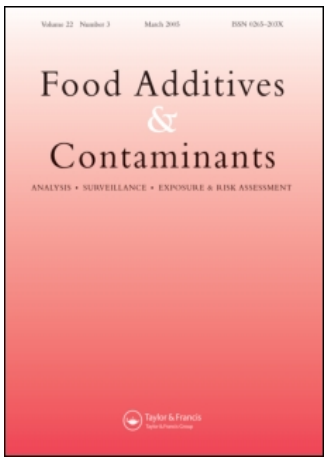

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Safe apples for baby-food production: Survey of pesticide treatment regimes leaving minimum residues

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Abstract
A total of 19 pesticide preparations were used according to agricultural practice in six trials in apple orchards. Using gas chromatography–mass spectrometry (GC–MS) and liquid chromatography–tandem mass spectrometry (LC–MS/MS), premature Golden Delicious apples collected 64, 50, 36 days before harvest and mature fruit were examined for residues of active ingredients. No residues of triflumuron, triazamate, chlorpyrifos, etofenprox, fenoxycarb, kresoxim-methyl, cyprodinyl, difenoconazole or 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 producers in any raw material. The only residues exceeding this concentration were captan and teflubenzuron. Based on the data, farmers can choose pesticides for optimal treatment of plants, while enabling growth of a 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 and Crossley 2004). To maintain healthy, productive plants and prevent losses of stored crops, producers should recognize pests, understand pest biology, use appropriate preventive measures and, last but not least, apply timely effective pesticide preparations when needed. In general, after elimination of targeted pests, modern pesticides are designed to dissipate via various environmental factors and detoxification 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). Consumer-exposure to such hazardous chemicals is a growing health concern, specifically in the case of infants and young children, which represent the most vulnerable population group – not only because of their high food intake per body weight unit but also due to, as yet, undeveloped detoxification 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 (European Commission 1999).

It should be noted that EU MRLs applying to common products of plant origin are often higher

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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 available 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 (European Commission 2006a,b).

A significant decrease in pesticide residues typically occurs during technical operations, such as washing, steam boiling, thermal sterilisation, etc., employed for industrial/household fruits and/or vegetable processing (Cano and De LaPlaza 1987; Cabras et al. 1990, 1993, 1997, 1998a, b, c, 2000; Mahajan and Chopra 1992; Holland et al. 1994; Ong et al. 1996; Burchat et al. 1998; Will and Krüger 1999; Panagiotis and Pappas 2000; Rassmussen et al 2003; Yirong-Su et al. 2003). Despite this fact and 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 a 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 damaging residues is minimized. Strategies relying on ''dilution'' of contaminated batches by residue-free raw material and/or degradation of pesticides during processing are not a representative approach.

Under these conditions, close collaboration between industry and farmers was established to facilitate a supply of crops containing low residues or residue-free. 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. It should be noted that, in addition to documentation submitted by producers within the pesticides registration process, there is a large number of papers documenting a decline in various pesticide residues following respective food plant/crop treatment. However, due to largely variable climatic conditions, as well as differences in application practices, such information is only tentative and cannot be considered 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 apple 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, Czech Republic. The field experiment was performed at the orchard at Sady Rokos Petrovicky, Jicín, Czech Republic. Standard agricultural practices (pruning, fertilizing and soil management) were carried out on the plots during the season. Golden Delicious apple trees (24 years old) were spaced at 5 × 3 m (approximately 650 trees per ha) in an experimental area of 0.25 ha. An overview of pesticide preparations, including some relevant characteristics, are summarized in Table I. A tractor-mounted sprayer Tifone Vanguard 1070 equipped with Albuz ATR nozzles was used for the pesticide application; the volume was 400 L ha^{-1} and operating pressure 1.3 MPa.

In April 2004, pesticide treatment started on the whole experimental orchard. In June 2004, the experimental orchard was divided into six field experiments, representing six different pesticide preparation treatments (the frequency of applications was higher compared to standard practice). A detailed overview of pre-harvest treatment of apple trees performed in the year 2004 is summarized in Table II.

Four samplings of fresh apples were carried out, 14, 28, 42 and 78 days (i.e. on August 2, August 16, August 30 and on October 5) after the last application of pesticide preparations. In total, 24 samples were obtained. Sampling was performed by randomly hand-picking fruit (approximately 3 kg of apples per sample) from various places of the experimental fields, according to the standard operating procedure elaborated in compliance with Commission Directive 2002/63/EC (European Commission 2002).

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)
		coleseed oil	75%	–	–			
	Trebon10F	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)
	Sanmite 20 WP	pyridaben	20%	0.1	42	364.9	6.37 (25°C)	0.012 (24°C)
Fungicides	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	tolylfluanid	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)
		tolylfluanid	40%	1	28			
	Champion 50 W	$Cu(OH)_2$	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	$Na_2B_8O_{13}{\cdot}4H_2O$	>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 preparation use and treatment rates.

	Codes of experiments (Field Experiment FE 1–6)					
Application date 2004	FE1	FE2	FE3	FE4	FE5	FE6
April 7	Champion 50 WP (4.0 kg ha^{-1})*					
April 19	Chorus 75 WG (0.2 kg ha^{-1}), Domark 10 EC (0.25 l ha^{-1}), Oleoekol (10.0 l ha^{-1})					
April 26	Zato 50 WG (0.15 kg ha^{-1})					
May 5	Thiram (3.0 kg ha^{-1}), Domark 10 EC (0.25 l ha^{-1}), Solubor (1.5 kg ha^{-1})					
May 11	Discus (0.2 kg ha^{-1}), Calypso 480 SC (0.2 l ha^{-1})					
May 20	Zato 50 WG (0.15 l h^{-1}), Foligreen (1.0 l ha^{-1})					
May 28	Merpan 80 WG (2.0 kg ha^{-1}), Foligreen (1.0 l ha^{-1})					
June 9	Score 250 EC (0.2 1) Insegar 25 (0.6 l kg)	Score 250 EC (0.2 1) Nomolt (1.0kg)	Merpan 80 WG (2.0 kg) Trebon10 F(0.5 l)	Domark 10 EC (0.41) Alsystin (0.25 kg)	Thiram (3.0 kg) Calypso 480 (0.2 l)	Hattrick (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 (0.41)	Hattrick (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)	Hattrick (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)	Reldan40EC (1.25 1)	Aztec 140 EW (0.5 l)	Reldan 40 (1.25 l)	Calypso 480 (0.2 l)

*Per hectare dosage of applied pesticide preparation.

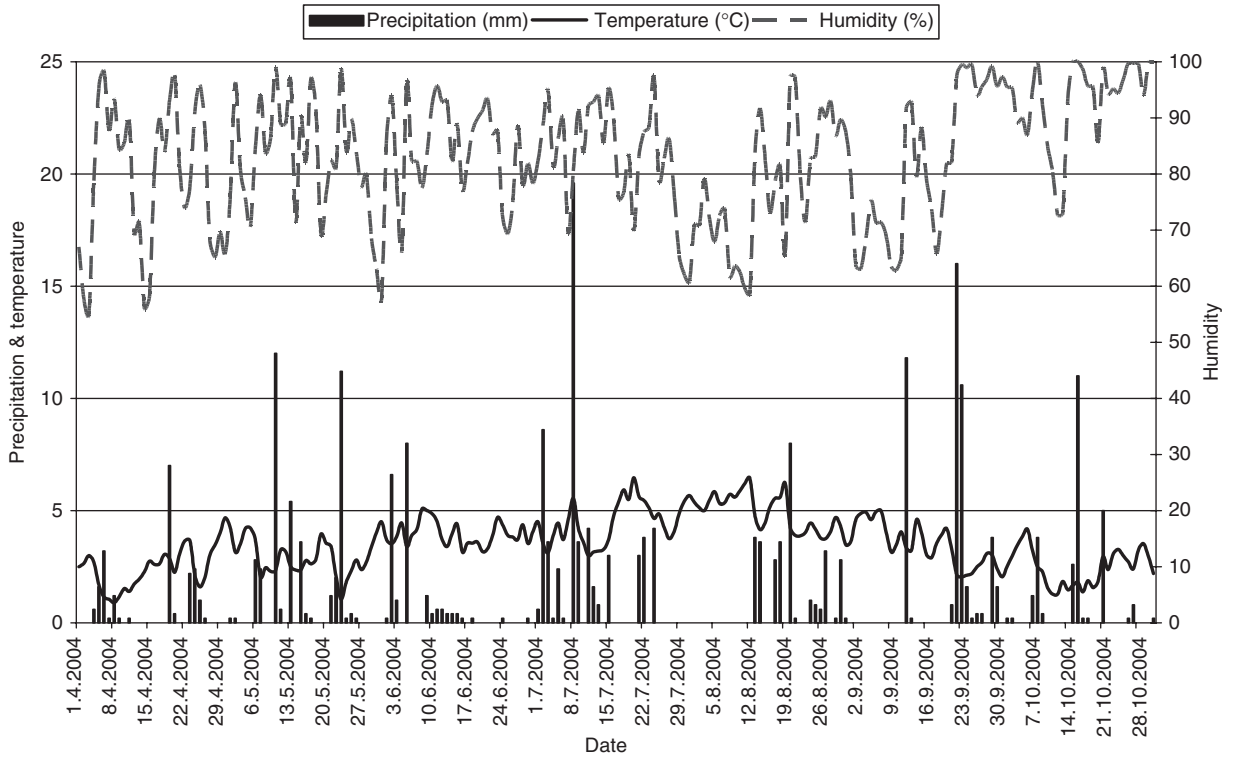


Figure 1. Weather conditions in the pre-harvest period.

Weather

Weather conditions were monitored at 15 min intervals over the whole season by an automatic weather-station. Temperatures, humidity and precipitations were recorded between April 2004 and October 2004. See Figure 1 for an overview of average values recorded during the pre-harvest interval.

Chemicals

(i) Certified pesticide standards (purity 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 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 $1\,\mu g\,ml^{-1}$). 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 2×, 10×, 20×, 100× and 200× dilutions of S_{1G} and S_{1L}, respectively. For spiking, solutions in ethyl acetate 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: ethyl acetate (Scharlau, Spain), cyclohexane, toluene and methanol (Merck, Germany) and acetonitrile (Sigma–Aldrich, USA).

(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) A Waring blender (Waring, USA) was used to homogenize fresh apples.

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

(iii) An automated high-performance gel permeation chromatography system (HP GPC) Aspec XL (Gilson, France), equipped with a PL gel column (600 × 7.5 mm, particle size 10 μm, 50 Å; 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) A Büchi Rotavor vacuum rotary evaporator (Büchi, Switzerland) was used for removing organic solvents from crude extracts and ''pesticide fraction'' after HP GPC clean-up.
(v) A high-performance liquid chromatograph (HPLC) 2695 Alliance module (Waters, UK), coupled to a tandem mass spectrometric detector (Quattro Premier XE; Waters) was used for determination of more polar pesticides.
(vi) A 6890N gas chromatograph (GC) (Agilent, USA), equipped with a 5975 Inert XL mass-selective detector with quadrupole analyzer and 7683 Series autosampler (Agilent) was used for determination of GC amenable pesticides.

Analytical methods

The scope of the two multi-residue methods described below (LC–MS/MS and GC–MS) covered all pesticides involved in this study, with the exception of thiram representing the group of ethylene bisdithiocarbamates (EBDCs). According to legislation requirements, these contact fungicides are determined as carbon disulphide (CS_2). An accredited method (ISO 17025), consisting of the following steps, was used for examination of apples: (i) solid-phase micro-extraction (SPME) for absorption of CS_2 (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 no thiram residues were detected in samples (LOD of method: $0.5\,\mu g\,kg^{-1}$), no further detailed description is provided.

LC–MS/MS method

Sample preparation. A 3 kg sample of unwashed fresh apples (representing field sample), delivered to the laboratory, was homogenized using a Waring blender. Then, 12.5 g of homogenate was mixed with 50 ml of acetonitrile and extracted for 2 min with an Ultra-Turrax homogenizer. The suspension was filtered under vacuum; the filtrate cake was washed with 3×10 ml of acetonitrile and then evaporated on a vacuum rotary evaporator. A 15 ml aliquot of methanol was added to the evaporation flask and the volume quantitatively transferred to a 50-ml volumetric flask and made-up with methanol. Samples were filtered through PTFE filters prior to injection. 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 Discovery C_{18} column (150×3 mm, 5 μm). The sample and column temperatures were maintained at 25°C. The mobile phase contained water (A) and methanol (B) and the flow rate was $0.3\,ml\,min^{-1}$. A gradient was employed with a starting composition of 50% B, rising linearly to 100% B over 6 min, then held for 11 min at 100% B followed by 10 min re-equilibration to initial mobile phase composition. An injection volume of 20 μl 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 positive electrospray (ES+) ionisation mode. Multiple reaction monitoring (MRM) conditions (collision energy and cone voltage) were optimised for each pesticide during infusion ($5\,\mu l\,min^{-1}$) of individual pesticide solution ($1–5\,\mu g\,ml^{-1}$) into the mobile phase flow (A/B, 50:50 v/v, $0.3\,ml\,ml^{-1}$). 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 (3.3×10^{-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 of 0.005 s, inter-channel 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).

Table III. Optimised MS/MS transition 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

GC–MS method

Sample preparation. First, 3 kg of unwashed fresh apples (representing field sample) delivered to laboratory were homogenized using a Waring blender. A 25 g sample of homogenized apples were weighed in a glass beaker and, after addition of 100 ml ethyl acetate and 75 g of anhydrous sodium sulphate, the sample was extracted for 2 min with the Ultra-Turrax homogenizer at 10 000 rpm. The suspension was filtered under vacuum through a layer of anhydrous sodium sulphate and the beaker and filtrate cake rinsed with 3×25 ml of ethyl acetate. Combined filtrates were evaporated using a vacuum rotary evaporator (max temperature 40°C, pressure 220 mbar) to a volume of ~25 ml. Concentrated crude extract was transferred into a volumetric flask and the final volume made up with cyclohexane to 50 ml (matrix concentration 0.5 g ml^{-1}).

HP GPC purification

Samples were filtered through PTFE filters prior to purification. Crude extracts were purified employing automated high-performance gel permeation chromatography system. The following conditions were used for sample clean-up: mobile phase ethyl acetate/cyclohexane (1:1, v/v), flow rate 1 ml min^{-1}, injection volume 2 ml, collected ''pesticide'' fraction 14.5–31 ml. The purified pesticide fraction was evaporated with a vacuum rotary evaporator (max temperature 40°C, pressure 220 mbar) almost to dryness and the residual solvent removed with a gentle stream of nitrogen. After addition of 1 ml of toluene, the sample was ready for GC–MS analysis. The content of original matrix was 1 g ml^{-1}.

GC–MS identification/quantification

All separations of GC amenable pesticides were carried out on a DB-5MS capillary column (60 m × 0.25 mm × 0.25 μm). Pulsed splitless injection was used (pressure pulse 60 psi, pulse period 2 min, inlet temperature 250°C, injection volume 1 μl). The oven temperature program was: initial temperature 90°C (hold 2 min), 5°C min^{-1} to 180°C, then 2°C min^{-1} to 280°C (hold 5 min). Helium was used as a carrier gas at a constant rate of 19 cm s^{-1}. The MSD detector was in electron ionization mode (EI), ion source temperature was 230°C and MS Quad temperature 150°C. Identification/quantification was performed in the selected ion monitoring mode (SIM) (Table IV). All GC–MS chromatographic data were processed using ChemStation® Software (D.02.00 SP1; Agilent).

Matrix-matched standards

Matrix-matched standards used for calibration were prepared from untreated apples. Blank extracts were prepared according to the above described LC–MS/MS and GC–MS procedures. For LC–MS/MS, 100 μl of working standard solution S_{1L}–S_{6L} were added to 900 μl of blank apple extract prior to analysis. As for GC–MS, after evaporation of solvent from purified blank apple extract, the residue was

Table IV. Performance characteristics and monitored ions (*m/z*) of the methods employed for apples analyses.

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
Cyprodinil	GC–MS	224	210, 225	0.002	17	87
Difenoconazole	GC–MS	323	207, 267, 281	0.002	4	95
Etofenprox	LC–MS/MS	394 → 177	394 → 135	0.004	8	94
Fenoxycarb	GC–MS	255	116, 186	0.003	10	97
Chlorpyrifos	GC–MS	314	199, 258	0.003	8	94
Chlorpyrifos-methyl	GC–MS	286	125, 288	0.003	7	93
Kresoxim-methyl	GC–MS	206	116, 131	0.001	9	94
Penconazole	GC–MS	248	159, 161	0.002	10	110
Pyridaben	GC–MS	147	117, 309	0.003	5	72
Pyrimethanil	LC–MS/MS	200 → 107	200 → 82	0.004	9	87
Tebuconazole	GC–MS	250	125, 163, 252	0.002	8	89
Teflubenzuron	LC–MS/MS	381 → 158	381 → 141.05	0.004	7	86
Tetraconazole	GC–MS	336	159, 338	0.001	5	90
Thiacloprid	LC–MS/MS	253 → 126	253 → 186	0.004	6	94
Tolylfluanid	GC–MS	137	181, 238	0.003	5	100
Triazamate	GC–MS	314	227, 242, 262	0.002	8	103
Trifloxystrobin	GC–MS	131	116, 222	0.001	8	98
Triflumuron	LC–MS/MS	359 → 156	359 → 139	0.004	6	89

re-dissolved in 1 ml of appropriate standard working solution S_{1G}–S_{6G} for the following analysis.

Quality assurance

For recovery tests, apple homogenates were spiked with pesticide mixture (spike concentrations 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 residue analyses (European Commission 2006c) were applied in setting LOD and LOQ values. The latter was the lowest calibration level (LCL) and corresponded for a particular analyte to $3 \times$ LOD. Under these conditions, LOQs were the minimum concentration of analyte quantifiable with acceptable accuracy and precision.

The methods described above are accredited according to ISO/IEC 17025. As part of an external quality assurance program, the laboratory has successfully participated in proficiency tests (Food Analysis Performance Assessment Scheme (FAPAS®) and European Proficiency testing) within the pesticide monitoring program. The performance characteristics of applied analytical methods are summarized in Table IV.

Results and discussion

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

Monitoring of fresh apples

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

The most problematic pesticides in this respect were phosalone, chlorpyrifos-methyl, captan, tolylfluanid and fenitrothion. Following analysis of a data-set generated within this 3-year pilot study, farmers were advised to find replacement preparations. As a consequence of modified treatment practices, a significant decrease of organophosphorus insecticide residues (phosalone, and fenitrothion) were observed, but no changes in the incidence of captan and tolylfluanid. The search for ''new'' preparations resulted in increased trifloxystrobin residues. Overall, farmers' effort to improve treatment regimes resulted in a decrease of positive samples to 54% (i.e. only 15% exceeded baby food MRL of 0.01 mg kg^{-1} in 2004). It should be noted that climatic conditions did not differ significantly in this crop year from previous years.

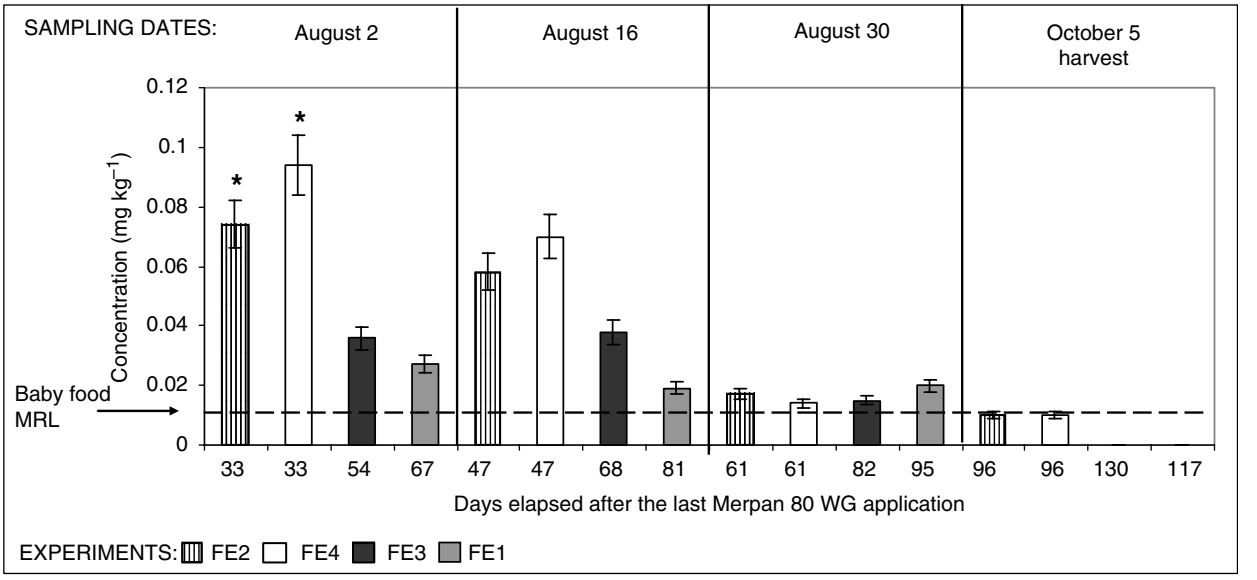
Considering the large variation (both qualitative and quantitative) in contamination patterns among apples supplied by individual farmers, treatment harmonization was needed to further improve crops quality.

Degradation of pesticide residues in the pre-harvest interval

To investigate the fate of individual pesticides, extensive experiments were carried out in 2004 (Table II). The 19 pesticides covered the range of preparations commonly applied during the growing season. However, there are other registered pesticide preparations, which could conceivably be used for apple-tree protection.

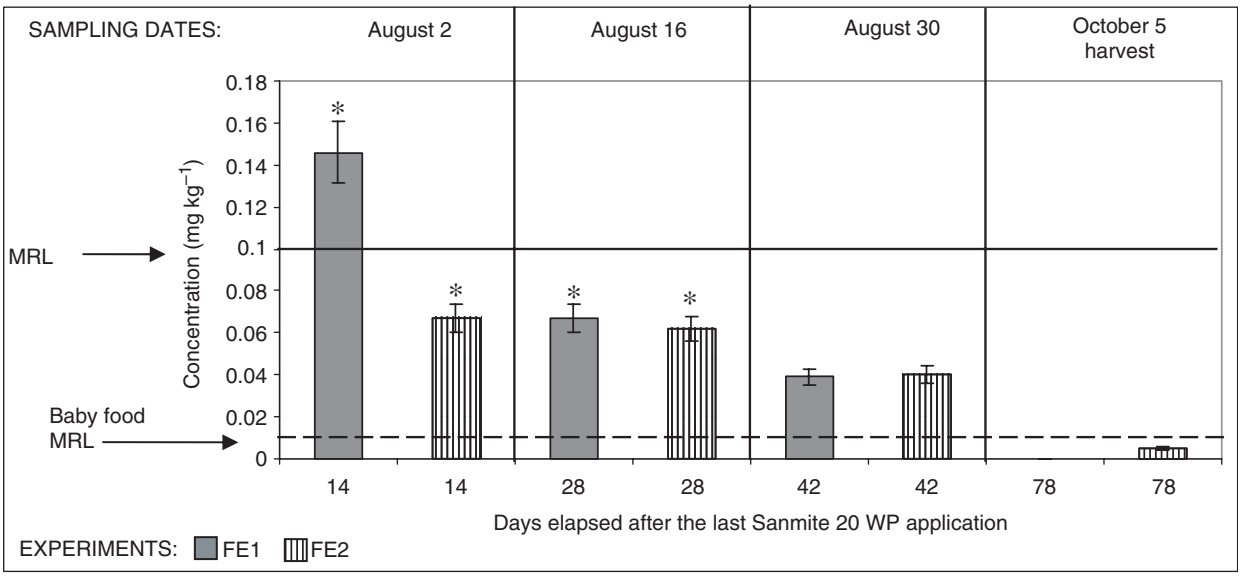
Very few studies are available in the literature, none covering the spectrum of pesticides involved in our study. The only available relevant data are summarized in reports (http://www.who.int/ipcs/publications/jmpr/en/) of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and Environment and the WHO Core Assessment Group of Pesticide Residues, JMPR (similar data have to be submitted for an active ingredients review process, currently handled by European Food Safety Authority, EFSA) (JMPR 2006).

Figures 2–10 document the pesticide dynamics in our study. In sampling 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 the pre-harvest interval, a successive decline in residues was observed, although the rate of dissipation varied greatly. Since it is impossible to generalize on the fate of the whole set of pesticides investigated, the behavior of individual compounds is discussed separately (Figures 2–9). Figure 10 summarizes the concentration levels of pesticides found in harvested apples within all six field experiments (FE1, FE2, FE3, FE4, FE5 and FE6).



*Safety period (35 days) has not elapsed yet.

Figure 2. Dynamics of captan decrease between samplings in FE1, FE2, FE3 and FE4 (error bars express the expanded uncertainty of respective results).



*Safety period (42 days) has not elapsed yet.

Figure 3. Dynamics of pyridaben decrease between samplings in FE1 and FE2 (error bars express the expanded uncertainty of respective results).

Captan

As shown in Figure 2, residues of captan, the main fungicide used for control of apple scab (*Venturia inaequalis*), were either not detected (FE5 and FE6) or well below the MRL (3 mg kg^{-1}) at the first sampling, even in an experiment with two treatments (FE2, FE3 and FE4). Successive decrease of captan occurred and, at the time of harvest, residues at 0.01 mg kg^{-1} level were detected in only two samples (FE2 and FE4).

It is worth noting that the relative decrease rate was higher in FE2, FE3 and FE4 compared to FE5 and FE6, with a longer time gap since the last treatment with Merpan 80 WG preparation. No significant differences in residue levels were found in the third sampling, regardless of the application rate

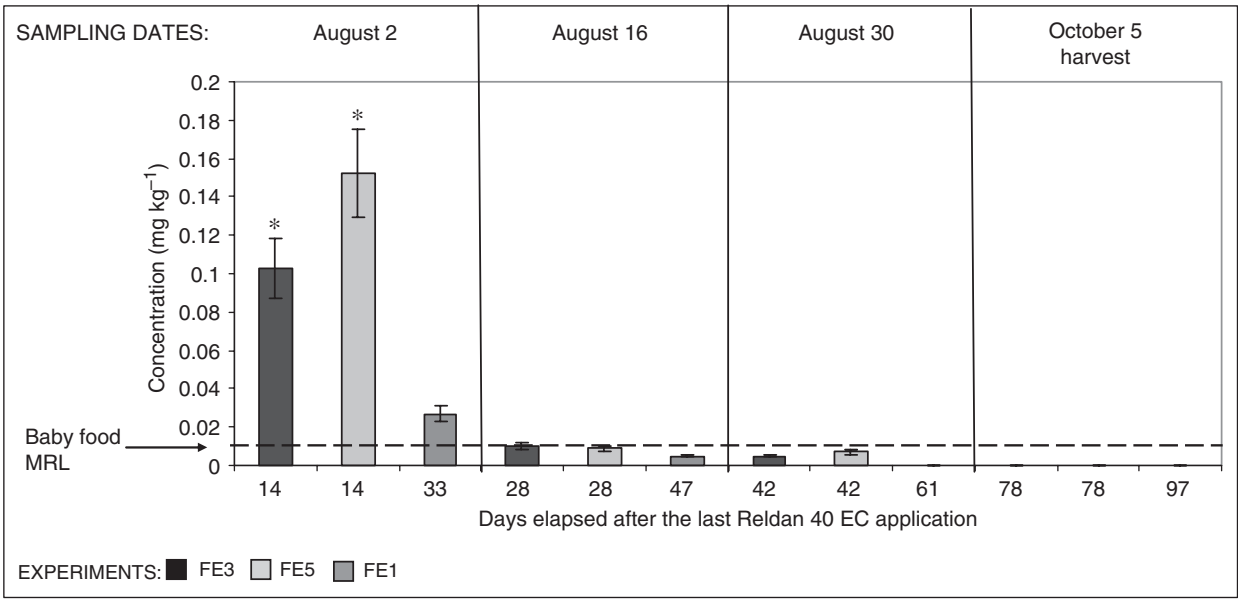


Figure 4. Dynamics of chlorpyrifos-methyl decrease between samplings in FE1, FE3 and FE5 (error bars express the expanded uncertainty of respective results).

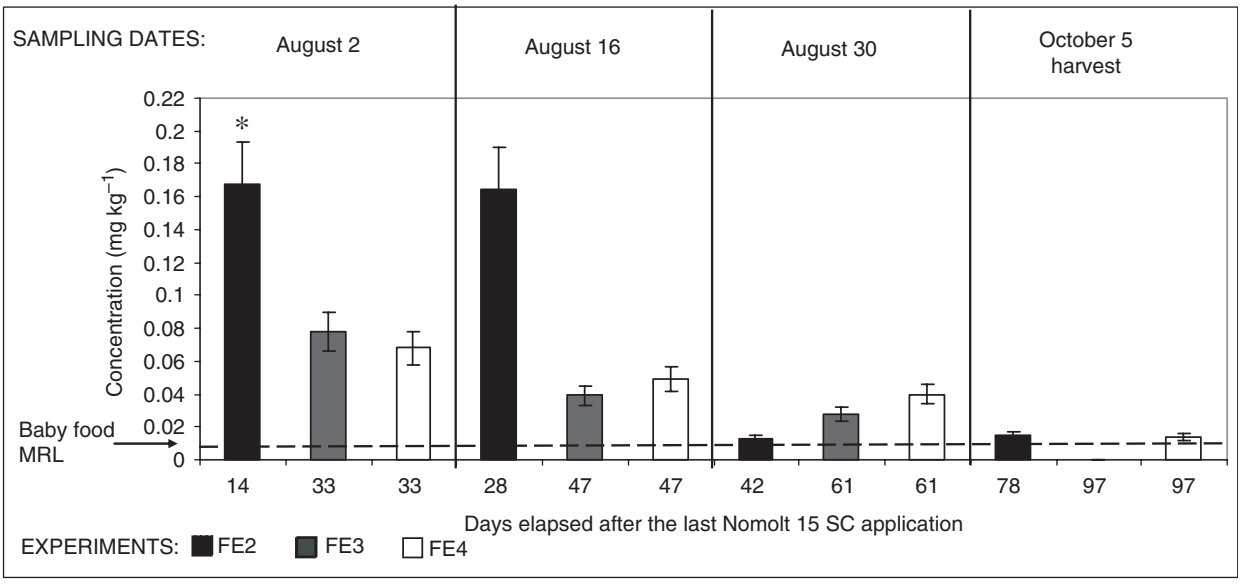


Figure 5. Dynamics of teflubenzuron decrease between samplings in FE2, FE3 and FE4 (error bars express the expanded uncertainty of respective results).

or date of application; residues were between 0.01 and 0.02 mg kg^{-1}.

Compared to data from a Canadian study for JMPR (Inchem.org 1984), we obtained significantly lower levels of captan residues. Captan levels in Canadian apples ranged 0.6–2.9 mg kg^{-1} 30 days after application; in our experiments the concentration did not exceed 0.1 mg kg^{-1} 33 days after treatment.

Pyridaben

Although applied at the same rate and on the same date, pyridaben residues from individual experiments differed greatly at the first sampling.

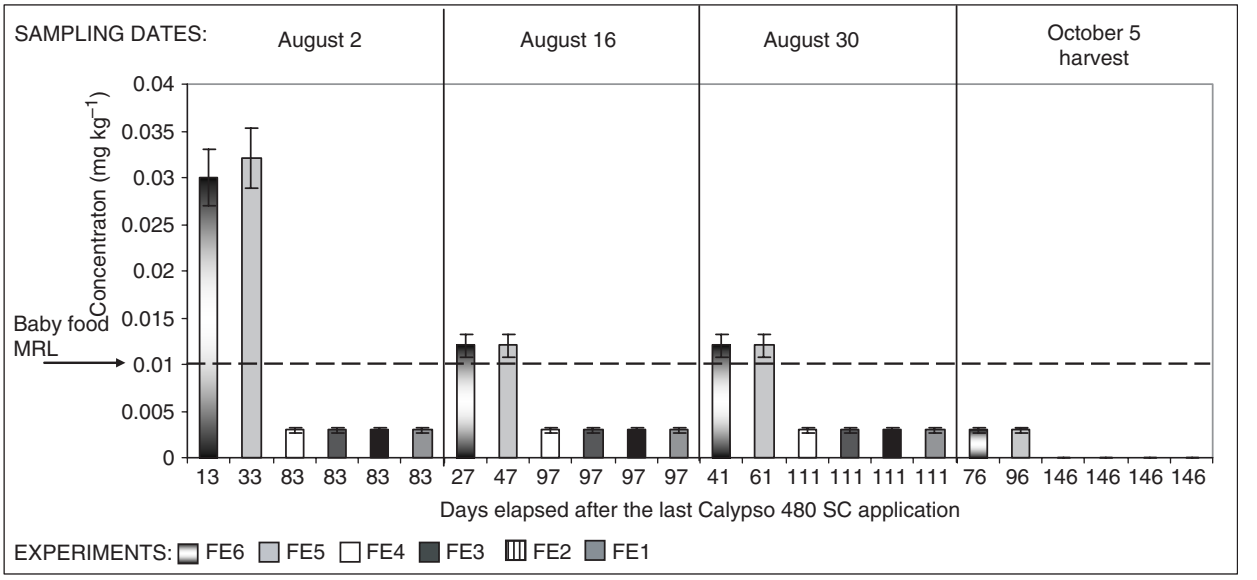


Figure 6. Dynamics of thiacloprid decrease between samplings in FE1, FE2, FE3, FE4, FE5 and FE6 (error bars express the expanded uncertainty of respective results).

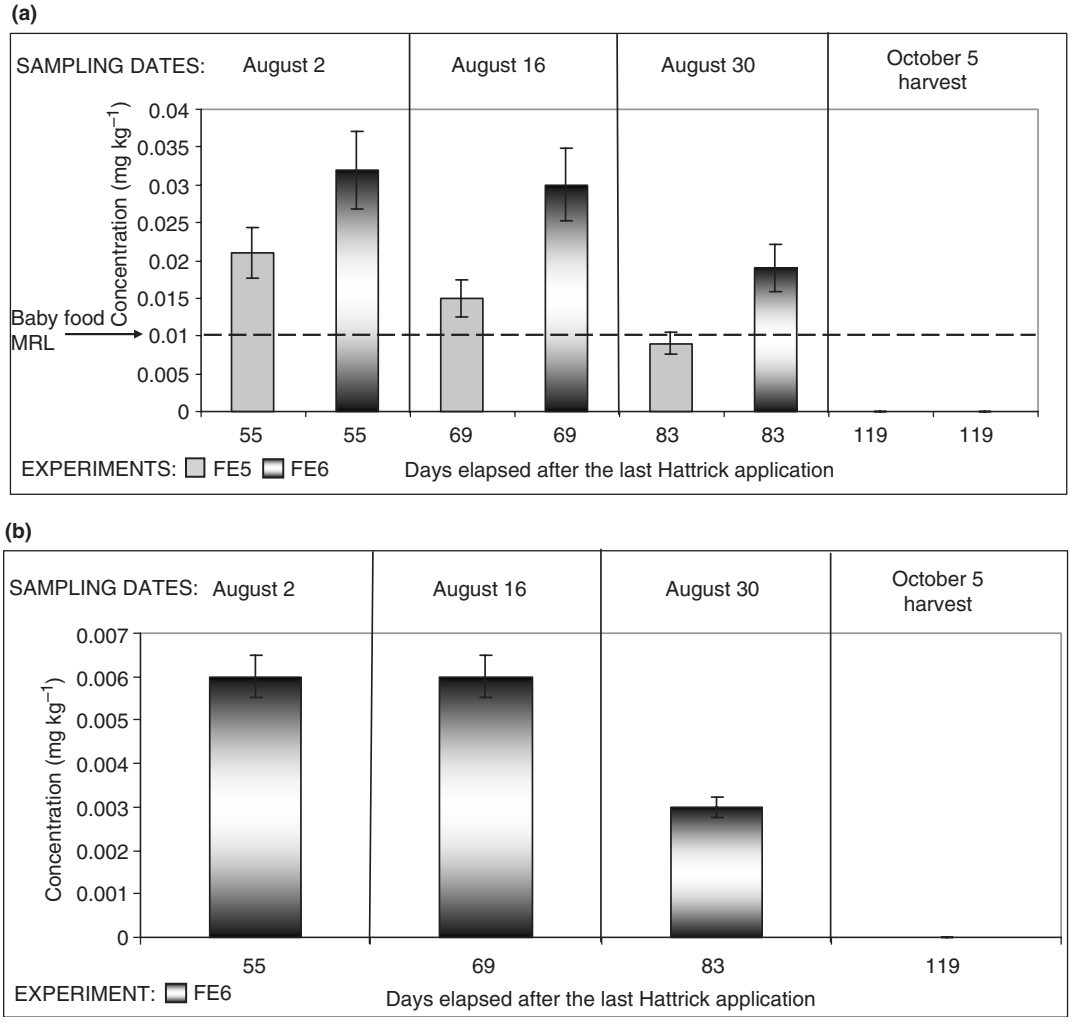


Figure 7. Dynamics of (a) tebuconazole decrease between samplings in FE5 and FE6, and (b) tolylfluanid decrease between samplings in FE 6 (error bars express the expanded uncertainty of respective results).

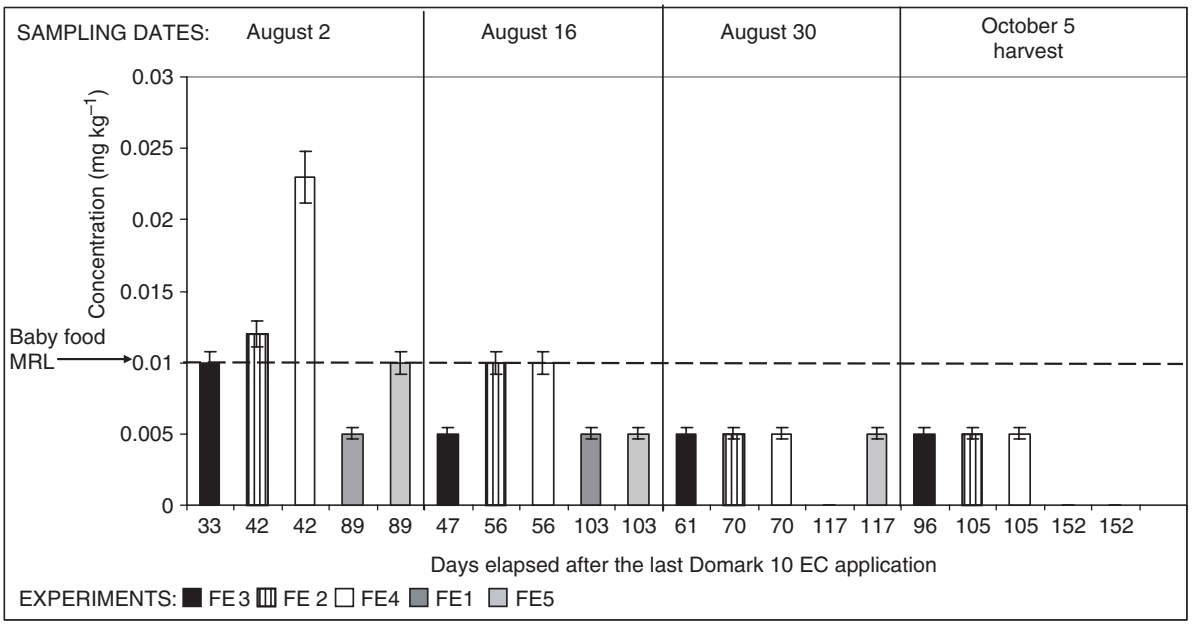


Figure 8. Dynamics of tetraconazole decrease between samplings in FE1, FE2, FE3, FE4 and FE5 (error bars express the expanded uncertainty of respective results).

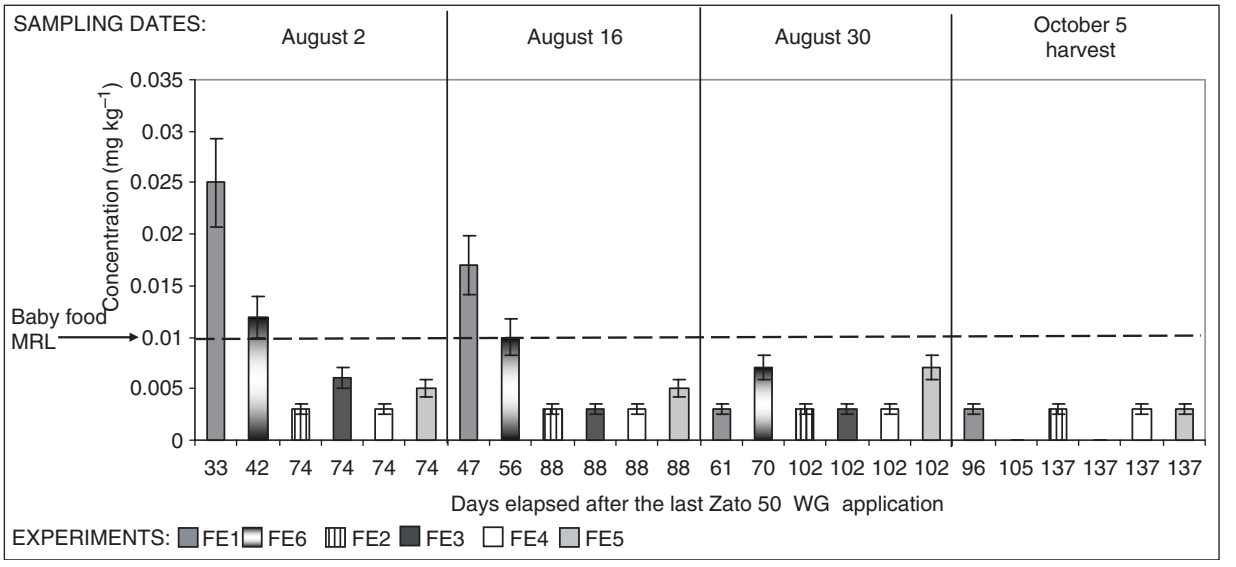


Figure 9. Dynamics of trifloxystrobin decrease between samplings in FE1, FE2, FE3, FE4, FE5 and FE6 (error bars express the expanded uncertainty of respective results).

While a relatively rapid decrease in residues occurred in FE1 with no detectable residues at harvest time, in FE2, despite the lower concentration in the first sampling, traces of pyridaben were detectable even in harvested apples (Figure 3).

Chlorpyrifos-methyl

Residues of the insecticide, chlorpyrifos-methyl, used for crop protection against codling moth (*Cydia pomonella*) and sawfly (*Hymenoptera, Haplocampa testudinea*), dropped in both FE3 and FE5 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 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 FE3 and FE5, did not contain any chlorpyrifos-methyl residues. In apples from experiment FE1, residues

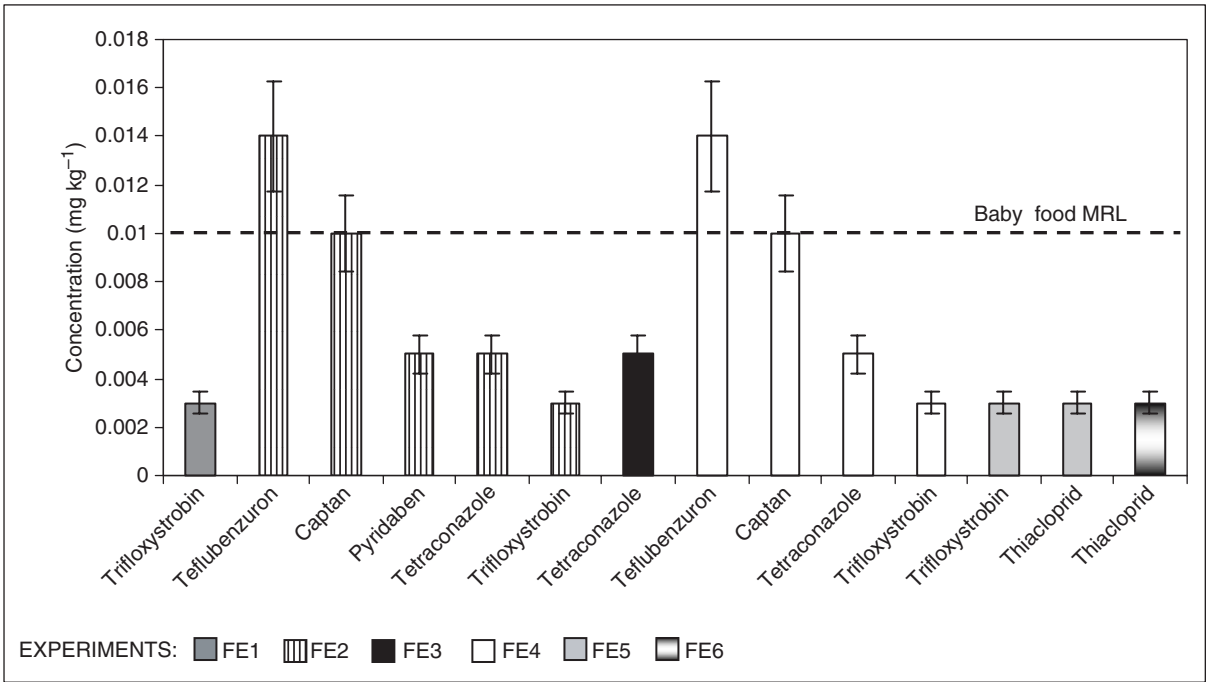


Figure 10. Differences pesticide residue levels in FE1, FE2, FE3, FE4, FE5 and FE6 at day of harvest (error bars express the expanded uncertainty of respective results).

were not detected, even during the pre-harvest interval (61 days).

Teflubenzuron

Teflubezuron is an insecticide recommended against tortricid (*Tortricidae*), which can cause mechanical damage to ripe fruits and, consequently, be a "gate" for secondary putrefactive diseases (*Alternaria, Nectria, Phytophtora*) during apple storage. As shown in Figure 5, repeated application of Nomolt 15 SC resulted in a high level of teflubenzuron in FE2, contrary to single application in FE3 and FE4, in the first and second samplings. In no 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 surface residues (Figure 1). In the following pre-harvest time, the level of teflubenzuron declined during apple maturation. No residues were detected in FE3 harvested apples. However, in FE2 and FE4 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 (Tomlin 2002).

Thiacloprid

Thiacloprid belongs to the very limited group of in-season insecticides approved for application at the early pink stage of tree development to control beetles (e.g. *Anthonomus pomorum*), codling moth (*Cydia pomonella*), rosy apple aphids, green aphids and help mite suppression (IPM 2006). Thiacloprid was applied once in all FE1, FE2, FE3 and FE4, three times in FE5 and twice in FE6. Since treatment was carried out in early May, as expected, low concentrations, correlated to application rate, were obtained in FE1, FE2, FE3 and FE4. Interestingly, these were detected even 111 days after Calypso 480 SC application (i.e. 97 days after the safe period elapsed). Nevertheless, no residues were detected at time of harvest. At the first sampling date, significant differences in thiacloprid levels was found with repeated applications carried out in FE5 and FE6 (Figure 6). However, levels rapidly declined during the pre-harvest interval and only trace amounts (0.003 mg kg^{-1}) were found in ripe apples.

Tebuconazole and tolylfluanid

Tebuconazole and tolylfluanid were applied together as the Hattrick pesticide preparation (Table I) against apple scab (*Venturia inaequalis*) in FE5 and FE6. Relatively high persistence was found for tebuconazole. Residues of this fungicide exceeded the baby food limit, even at the third sampling (55 days after the safety period). Nevertheless, residues were not detected in harvested fruit (Figure 7a).

Tolylfluanid was found only in FE6 at very low concentrations in the first sampling (Figure 7b) and dissipated during the growing season, in harvested apples no tolylfluanid residues were detected.

Tetraconazole

As shown in Figure 8, residues of tetraconazole, used against apple scab and powdery mildew, were well below the MRL (0.5 mg kg^{-1}) at the first sampling in experiments with three (FE2 and FE3) and four (FE4) treatments; in FE6, tetraconazole was not detected. A decline of tetraconazole residues occurred during pre-harvest; in harvested apples, only low concentrations (0.005 mg kg^{-1}) were observed in field experiments repeatedly treated (FE2, FE3 and FE4).

Trifloxystrobin

As shown in Figure 9, residues of trifloxystrobin were detected in FE1, FE2, FE3, FE4, FE5 and FE6 at first sampling. Since date and rate of application were the same in FE2, FE3, FE4 and FE5, similar concentrations were found compared to the repeated applications in FE1 and FE6, which resulted in higher levels of trifloxystrobin in the first sampling. While a relatively continuous decline in residue levels was identified in FE1 and FE6, with no (FE6) or trace (FE1) residues in harvested apples, in samples with lower treatment (FE2, FE3, FE4 and FE5), the reduction in residue levels was not as distinct, although the baby food limit was not exceeded.

Penconazole

The fungicide penconazole, applied only in FE5, was detected at the first sampling (33 days after the last Topas 100 EC application) at trace levels (0.005 mg kg^{-1}). No penconazole residues were found in harvested apples.

Overview of pesticide degradation in field experiments FE1–FE6

Field experiments were aimed at identifying pesticide preparations that ensured effective crop protection and, yet, left very low residues. Based on the six field experiments, a data-set, evaluating both degradation rates and levels of terminal residues in mature apples, was obtained. The main observations are summarized as follows.

- At first sampling, carried out before or immediately after the pesticide safety period elapsed, only 10 pesticides (46% of 22 active ingredients applied) were detected; eight at levels of $\geq$0.01 mg kg^{-1}. These were: fungicides – captan in FE1, FE2, FE3 and FE4, tetraconazole in FE2, FE3, FE4 and FE5, trifloxystrobin in FE1 and FE6, tebuconazole in FE5 and FE6; insecticides – pyridaben in FE1 and FE2, teflubenzuron in FE2, FE3 and FE4, chlorpyrifos-methyl in FE1, FE3 and FE5 and thiacloprid in FE5 and FE6. On the other hand, the levels of penconazole in FE5 and tolylfluanid in FE6 were below the baby food MRL; thiacloprid, tetraconazole and trifloxystrobin were also at low levels in some experiments. Residues of triflumuron, triazamate, chlorpyrifos, etofenprox, fenoxycarb, kresoxim-methyl, cyprodinyl, difenoconazole and thiram were not detected.
- At second sampling, nine pesticides were found (penconazole in FE5 was not detected); eight 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, levels of chlorpyrifos-methyl in FE1 and tetraconazole in FE3 and FE5 declined well below the baby food MRL.
- At third sampling, nine pesticides were also detected; nevertheless, only five exceeded 0.01 mg kg^{-1} (captan, pyridaben, teflubenzuron, thiacloprid and tebuconazole). Residues of chlorpyrifos-methyl in FE3 and FE5, tebuconazole in FE5, trifloxystrobin in FE1, FE5 and FE6 and tetraconazole in FE2 and FE4 dropped below this value since the second sampling.
- At fourth sampling, i.e. residues levels at harvest time, each treatment regimes showed detectable residues (Figure 10). In total, six pesticides, i.e. 27% of active ingredients applied for the treatment of apple trees, were detected.

The lowest contamination was observed in FE1, FE3 and FE6, where a residue of only one pesticide, well below 0.01 mg kg^{-1}, was found (trifloxystrobin in FE1, tetraconazole in FE3 and thiacloprid in FE6). Field treatment FE5 resulted in the occurrence of two detectable pesticides (trifloxystrobin and thiacloprid) in mature apples. Similarly to FE1 and FE6, the level 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 FE2 and FE4, where the worst contamination 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. Additional pesticide residues

were also found; these were pyridaben, tetraconazole, trifloxystrobin in FE2 and tetraconazole and trifloxystrobin in FE4 – all below 0.01 mg kg^{-1}.

Regarding the effectiveness of crop protection against pests, all six treatment regimes were evaluated for the occurrence of apple scab (*Venturia inaequalis*). No significant differences among the quality of apples from individual field experiments were recognized. The extent of apple scab was 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, in some cases, higher than 0.01 mg kg^{-1}).

Conclusion

Considering the requirements of baby-food producers for a safe raw material with pesticide residues not exceeding 0.01 mg kg^{-1}, treatment regimes FE1, FE3, FE5 and FE6 can be considered an appropriate apple protection strategy, since only traces of active ingredients (tetraconazole, thiacloprid and trifloxystrobin) were found at time of harvest. Residues of captan and teflubenzuron, active ingredients of pesticide preparations used for treatment in experiments FE2 and FE4, 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, difenoconazole, etofenprox, fenoxycarb, kresoxim-methyl, penconazole, pyridaben, tebuconazole, thiram, triazamate, triflumuron, and tolylfluanid in apple orchards seems – in terms of contamination – problem-free, i.e. no residues were detected in harvested fruit. It should be noted that employing treatment regimes aimed at minimization residues in harvested crops did not result in lower quality in terms of pest damage.

Validation of these recommendations needs to be carried out under conditions specific for a particular locality. Thus, it is believed that after long-term experimentation, specific guidelines for farmers supplying apples to baby-food producers will be developed.

Acknowledgements

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