-exposure to azole fungicides for developing mammalian embryos. These results suggest that azoles causing this effect should be grouped together for risk assessment and show that all the molecules in the class of triazole derivatives have common intrinsic teratogenic activity whose specific targets are the embryonic structures involved in

**Table 7**
Active substances of plant-protection products found in apples with corresponding health effects.

| Pesticide | Substance group | Mode of action | Carcinogen | Mutagen | Endocrine disruptor | Reproduction/ development effects | Acetyl cholinesterazy inhibitor | Neurotoxicant | Respiratory tract irritant | Skin irritant | Eye irritant |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Acaricides* | | | | | | | | | | | |
| Bifenthrin | Pyrethroid | Contact and stomach action with some residual effect. Sodium channel modulator. | ? | ? | V | ? | X | V | – | X | X |
| Propargite | Sulphite ester | Non-systemic with contact action. Inhibits oxidative phosphorylation. | ? | – | – | V | X | X | X | V | V |
| *Fungicides* | | | | | | | | | | | |
| Boscalid | Carboxamide | Protectant. Foliar absorption. Translocates. Inhibits spore germination and germ tube elongation. | ? | – | X | ? | X | X | X | X | ? |
| Captan | Phthalimide | Non-systemic with protective and curative action. | V | X | X | – | X | X | – | V | V |
| Carbendazim | Benzimidazole | Systemic with curative and protectant activity. Inhibition of mitosis and cell division. | ? | – | ? | V | X | X | X | X | X |
| Chlorothalonil | Chloronitrile | Non-systemic. Broad spectrum. Foliar action with some protectant properties. Acts by preventing spore germination and zoospore motility | ? | X | X | – | X | X | V | V | V |
| Cyprodinil | Anilinopyrimidine | Systemic. Absorbed through foliage. Inhibits protein synthesis. | X | X | – | ? | X | X | V | V | V |
| Diniconazole | Triazole | Systemic with curative and protective action. Inhibits the demethylation of steroids distrupting ergosterol biosynthesis. | X | – | – | – | X | X | – | – | – |
| Fludioxonil | Phenylpyrrole | Non-systemic with long residual activity. Inhibits transport-associated phosphorylation of glucose. Reducing mycelial growth. | ? | – | – | ? | X | X | X | V | V |
| Flusilazole | Triazole | Broad spectrum. Systemic with protective and curative action | ? | – | – | V | X | X | ? | ? | ? |
| Folpet | Phthalimide | Foliar applied with protective action. Acts by inhibiting normal cell division of many microorganisms. | V | ? | – | – | X | X | ? | V | V |
| Iprodione | Dicarboximide | Contact action with protectant and some eradicant activity. Signal transduction inhibitor. | V | – | ? | – | X | X | V | V | V |
| Myclobutanil | Triazole | Broad spectrum. Systemic with protective. Eradicative and curative action. Disrupts membrane function by inhibiting sterol biosynthesis. | X | – | – | ? | X | X | X | X | X |
| Procymidone | Dicarboximide | Systemic with protective and curative properties | V | – | V | V | X | – | ? | X | X |
| Propiconazole | Triazole | Systemic with curative and protective action. Works via the demethylation of C-14 during ergosterol biosynthesis. | ? | – | – | – | – | – | X | X | X |
| Pyraclostrobin | Strobilurin | Protective and curative action. Respiration inhibitor (QoL fungicide). | X | – | – | ? | X | X | X | V | ? |
| Pyrimathanil | Anilinopyrimidine | Protective action with some curative properties | X | – | ? | X | X | X | – | X | ? |
| Tebuconazole | Triazole | Systemic with protective. Curative and eradicant action. Disrupts membrane function. | ? | – | – | V | X | X | X | X | V |
| Tetraconazole | Triazole | Systemic with protectant. Eradicant and curative properties | X | – | – | ? | X | X | X | X | X |
| Tolylfluanid | Sulphamide | Broad spectrum. Multi-site with protective action. | ? | – | – | X | X | X | – | V | V |
| Trifloxystrobin | Strobilurin | Broad spectrum with preventative and curative action. Respiration inhibitor. | | | | | | | | | |
| (QoL fungicide) | X | – | – | V | X | X | – | V | X | | |

*(continued on next page)*

**Table 7** *(continued)*

| Pesticide | Substance group | Mode of action | Carcinogen | Mutagen | Endocrine disruptor | Reproduction/ development effects | Acetyl cholinesterazy inhibitor | Neurotoxicant | Respiratory tract irritant | Skin irritant | Eye irritant |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Metabolite* | | | | | | | | | | | |
| Dithiocarbamate | Unclassified | Not applicable. | – | – | – | – | – | – | – | – | – |
| *Insecticides* | | | | | | | | | | | |
| Acetampirid | Neonicotinoid | Systemic with translaminar activity having both contact and stomach action. Acetylcholine receptor (nAChR) agonist. | X | – | – | – | X | X | X | V | V |
| Bifenthrin | Pyrethroid | Contact and stomach action with some residual effect. | | | | | | | | | |
| Sodium channel modulator. | ? | ? | V | ? | X | V | – | X | X | | |
| Chlorpyrifos ethyl | Organophosphate | Non-systemic with contact and stomach action. Acetylcholinesterase (AChE) inhibitor. | X | X | ? | V | V | V | X | V | V |
| Chlorpyrifos metyl | Organophosphate | Non-systemic with contact. Stomach and respiratory action. Acetylcholinesterase (AChE) inhibitor. | X | – | X | – | V | V | X | V | X |
| Cypermethrin | Pyrethroid | Non-systemic with contact and stomach action. | | | | | | | | | |
| Sodium channel modulator. | ? | X | ? | ? | X | X | V | V | V | | |
| Diazinon | Organophosphate | Non-systemic with respiratory. Contact and stomach action. Acetylcholinesterase (AChE) inhibitor. | X | ? | ? | ? | V | V | V | V | V |
| Dimethoate | Organophosphate | Systemic with contact and stomach action. | | | | | | | | | |
| Acetylcholinesterase (AChE) inhibitor | ? | X | ? | V | V | X | – | X | V | | |
| Esfenvalerate | Pyrethroid | Contact and stomach action. Sodium channel modulator. | X | X | ? | ? | X | X | – | X | X |
| Fenazaquin | Unclassified | A mitochondrial electron transport inhibitor with contact action. | X | – | X | ? | X | – | V | X | X |
| Fenitrothion | Organophosphate | Non-systemic. Broad spectrum with contact and stomach action. Acetylcholinesterase (AChE) inhibitor. | X | X | V | – | V | X | – | V | X |
| Lambda-cyhalothrin | Pyrethroid | Non-systemic. Contact and stomach action. Some repellant properties. Sodium channel modulator. | – | X | – | X | X | – | V | V | V |
| Phosalone | Organophosphate | Non-systemic with contact and stomach action. Acetylcholinesterase (AChE) inhibitor. | X | – | – | – | V | V | V | V | V |
| Pirimicarb | Carbamate | Selective. systemic with contact. Stomach and respiratory action. Acetylcholinesterase (AChE) inhibitor. | ? | – | – | X | V | V | X | ? | V |

V: yes, known to cause a problem; X: no, known not to cause a problem; ?: possibly, status not identified; −: no data, http://sitem.herts.ac.uk/aeru/ppdb/en/573.htm.

craniofacial and palate formation. This evidence leads to the problem of risk assessment and management of these compounds.

The acute risk, especially from dimethoate at a concentration of 0.3 mg/kg, is high for UK toddlers, and the aHI value is equal to 312.3%, approaching the limiting value. Other fungicides such as tebuconazole (173.5% ARfD) and captan (104% ARfD) create a hazard for small children. Carbendazim (99% ARfD) and pyraclostrobin (73% ARfD) also have high aHIs. Among the group of insecticides, chlorpyrifos methyl slightly exceeds the value of 104%.

Due to the widespread presence of pesticide residues, various processes having the goal of reducing pesticide content have been the subject of many studies. The effects of washing, storing, boiling, peeling, coring and juicing on some pesticide residues on apples were investigated (Kong et al., 2012). The application of these processes may have the effect of reducing pesticide content in apples, which may have a beneficial impact on health.

## 4. Conclusions

These studies indicate that apples contain pesticide residues in more than half of all samples. The most frequently detected group of active pesticidal substances in apples originating from the northeastern and central part of Poland were fungicides, which comprised 70% of all detections (captan, dithiocarbamates, tolylfluanid, pyrimethanil, flusilazole, procymidone, and chlorothalonil); however, organophosphate insecticides (fenitrothion, chlrpyrifos ethyl, diazinon, dimethoate, and fozalon) were detected above the acceptable limits more often. During the studies, samples with multiple residues were noted: 16.4% contained more than two compounds, including 9.6% with three compounds and 4.3% with three active substances. From a food-safety perspective, such samples carry a greater health risk to consumers. However, despite the annually increasing number of samples with multiple residues, their percentage still remains lower than the average percentage of multiresidue samples, e.g., for the European Union (26%). The estimated risk of acute exposure was highest for the triazoles flusilazole and tebuconazole. The group that was most exposed to the systematic activity of pesticide residues in apples is children, and this exposure significantly exceeds the acceptable value of 100% ADI. The contamination of apples with agricultural pesticide residues is an obvious pathway of human exposure, and it is strongly influenced by age and dietary preferences. This contamination is why special attention must be paid to the consumption of apples by children.

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