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Jana Ticha, Jana Hajslova, Tomas Kovalczuk, Martin Jech, Jiri Honzicek, et al.. Safe apples for baby food production:search for pesticide preparations leaving minimum residues. Food Additives and Contaminants, 2007, 24 (06), pp.605-620. 10.1080/02652030601013703 . hal-00577510

HAL Id: hal-00577510

<https://hal.science/hal-00577510>

Submitted on 17 Mar 2011

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Safe apples for baby food production: search for pesticide preparations leaving minimum residues

Journal:	<i>Food Additives and Contaminants</i>
Manuscript ID:	TFAC-2006-116.R1
Manuscript Type:	Original Research Paper
Date Submitted by the Author:	23-Aug-2006
Complete List of Authors:	Ticha, Jana; Institute of Chemical Technology, Food Chemistry and Analysis Hajslova, Jana; Institute of Chemical Technology, Food Chemistry and Analysis Kovalczuk, Tomas; Institute of Chemical Technology, Food Chemistry and Analysis Jech, Martin; Institute of Chemical Technology, Food Chemistry and Analysis Honzicek, Jiri; Institute of Chemical Technology, Food Chemistry and Analysis Kocourek, Vladimir; Institute of Chemical Technology, Food Chemistry and Analysis Lansky, Miroslav; Research and Breeding Institute of Pomology Holovousy Ltd. Kloutvorova, Jana; Research and Breeding Institute of Pomology Holovousy Ltd. Falta, Vladan; Research and Breeding Institute of Pomology Holovousy Ltd.
Methods/Techniques:	Clean-up, Extraction, GC/MS, LC/MS
Additives/Contaminants:	Pesticide residues, Pesticides - spraying regimes
Food Types:	Baby food, Fruit, Processed foods

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Safe apples for baby food production: search for pesticide preparations leaving minimum residues

Abstract

Altogether 19 pesticide preparations were used according to agricultural practice within 6 trials in apple orchards. Using gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods, premature Golden Delicious apples collected 64, 50, 36 days before harvest and matured fruit were examined for residues of active ingredients. No residues of triflumuron, triazamate, chlorpyrifos, etofenprox, fenoxycarb, kresoxim-methyl, cyprodinyl, difenoconazole and thiram were detected in the first sampling, also the levels of chlorpyrifos-methyl, penconazole, tebuconazole and tolylfluanid dropped during the pre-harvest interval. Detectable, residues of pyridaben, thiacloprid, trifloxystrobin and tetraconazole in harvested fruits were below 0.01 mg kg^{-1} , which is the maximum concentration of residues acceptable by baby food producer in any raw material to be processed. The only residues exceeding this concentration were captan and teflubenzuron. Based on these data, farmers may choose the pesticides for optimal treatment plants whilst enabling production of safe crop suitable for baby food production.

Keywords: apples, pesticides, baby food, fruit production, gas-chromatography-mass spectrometry, liquid-chromatography- tandem mass spectrometry

Introduction

Various abiotic factors such as unfavourable temperature, moisture, nutrients, soil conditions and/or phytotoxic environmental chemicals as well as several infectious disease agents represented mainly by biotic pathogens such as fungi, bacteria, viruses, nematodes, mycoplasmas etc. may cause damage to food crops (Hamilton 2004). To maintain healthy, productive plants and prevent losses of stored crops, producers should recognize what pests to look for, understand pest biology, use appropriate preventive measures, and apply last but not least timely efficient pesticide preparations when needed. In general, after

elimination of targeted pests, modern pesticides are designed to dissipate due to various environmental factors and detoxication processes taking place in treated plants.

However, under certain circumstances, even if applied in accordance with Good Agriculture Practice (GAP), residues of pesticides can still be detected in treated crops (Hajslová 1999). Consumers' exposure to such hazardous chemicals is of growing health concern, specifically in the case of infants and young children that represent the most vulnerable population group – not only because of their high food intake per body weight unit but also due to yet undeveloped detoxication mechanisms. With respect to these facts, a uniform Maximum Residue Limit (MRL) as low as 0.01 mg kg⁻¹ was established by EU Directive for any pesticide residue in processed cereal-based foods and baby foods for infants and young children (Commission Directive 1999/39/EC).

It should be noted that EU Maximum Residue Limits (MRLs) applying to common products of plant origin are often higher than 0.01 mg kg⁻¹. According to an internationally accepted strategy, their values are based not only on toxicologically acceptable levels, but also on evaluation of residue data from supervised field trials carried out according to the principles of Good Agricultural Practice (GAP). Experience shows that GAP-based MRLs are generally lower than MRLs derived from toxicological end-points (Hamilton 2004). The overview of EU legislation concerned with pesticides and their MRLs is shown at http://europa.eu.int/comm/food/plant/protection/pesticides/legislation_en.htm and http://ec.europa.eu/food/plant/protection/pesticides/index_en.htm, respectively.

A significant decrease in pesticide residues typically occurs during technological operations such washing, steam boiling, thermal sterilisation etc. employed for industrial/household fruits and/or vegetable processing (Cano et al. 1987; Cabras et al. 1990; Mahajan et al. 1992; Cabras et al. 1993; Holland et al. 1994; Ong et al. 1996; Cabras et al. 1997; Burchat et al. 1998; Cabras et al. 1998a, 1998b, 1998c; Will et al. 1999; Cabras et al. 2000; Panagiotis EA et al. 2000; Rassmussen et al. 2003; Yirong-Su et al. 2003). Despite this fact, in view of the large number of registered pesticides representing various chemical classes and (consequently) a wide range of physico-chemical properties, the possible transfer of some (more persistent) residues into the final product cannot be

avoided. To prevent such a risk and to ensure the quality of fruit/vegetable baby food, baby food producers are nowadays reluctant to process any raw materials containing residues exceeding 0.01 mg kg^{-1} limit (even though the “common” MRL for particular crop is not exceeded). To our knowledge, the current practice of major baby food producer is to check all batches of components prior to processing; thus the risk of marketing a product (baby food) containing violative residues is minimized. Thus, strategies relying on “dilution” of contaminated batch by residues free raw material and/or degradation of occurring pesticides during processing chain are not represent common approach.

Under these conditions, close collaboration between industry and farmers was established to facilitate a supply of crops containing low residue or residue free requirements. Although the use of organic crops might represent a conceivable option, this may not be a realistic solution in view of the global demands for raw materials. Therefore, a careful choice of agricultural practices namely selection of low input, non-persistent pesticide preparations is a critical issue in this context. It should be noted that in addition to documentation submitted by producers within the pesticides registration process, there are a large number of papers documenting a decline of various pesticide residues following respective food plant/crop treatment, however, due to a largely variable climatic conditions as well as with respect to differences in application practices, such information is only tentative and cannot be considered as fully generic (Holland et al. 1994, Rasmussen et al. 2003, Gambacorta et al. 2005, Cengiz et al. 2006).

In our study, extensive field experiments concerned with optimisation of apples production are presented. The aim of this work was to identify the optimal strategy for apple tree protection providing efficient control of pests (plant diseases) and, at the same time leaving undetectable or very low pesticide residues not exceeding 0.01 mg kg^{-1} .

Materials and methods

Field work

Golden Delicious apples examined in this study for pesticide residues were obtained from our project partner Research and Breeding Institute of Pomology, Holovousy Ltd., Czech Republic. The field experiment was performed at the orchard Sady Rokos Petrovicky,

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3 1 Jicín, Czech Republic. The standard agricultural practices (pruning, fertilizing, and soil
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5 2 management) were carried out on the plots during the season. Golden Delicious apple trees
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7 3 (24 years old) were situated in spacing 5 x 3 m (approximately 650 trees per ha),
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9 4 experimental area was 0.25 ha. The overview of pesticide preparations including some
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11 5 relevant characteristics are summarized in Table I. A tractor mounted sprayer Tifone
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13 6 Vanguard 1070 equipped with the Albuz ATR nozzles was used for the pesticide
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15 7 applications; the volume was 400 L per ha and the operating pressure 1.3 MPa.
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17 9 In April 2004, pesticide treatment started on the whole experimental orchard. In June 2004,
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19 10 experimental orchard was divided into six field experiments representing six differences in
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21 11 pesticide preparation treatments (the frequency of applications was higher compared to
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23 12 standard practice). The detailed overview of pre-harvest treatment of apple trees performed
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25 13 in the year 2004 is summarized in Table II.
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28 15 Four samplings of fresh apples were carried out, 14, 28, 42 and 78 days (i.e. in August 2,
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30 16 August 16, August 30 and in October 5, respectively) after the last application of pesticide
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32 17 preparations. In total 24 samples were obtained. Sampling was performed by randomly
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34 18 hand-picking of fruits (approximately 3 kg of apples per sample) from various places of the
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36 19 experimental fields according to the standard operating procedure elaborated in compliance
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38 20 with Commission Directive 2002/63/EC.
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40 22 [Insert Table I about here]
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44 24 [Insert Table II about here]
45 25
46
47 26 *Weather*
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49 27 Weather conditions were monitored during the whole season by automatic weather station
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51 28 at 15 min intervals. Temperatures, humidity and precipitations since April 2004 till October
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53 29 2004 were recorded. For an overview of average values counted during the pre-harvest
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55 30 interval, see Figure 1.
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58 32 [Insert Figure I about here]
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Chemicals

(i) Certified pesticide standards (purity in the range 94-99.5%) were obtained from Dr. Ehrenstorfer GmbH (Germany). Pesticides stock solutions were prepared by dissolving neat standards in toluene for analyses carried out by gas chromatography - mass spectrometry (GC-MS) and acetonitrile for examinations employing liquid chromatography - tandem mass spectrometry (LC-MS/MS). Working standards S_{1G} and S_{1L} consisting of mixtures of target pesticides were prepared from stock solutions of individual pesticides (concentrations of individual pesticides were $1\mu\text{g ml}^{-1}$). Other working standards S_{2G} , S_{3G} , S_{4G} , S_{5G} , S_{6G} and S_{2L} , S_{3L} , S_{4L} , S_{5L} , S_{6L} were obtained by dilution 2x, 10x, 20x, 100x, 200x of S_{1G} and S_{1L} respectively. For spiking, solutions in ethylacetate for GC-MS and in acetonitrile for LC-MS/MS with concentration corresponding to S_{3G}/S_{3L} were prepared.

(ii) Organic solvents for pesticide residue analysis were purchased from Scharlau, Spain (ethyl acetate), Merck, Germany (cyclohexane, toluene and methanol) and Sigma-Aldrich (acetonitrile).

(iii) Anhydrous sodium sulphate obtained from Penta, Czech Republic was dried at 600°C for 7 h and then stored in a tightly closed glass container prior to use.

Apparatus

(i) Warring Blender (Warring, USA) was used to homogenize fresh apples.

(ii) Ultra-Turrax homogenizer (IKA, Werk, Germany) was used for extraction of apple homogenate.

(iii) Automated high performance gel permeation chromatography system (HP GPC) Aspec XL (Gilson, France) equipped with PL gel column (600 x 7.5 mm, particles size 10 μm , 50 $\text{\AA}$; Polymer Laboratories Ltd., UK) was used for purification of crude apple extracts. Polytetrafluoroethylene (PTFE) filters (5 μm ; National Scientific, USA) were used for filtration of crude extracts prior to the clean-up.

(iv) Vacuum rotary evaporator Büchi Rotavor (Büchi, Switzerland) was used for removing of organic solvents from crude extracts and "pesticide fraction" after HP GPC clean-up.

(v) High Performance Liquid Chromatograph (HPLC) 2695 Alliance module (Waters, UK) coupled to tandem mass spectrometric detector Quattro Premier XE (Waters, UK) was used for determination of more polar pesticides.

(vi) Gas chromatograph (GC) 6890N (Agilent, USA) equipped with a mass-selective detector 5975 Inert XL with quadrupole analyzer (Agilent, USA) and autosampler (7683 Series) was used for determination of GC amenable pesticides.

Analytical methods

The scope of two multi-residue methods described below (LC-MS/MS and GC-MS) covered all the pesticides involved in this study with the exception of thiram compound representing the group of ethylene bisdithiocarbamates (EBDCs). These contact fungicides are according to legislation requirements determined as a carbon disulphide (CS₂). Accredited method (ISO 17025) consisting of the following steps was used for examination of apples: (i) solid phase micro extraction (SPME) for absorption of CS₂ (degradation product of EBDCs) from headspace of sample digested by hydrochloric acid in the presence of stannous chloride and (ii) GC-MS identification/quantification of analyte thermally desorbed in GC injector port. Since in none of samples residues of thiram were detected (LOD of method was 0.5 µg kg⁻¹), no more detailed description is provided here.

LC-MS/MS method

Sample preparation

3 kg of unwashed fresh apples (representing field sample) delivered to laboratory were homogenized using a Warring Blender homogenizer. 12.5 g of homogenate were mixed with 50 ml of acetonitrile and extracted for 2 min with Ultra-Turrax homogenizer. The suspension was filtered under vacuum; the filtrate cake was washed with 3 x 10 ml of acetonitrile and then evaporated on a vacuum rotary evaporator. 15 ml of methanol were added into the evaporation flask and the volume was quantitatively transferred into 50 ml volumetric flask and made-up with methanol. Samples were prior to injection filtered through PTFE filters. The matrix content in crude extract was 0.25 g ml⁻¹.

LC-MS/MS identification/quantification

LC separations were carried out on a reversed phase column Discovery C₁₈ (150 x 3 mm, 5 µm). The sample temperatures as well as column temperature were maintained at 25°C. The mobile phase contained water (A) and methanol (B) and the flow rate was 0.3 ml min⁻¹. Gradient was employed, starting composition of 50% B, rising linearly 100% B over 6

1 min then held for 11 min at 100% B followed by 10 min re-equilibration to initial mobile phase composition. Injection volume 20 μ l was used in all separations.

Identification/quantification of target analytes was performed using a Quattro Premier tandem quadrupole mass spectrometer. The detector was operated in a positive electrospray (ES+) ionisation mode. Multiple reaction monitoring (MRM) conditions (collision energy and cone voltage) were optimised for each pesticide during infusion (5 μ l min⁻¹) of individual pesticide solution (1-5 μ g ml⁻¹) into the mobile phase flow (A:B 50:50, (v/v), 0.3 ml min⁻¹). All experiments were realised employing the following parameters: capillary voltage 3.5 kV, extractor voltage 4 V, source temperature 120°C, desolvation temperature 250°C, cone gas flow 100 L h⁻¹ and desolvation gas flow 700 L h⁻¹ (both gasses were nitrogen). Argon was used as a collision gas (3.3 x 10⁻³ mbar). Tuned and optimised MS/MS transitions as well as specific cone voltages and collision energies are summarized in Table III. Analytes were divided into time segments based on their elution characteristics. The MS/MS transitions were monitored in the multiple reaction-monitoring (MRM) mode at the same dwell time 0.005 s, inter-channel delays, and inter-scan delays of 10 ms for all transitions.

Generated experimental data were processed using MassLynx software version 4.0 Service Pack 4, Software Change Note #462.

[Insert Table III about here]

GC-MS method

Sample preparation

3 kg of unwashed fresh apples (representing field sample) delivered to laboratory were homogenized using a Warring Blender homogenizer. A 25 g of homogenized apples were weighed in a glass beaker, after addition of 100 ml ethyl acetate and 75 g of anhydrous sodium sulphate the sample was extracted for 2 minutes with the Ultra-Turrax homogenizer at 10 000 rpm. The suspension was filtered under vacuum through the layer of anhydrous sodium sulphate, the beaker and filtrate cake were rinsed with 3 x 25 ml of ethyl acetate. Combined filtrates were evaporated using vacuum rotary evaporator (temperature max.

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3 1 40°C, pressure 220 mbar) to volume circa 25 ml. Concentrated crude extract was
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5 2 transferred into volumetric flask and the final volume was made up with cyclohexane to 50
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7 3 ml (matrix concentration 0.5 g ml⁻¹).

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9 4 *HP- GPC purification*

10 5 Samples were filtered through PTFE filters prior to purification. Crude extracts were
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12 6 purified employing automated high performance gel permeation chromatography system.
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14 7 The following conditions were used for sample clean-up: mobile phase ethyl acetate-
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16 8 cyclohexane (1:1), flow rate 1 ml min⁻¹, injection volume 2 ml, collected “pesticide”
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18 9 fraction 14.5-31 ml. Purified pesticide fraction was evaporated using vacuum rotary
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20 10 evaporator (temperature max. 40°C, pressure 220 mbar) almost to dryness, the residual
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22 11 solvent was removed with a gentle stream of nitrogen. After addition of 1 ml of toluene,
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24 12 sample was ready to GC-MS analysis. The content of original matrix was 1 g ml⁻¹.

25 13 *GC-MS identification/quantification*

26 14 All separations of GC amenable pesticides were carried out on capillary column DB-5MS
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28 15 (60 m x 0.25 mm x 0.25 µm). Pulsed splitless injection was used (pressure pulse 60 psi,
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30 16 pulse period 2 min, inlet temperature 250°C, injection volume 1 µl). Oven temperature
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32 17 program started at initial temperature 90°C (hold 2 min), 5°C min⁻¹ to 180°C then 2°C min⁻¹
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34 18 to 280°C (hold 5 min). Helium was used as a carrier gas at a constant rate 19 cm s⁻¹. MSD
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36 19 detector was working in electron ionization mode (EI), ion source temperature 230°C, MS
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38 20 Quad temperature 150°C. Identification/quantification was performed in the Selected Ion
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40 21 Monitoring mode (SIM), see Table IV. All GC-MS chromatographic data were processed
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42 22 using ChemStation® Software (D.02.00 SP1, Agilent, USA)

43 23 *Matrix - matched standards*

44 24 Matrix-matched standards used for calibration were prepared from untreated apples. Blank
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46 25 extracts were prepared according to the above described LC-MS/MS and GC-MS
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48 26 procedures. For LC-MS/MS, 100µl of working standard solution S_{1L}-S_{6L} were added to
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50 27 900µl of blank apple extract prior to analysis. As for GC-MS, residue after evaporation of
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52 28 solvent from purified blank apple extract was re-dissolved in 1ml of appropriate standard
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54 29 working solution S_{1G}-S_{6G} for following analysis.

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57 31 *Quality Assurance*
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For recovery tests, apple homogenates were spiked by pesticide mixture (spikes concentration corresponded to 0.1 mg kg^{-1}) and then processed as described for LC-MS/MS and GC-MS analyses. Quality control procedures for pesticide residues analysis (Document N° SANCO/10232/2006) were applied in setting LOD and LOQ values. The latter one was the lowest calibration level (LCL) and corresponded for particular analyte to $3 \times \text{LOD}$. Under these conditions, LOQs were the minimum concentrations of analytes that was possible to quantify with acceptable accuracy and precision.

The methods described above are accredited according to ISO/IEC 17025. As a part of external Quality Assurance, laboratory has been successfully participating in proficiency tests - Food Analysis Performance Assessment Scheme (FAPAS®) and European proficiency testing within the pesticide monitoring programme. Performance characteristics of applied analytical methods are summarized in Table IV.

[Insert Table IV about here]

Results and discussion

As mentioned in the introduction, where fruit is intended for baby food production, careful attention has to be paid to selection of pesticide preparations matching specific requirements for obtaining low or residue-free crop. Since baby food producers tolerate only residues not exceeding 0.01 mg kg^{-1} in the raw material, fundamental changes of treatment strategy might have to be adopted in this aspect to produce acceptable raw material. To identify “high residue” pesticides that should be in particular case eliminated from the further use, monitoring of apples grown by several farmers under common conventional practices was carried out in the first phase of our experiments.

Monitoring study of fresh apples

As shown in our previous study conducted in years 2001-2003 (Stepán et al. 2005), almost no violation of MRLs occurred. Nevertheless, 60 % from 220 batches of apples delivered by farmers contained detectable pesticide residues, 48% of them exceeded baby food MRL 0.010 mg kg^{-1} and therefore had to be used for other purposes than baby food production.

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5 2 The most troublesome pesticides in this respect were phosalone, chlorpyrifos-methyl,
6 3 captan, tolylfluanid and fenitrothion. Following the analysis of a data set generated within
7 4 this 3-year pilot study, farmers were advised to search for replacing preparations containing
8 5 these with other ones. As a consequence of modified treatment practices, significant
9 6 decrease of organophosphorus insecticide residues (phosalone, and fenitrothion) were
10 7 observed, while no changes in captan and tolylfluanid incidence were found. The search for
11 8 “new” preparation resulted in increased occurrence of trifloxystrobin residues. Overall, the
12 9 farmers` effort to improve treatment plans was documented by decrease of positive samples
13 10 number to 54% (i.e. only 15% exceeded baby food MRL of 0.01 mg kg⁻¹ in 2004). It
14 11 should be noted that climatic conditions did not differ significantly in this crop year from
15 12 previous ones.
16 13

17
18 14 Considering large variation (both qualitative and quantitative) in contamination patterns
19 15 among apples supplied by individual farmers, the need to harmonize treatment plans with
20 16 the aim to further improve crops quality become evident.
21 17

22 18 *Degradation of pesticide residues in pre-harvest interval*

23 19 To get more knowledge on the fate of individual pesticides, extensive experiments were
24 20 carried out in 2004, see Table II. All 19 pesticides studied covered the range of
25 21 preparations most commonly applied within apple trees treatment during the pre-harvest
26 22 interval. However, there are also other registered pesticide preparations which are
27 23 conceivably used for apple trees protection.
28 24

29 25 It should be noted that only very few studies are available in the scientific literature, none
30 26 of them covering the spectrum of pesticides involved in our study. Actually, the only
31 27 available data relevant to our experiment are those summarized in reports
32 28 (<http://www.who.int/ipcs/publications/jmpr/en/>) of the Joint Meeting of the FAO Panel of
33 29 Experts on Pesticide Residues in Food and Environment and the WHO Core Assessment
34 30 Group of Pesticide Residues, JMPR (similar data have to be submitted by applicants for
35 31 active ingredients review process currently dealt by European Food Safety Authority,
36 32 EFSA).
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Figures 2-10 document the dynamics of pesticides involved in our study. In the sampling carried out 14 days after the last pesticide application, residues of fungicides (captan, tetraconazole, trifloxystrobin, penconazole, tebuconazole and tolylfluanid) and insecticides (pyridaben, chlorpyrifos-methyl, thiacloprid and teflubenzuron) were detected. In following pre-harvest interval successive decline of residues was observed, although the rate of dissipation varied largely. Since it is impossible to generalize the fate of the whole set of pesticides investigated, behavior of individual compounds is discussed in separated paragraphs below (Figures 2-9). Figure 10 summarizes concentration levels of pesticides found in harvested apples within all 6 field experiments (FE 1, FE 2, FE3, FE 4, FE 5 and FE 6).

Captan

As shown in Figure 2, the residues of captan, the main fungicide widely used for control of apple scab (*Venturia inaequalis*), were either not detected (FE 5 and FE 6) or already fairly well below the MRL (3 mg kg^{-1}) at the first sampling, even in an experiment with two treatments (FE 2, FE 3 and FE 4). Successive decrease of captan occurred and at the time of harvest its residues at 0.01 mg kg^{-1} level were detected in only two samples (FE 2 and FE 4).

It is worth noting that the relative decrease rate was higher in FE 2, FE 3 and FE 4 compared to FE 5 and FE 6 in which longer time elapsed since the last treatment with Merpan 80 WG preparation. No significant differences in residue levels were found in the third sampling regardless the application rate and date of application, residues were between $0.01 - 0.02 \text{ mg kg}^{-1}$.

Considering the data obtained from the Canadian study for JMPR (Captan residues in food: 1984 evaluation), we obtained significantly lower content of captan residues. Whereas content of captan in Canadian apples was in the range $0.6 - 2.9 \text{ mg kg}^{-1}$ some 30 days after its application, in apples carried out within our experiments its concentration did not exceed 0.1 mg kg^{-1} in 33 days after captan treatment.

[Insert Figure 2 about here]

Pyridaben

Although applied at the same rate and the same date, pyridaben residues in apples from individual experiments differed largely at the time of the first sampling. While relatively rapid decrease of residues occurred in FE 1 with no detectable residues at the harvest time, in FE 2 despite the lower concentration in the first sampling, traces of pyridaben were detectable even in harvested apples (see Figure 3).

[Insert Figure 3 about here]

Chlorpyrifos-methyl

Residues of insecticide chlorpyrifos-methyl used for crop protection against codling moths (*Cydia pomonella*) and sawfly insects (*Hymenoptera*, *Haplocampa testudinea*) dropped in both FE 3 and FE 5 below the MRL (0.50 mg kg^{-1}) in all experiments even before the end of the safety period. As can be seen from Figure 4, further degradation of residues occurred and residues were close to or below the baby food MRL. Harvested apples collected 78 days after the last Reldan 40 EC application in FE 3 and FE 5 did not contain any chlorpyrifos-methyl residues. In apples from experiment FE 1, residues were not detected even during the pre-harvest interval (61 days).

[Insert Figure 4 about here]

Teflubenzuron

Teflubenzuron is an insecticide recommended against tortricid (*Tortricidae*) that can cause mechanical damage to ripe fruits, which can consequently be a “gate” for secondary putrefactive diseases (*Alternaria*, *Nectria*, *Phytophthora*) during apple storage. As shown in Figure 5, repeated application of Nomolt 15 SC resulted in a fairly high content of teflubenzuron in FE 2 contrary to single application in case of FE 3 and FE 4 in the first and second samplings. In none of the experiments was the MRL (0.5 mg kg^{-1}) exceeded even in the first sampling. Among the first and second samplings only a slight decrease of teflubenzuron content was observed, probably due to lack of precipitation that might remove some surface residues (see Figure 1). In the following pre-harvest time the content

of teflubenzuron declined during apple maturation. No residues were detected in FE 3 harvested apples. However, in FE 2 and FE 4 ripe apples, teflubenzuron at concentration levels exceeding the baby food MRL was found. This can be attributed to the relatively high stability of this compound (The Pesticide Manual 2002).

[Insert Figure 5 about here]

Thiacloprid

Thiacloprid belongs to the very limited group of in-season insecticides allowed to be applied at the early pink stage of trees development to control beetles (*e.g. Anthonomus pomorum*), codling moths (*Cydia pomonella*), rosy apple aphids, green aphids and to help mite suppression (IPM Commercial Apples, Insect, Disease and Weed Control Recommendations for 2006). Thiacloprid was applied in all FE 1, FE 2, FE 3 and FE 4 once, three times in FE 5 and twice in FE 6. Since the treatment was carried out in early May, in line with expectation low concentrations correlating with application rate were obtained in FE 1, FE 2, FE 3 and FE 4. Interesting is that these were detected even 111 days after Calypso 480 SC application (i.e. 97 days after safe period elapsed). Nevertheless, in time of harvest, no residues were detected. Significant differences in thiacloprid contents in the first sampling date was found in the case of repeated applications carried out in FE 5 and FE 6 (see Figure 6). However, its content rapidly declined during pre-harvest interval and only trace amounts (0.003 mg kg^{-1}) were found in ripe apples.

[Insert Figure 6 about here]

Tebuconazole and tolylfluanid

Tebuconazole and tolylfluanid were applied together as Hattrick pesticide preparation (see Table I) against apple scab (*Venturia inaequalis*) in FE 5 and FE 6. Relatively high persistency was recognized for tebuconazole. Residues of this fungicide exceeded the baby food limit even in the third sampling (55 days beyond safety period). Nevertheless, these were not detected in the harvested fruits (see Figure 7a). As compared to tolylfluanid, this was found only in FE 6 at very low concentration level already in the first sampling (see

Figure 7b) and dissipated during the pre-harvest time; in harvested apples no tolylfluanid residues were detected.

[Insert Figures 7a and 7b about here]

Tetraconazole

As shown in Figure 8, residues of tetraconazole used as a protection against apple scab and powdery mildew were well below the MRL (0.5 mg kg^{-1}) in the first sampling in experiments with three (FE 2 and FE 3) and four (FE 4) treatments; in FE 6 tetraconazole was not detected. A decline of tetraconazole residues occurred in following pre-harvest time; in harvested apples only low concentrations levels (0.005 mg kg^{-1}) were observed at field experiments repeatedly treated (FE 2, FE 3 and FE 4).

[Insert Figure 8 about here]

Trifloxystrobin

As shown in Figure 9, the residues of trifloxystrobin were detected in all samples FE 1, FE 2, FE 3, FE 4, FE 5 and FE 6 in the first sampling. Since applied at the same date and same application rate in FE 2, FE 3, FE 4 and FE 5, similar concentrations were found contrary to repeated applications in FE 1 and FE 6 resulting in higher contents of trifloxystrobin in the first sampling. While relatively continuous decline of residues was identified in FE 1 and FE 6 with no (FE 6) or trace (FE 1) residues in harvested apples, in samples with lower treatment (FE 2, FE 3, FE 4 and FE 5), the reduction in residue levels was not so distinct, although the baby food limit was not exceeded.

[Insert Figure 9 about here]

Penconazole

The fungicide penconazole applied only in FE 5, was detected in the first sampling (33 days after the last Topas 100 EC application) at trace concentration levels (0.005 mg kg^{-1}). In harvested apples no penconazole residues were found.

Overview of pesticide degradation in field experiments FE 1 – FE 6

Field experiments were carried out aimed at identifying pesticide preparations that ensure effective crop protection and yet at the same time leave very low residues. Based on 6 field experiments described in this study, a set of data enabling evaluation of both the degradation rate and levels of terminal residues in matured apples was obtained. The main observations resulting from this study are summarized below.

- In the *first sampling*, carried out before or immediately after the pesticides safety period has elapsed, only 10 pesticides (46% of 22 active ingredients applied) were detected; 8 of them exceeded or were at 0.01 mg kg^{-1} . Those were fungicides captan in FE 1, FE 2, FE 3 and FE 4, tetraconazole in FE 2, FE 3, FE 4 and FE 5, trifloxystrobin in FE 1 and FE 6, tebuconazole in FE 5 and FE 6; insecticides pyridaben in FE 1 and FE 2, teflubenzuron in FE 2, FE 3 and FE 4, chlorpyrifos-methyl in FE 1, FE 3 and FE 5 and thiacloprid in FE 5 and FE 6. On the other hand, the levels of penconazole in FE 5 and tolylfluanid in FE 6 were found below baby food MRL; similarly low were thiacloprid, tetraconazole and trifloxystrobin in some experiments. Residues of triflumuron, triazamate, chlorpyrifos, etofenprox, fenoxycarb, kresoxim-methyl, cyprodinyl, difenoconazole and thiram were not detected at all.

- In the *second sampling*, 9 pesticides were found (penconazole in FE 5 was not detected anymore); 8 residues of active ingredients still exceeded 0.01 mg kg^{-1} in some experiments (captan, pyridaben, teflubenzuron, thiacloprid, tebuconazole, trifloxystrobin, tetraconazole and chlorpyrifos-methyl). Since the previous sampling, contents of chlorpyrifos-methyl in FE 1 and tetraconazole in FE 3 and FE 5 declined fairly below baby food MRL.

- Also in the *third sampling*, 9 pesticides were detected, nevertheless only 5 of them exceeded 0.01 mg kg^{-1} (captan, pyridaben, teflubenzuron, thiacloprid and tebuconazole). Residues of chlorpyrifos-methyl in FE 3 and FE 5, tebuconazole in FE 5, trifloxystrobin in FE 1, FE 5 and FE 6 and tetraconazole in FE 2 and FE 4 dropped below this value since second sampling.

- When assessing the residues situation at the harvest time (the *fourth sampling*), each of the treatment regimes resulted in detectable residues (see Figure 10). In total, 6 pesticides i.e. 27% of active ingredients applied for the treatment of apple trees were detected.

The lowest contamination was observed in FE 1, FE 3 and FE 6, where residues of only one pesticide, well below 0.01 mg kg⁻¹ was found (trifloxystrobin in FE 1; tetraconazole in FE 3 and thiacloprid in FE 6). Field treatment FE 5 resulted in the occurrence of two detectable pesticides (trifloxystrobin and thiacloprid) in matured apples. Similarly to FE 1 and FE 6, the concentration levels of residues found in these samples were well below the baby food MRL. On the other hand, baby food producers' requirements were not fulfilled in FE 2 and FE 4, where "the worst" contamination situation was found. In both these field experiments tefubenzuron exceeded 0.01 mg kg⁻¹ and residues of captan were also present close to this critical concentration level. Besides, additional pesticide residues were found; these were pyridaben, tetraconazole, trifloxystrobin in FE 2 and tetraconazole and trifloxystrobin in FE 4 - all of them below 0.01 mg kg⁻¹

Regarding the effectiveness of crop protection against pests, all six treatment regimes were also evaluated for the occurrence of apple scab (*Venturia inaequalis*). No significant differences among the quality of apples obtained from individual field experiments were recognized. The extent of apple scab was only about 5% higher in our experiments compared to "conventionally" treated apples (regimes conducted in accordance with GAP leaving residues complying with MRL which may be, however, in some cases higher than 0.01 mg kg⁻¹).

[Insert Figure 10 about here]

Conclusion

Considering the requirements of baby food producers for safe raw material with pesticide residues not exceeding 0.01 mg kg⁻¹, treatment regimes FE 1, FE 3, FE 5 and FE 6 might be considered as an appropriate apple protection strategy, since only traces of active ingredients (tetraconazole, thiacloprid and trifloxystrobin) were contained in crops at the time of harvest. Residues of captan and teflubenzuron, active ingredients of pesticide preparations used for treatment in experiments FE 2 and FE 4, exceeded 0.01 mg kg⁻¹ and their use in farms supplying apples for baby food production should be carefully considered. On the other hand, the use of cyprodinyl, chlorpyrifos, chlorpyrifos-methyl,

1 difenoconazole, etofenprox, fenoxycarb, kresoxim-methyl, penconazole, pyridaben,
2 tebuconazole, thiram, triazamate, triflumuron, and tolylfluanid in apple orchards seems – in
3 terms of contamination - free of problems, no residues were detected in harvested fruit. It
4 should be noted that employing treatment regimes aimed at minimization of residues in
5 harvested crops did not result in its lower quality in terms of damage caused by pests.

6
7 In any case, follow-up validation of these recommendations has to be carried out under
8 conditions specific for particular locality. It is believed that at the end of long-term
9 experiments specific guidelines for farmers supplying apples to baby food producers will
10 be developed.

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Table I: Overview of pesticide preparations used in field experiments and physico-chemical properties of their active ingredients

Mode of action	Commercial product	Active ingredient (content)	Content in preparation	MRL (mg kg ⁻¹)	Safety period (days)	Physico-chemical properties of active ingredient		
						Molecular weight	log K _{OW}	Water solubility (mg l ⁻¹)
insecticides	Alsystin	triflumuron	480 g/l	1	28	358.7	4.91 (20 °C)	0.025 (20°C)
	Aztec 140 EW	triazamate	140 g/l	0.1	7	314.4	2.15 (pH 7, 25 °C)	399 (pH 7, 25°C)
	Calypso 480 SC	thiacloprid	480 g/l	0.3	14	252.7	-	185 (20 °C)
	Nomolt 15 SC	teflubenzuron	150 g/l	0.5	28	381.1	4.30 (20°C)	0.019 (23°C)
	Oleoekol	chlorpyrifos	30 g/l	0.5	-	350.6	4.7	1.4 (25°C)
		coarse oil	75%	-	-			
	Trebon 10 F	etofenprox	100 g/l	1	28	376.5	7.05 (25°C)	<1 (25°C)
	Insegar 25	fenoxycarb	25%	0.05	60	301.3	4.07 (25°C)	7.9 (pH 7.55-7.84, 25°C)
	Reldan 40 EC	chlorpyrifos-methyl	400 g/l	0.5	28	322.5	4.24	2.6 (20°C)
fungicides	Sanmite 20 WP	pyridaben	20%	0.1	42	364.9	6.37 (25°C)	0.012 (24°C)
	Discus	kresoxim-methyl	50%	0.2	35	313.4	3.40 (pH 7, 25°C)	2 (20°C)
	Domark 10 EC	tetraconazole	100 g/l	0.5	14	372.1	3.56 (20°C)	156 (pH 7, 20°C)
	Euparen Multi	tolyfluanid	50%	1	7	347.3	3.90 (20°C)	0.9 (20°C)
	Foligreen	agricultural micronutrient (N, P, K, B, Fe, Mn, Zn, Mg, Co, Cu, Mo)						
	Hattrick	tebuconazole	10%	0.5	28	307.8	3.70 (20°C)	36 (pH 5-9, 20°C)
		tolyfluanid	40%	1	28			
	Champion 50W	Cu(OH) ₂	77%	10	-	97.6	-	2.9 mg/l (pH 7, 25°C)
	Chorus 75 WG	cyprodinil	750g/kg	1	28	225.3	4.0 (pH 9.0, 25°C)	20 (pH 5.0, 25°C)
	Merpan 80 WG	captan	80%	3	35	300.6	2.80 (25°C)	3.3 (25°C)
	Score 250 EC	difenoconazole	250 g/l	0.02	49	406.3	4.20 (25°C)	15 (25°C)
	Solubor	Na ₂ B ₈ O ₁₃ ·4H ₂ O	> 98 %	-	-	412.52		9.7% (20°C)
	Thiram Granuflo (F)	thiram	80%		14	240.4	1.73	18 (room temperature)
	Topas 100 EC (F)	penconazole	100 g/l	0.2	35	284.2	3.72 (25°C)	73 (20°C)
	Zato 50 WG	trifloxystrobin	500 g/kg	0.5	14	408.4	4.50 (25°C)	610 (25 °C)

Table II: Field experiments: time-schedule of pesticide preparations use and treatment rates

Application date 2004	Codes of experiments (Field Experiment FE 1 - 6)					
	FE 1	FE 2	FE 3	FE 4	FE 5	FE 6
April 7	Champion 50 WP (4.0 kg ha ⁻¹)* Chorus 75 WG (0.2 kg ha ⁻¹), Domark 10 EC (0.25 l ha ⁻¹), Oleoekol (10.0 l ha ⁻¹) Zato 50 WG (0.15 kg ha ⁻¹) Thiram (3.0 kg ha ⁻¹), Domark 10 EC (0.25 l ha ⁻¹), Solubor (1.5 kg ha ⁻¹) Discus (0.2 kg ha ⁻¹), Calypso 480 SC (0.2 l ha ⁻¹) Zato 50 WG (0.15 l ha ⁻¹), Foligreen (1.0 l ha ⁻¹) Merpan 80 WG (2.0 kg ha ⁻¹), Foligreen (1.0 l ha ⁻¹)					
April 19						
April 26						
May 5						
May 11						
May 20						
May 28						
June 9	Score 250 EC (0.2 l) Insegar 25 (0.6 l kg)	Score 250 EC (0.2 l) Nomolt (1.0 kg)	Merpan 80 WG (2.0 kg) Trebon 10 F (0.5 l)	Domark 10 EC (0.4 l) Alsystin (0.25 kg)	Thiram (3.0 kg) Calypso 480 (0.2 l)	Hatrick (1.125 kg) Reldan 40 (1.25 l)
June 21	Score 250 EC (0.2 l)	Domark 10 EC (0.4 l)	Score 250 (0.2 l)	Domark 10 EC (0.4 l)	Hatrick (1.125 kg)	Zato 50 WG (0.15 kg)
July 1	Zato 50 WG (0.15 kg) Reldan 40 EC (1.25 l)	Merpan 80 WG (2.0 kg) Insegar 25 WG (0.6 kg)	Domark 10 EC (0.4 l) Nomolt 15 SC (1.0 kg)	Merpan 80 (2.0 kg) Nomolt 15 (1.0 kg)	Topas 100 (0.45 l) Calypso (0.2 l)	Hatrick (1.125 kg) Reldan 40 (1.25 l)
July 20	Sanmite 20 WP (0.75 kg) Insegar 25 WP (0.3 kg)	Sanmite 20 WP (0.75 kg) Nomolt 15 SC (1.0 kg)	Reldan 40 EC (1.25 l)	Aztec 140 EW (0.5 l)	Reldan 40 (1.25 l)	Calypso 480 (0.2 l)

* Per hectare dosage of applied pesticide preparation

Table III: Optimised MS/MS transitions parameters

Analyte	Transition (m/z)	Cone (V)	Colision (V)
diflubenzuron	311 > 158	25	10
	311 > 141	25	29
etofenprox	394 > 177	20	14
	394 > 135	20	26
pyrimethanil	200 > 107	54	24
	200 > 82	54	24
teflubenzuron	381 > 158	23	13
	381 > 141	23	13
thiacloprid	253 > 126	35	25
	253 > 186	35	13
triflumuron	359 > 156	29	16
	359 > 139	29	30

Table IV: Performance characteristics and monitored ions (m/z) of the analytical methods employed for apples analysis

Deleted: ¥

Analyte	Method	Quantitation ion (m/z)	Confirmation ions (m/z)	LOD (mg kg ⁻¹)	Repeatability (RSD, %) at 0.100 mg kg ⁻¹	Recovery (%)	
captan	GC-MS	149	79, 264	0.003	9	92	Deleted: 0.010%
cyprodinil	GC-MS	224	210, 225	0.002	17	87	Deleted: 0.007%
difenoconazole	GC-MS	323	207, 267, 281	0.002	4	95	Deleted: 0.008%
etofenprox	LC-MS/MS	394 > 177	394 > 135	0.004	8	94	
fenoxycarb	GC-MS	255	116, 186	0.003	10	97	Deleted: 0.009%
chlorpyrifos	GC-MS	314	199, 258	0.003	8	94	Deleted: 0.009
chlorpyrifos-methyl	GC-MS	286	125, 288	0.003	7	93	Deleted: 0.009
kresoxim-methyl	GC-MS	206	116, 131	0.001	9	94	Deleted: 0.004%
penconazole	GC-MS	248	159, 161	0.002	10	110	Deleted: 0.006%
pyridaben	GC-MS	147	117, 309	0.003	5	72	Deleted: 0.010%
pyrimethanil	LC-MS/MS	200 > 107	200 > 82	0.004	9	87	
tebuconazole	GC-MS	250	125, 163, 252	0.002	8	89	Deleted: 0.006%
teflubenzuron	LC-MS/MS	381 > 158	381 > 141.05	0.004	7	86	
tetraconazole	GC-MS	336	159, 338	0.001	5	90	Deleted: 0.004%
thiacloprid	LC-MS/MS	253 > 126	253 > 186	0.004	6	94	
tolyfluanid	GC-MS	137	181, 238	0.003	5	100	Deleted: 0.003
triazamate	GC-MS	314	227, 242, 262	0.002	8	103	Deleted: 0.006
trifloxystrobin	GC-MS	131	116, 222	0.001	8	98	Deleted: 0.003
triflumuron	LC-MS/MS	359 > 156	359 > 139	0.004	6	89	

Figure 1: Weather conditions in the pre-harvest period

Figure 2: The dynamics of captan decrease between samplings in FE 1, FE 2, FE 3 and FE 4 (error bars express the expanded uncertainty of respective results).

* Safety period (35 days) has not elapsed yet

Figure 3: The dynamics of pyridaben decrease between samplings in FE 1 and FE 2
(error bars express the expanded uncertainty of respective results).

* Safety period (42 days) has not elapsed yet

Figure 4: The dynamics of chlorpyrifos-methyl decrease between samplings in FE 1, FE 3 and FE 5 (error bars express the expanded uncertainty of respective results).

* Safety period (28 days) has not elapsed yet

Figure 5: The dynamics of teflubenzuron decrease between samplings in FE 2, FE 3 and FE 4 (error bars express the expanded uncertainty of respective results).

* Safety period (28 days) has not elapsed yet

Figure 6: The dynamics of thiacloprid decrease between samplings in FE 1, FE 2, FE 3, FE 4, FE 5 and FE 6 (error bars express the expanded uncertainty of respective results).

Figure 7a: The dynamics of tebuconazole decrease between samplings in FE 5 and FE 6 (error bars express the expanded uncertainty of respective results).

Figure 7b: The dynamics of tolylfluand decrease between samplings in FE 6 (error bars express the expanded uncertainty of respective results).

Figure 8: The dynamics of tetraconazole decrease between samplings in FE 1, FE 2, FE 3, FE 4 and FE 5 (error bars express the expanded uncertainty of respective results).

Figure 9: The dynamics of trifloxystrobin decrease between samplings in FE 1, FE 2, FE 3, FE 4, FE 5 and FE 6 (error bars express the expanded uncertainty of respective results).

Figure 10: Differences in the contents of pesticide residues in FE 1, FE 2, FE 3, FE 4, FE5 and FE 6 at the day of harvest (error bars express the expanded uncertainty of respective results).