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Journal of Chromatography A, 1040 (2004) 259–272
JOURNAL OF CHROMATOGRAPHY A
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Evaluation of common organic solvents for gas chromatographic analysis and stability of multiclass pesticide residues☆

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Received 6 January 2004; received in revised form 16 April 2004; accepted 16 April 2004

Abstract

In this study, we evaluated the suitability of six common organic solvents for gas chromatographic (GC) analysis of pesticides. Three of these, acetone, acetonitrile (MeCN) and ethyl acetate (EtAc), represent extraction solvents commonly used in multiresidue methods for determination of pesticides in produce. The other three, isooctane, hexane and toluene, often serve as exchange solvents before a GC analysis. An ideal solvent for GC analysis of multiclass pesticide residues should be compatible with: the analytes, sample preparation, and GC analysis. This study addresses each aspect with emphasis placed on stability of selected pesticides in the given solvents. In this respect, the exchange solvents proved to be superior to the more polar extraction solvents. Degradation of *N*-trihalomethylthio fungicides (e.g., captan, folpet, dichlofluanid) in MeCN was observed only in certain lots of the tested MeCN, but even if it occurred, the stability of these analytes as well as that of dicofol and chlorothalonil was dramatically improved by the addition of 0.1% (v/v) acetic acid. Dicofol and chlorothalonil were also unstable in acetone, and pesticides with a thioether group (e.g., fenthion, disulfoton) degraded in the tested EtAc. Formation of isomers of certain pyrethroids (deltamethrin, λ-cyhalothrin) was recorded in the chromatograms from MeCN and acetone solutions, but this effect more likely occurred during the GC injection than in solution. For several reasons, MeCN was found to be the most suitable solvent for extraction of a wide polarity range of pesticide residues from produce. After acidification, the stability of problematic pesticides in MeCN is acceptable, and MeCN can also serve as a medium for GC injection; therefore solvent exchange is generally not required before GC analysis. If sensitivity is an issue in splitless injection, then toluene was demonstrated to be the best exchange solvent due to its miscibility with MeCN and stronger responses of relatively more polar pesticides (e.g., acephate, methamidophos) as compared to hexane and isooctane.

Keywords: Stability studies; Pesticides

1. Introduction

An ideal solvent for gas chromatographic (GC) analysis of multiclass pesticide residues should be compatible with: (i) the analytes, (ii) sample preparation and (iii) GC multiresidue analysis. Basically, these three requirements mean that all analytes of interest should be sufficiently soluble and stable in the given solvent, the same solvent should be used in the extraction and/or clean-up step to avoid solvent exchange, and physicochemical properties of the solvent should permit an optimal GC analysis of a diverse range of pesticide residues. With respect to the GC analysis, an ideal solvent should allow optimum sample introduction and not adversely affect separation and detection of the analytes. Optimum sample introduction means highly sensitive, reproducible and fast, resulting in narrow initial band widths and symmetric peaks. Other important attributes of an ideal solvent include: low toxicity, flammability, environmental hazard, and cost.

Factors involved in selecting the extraction solvent(s) have been discussed in detail in a study describing development of a new sample preparation method for the analysis of pesticide residues in produce, called by the authors

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doi:10.1016/j.chroma.2004.04.017

"QuEChERS", which stands for quick, easy, cheap, effective, rugged, and safe [1]. The main aspects to be considered for extraction include: (i) compatibility with the analytes (extraction efficiency for the desired range of analyte polarities and stability of analytes during the sample preparation and storage of the final extracts); (ii) selectivity (ability to avoid potentially interfering matrix components in the final extract); (iii) ability to separate from water (miscibility with water or possibility to induce partitioning using additives); (iv) compatibility with techniques used in the clean-up and/or determinative step; (v) volatility if evaporation of the extract is desired; (vi) safety for the laboratory personnel and the environment; and (vii) cost.

Acetonitrile (MeCN) [1–4], acetone [5–8] and ethyl acetate (EtAc) [8–11] are three extraction solvents most commonly used in multiresidue methods (MRMs) for the determination of pesticide residues in produce. Moreover, they often serve as elution solvents in solid phase extraction (SPE) of pesticides from water samples and during clean-up steps. If these solvents are involved in post-extraction sample clean-up (alone or in a mixture with other solvents), or if no clean-up is performed, they also constitute the medium in which the final extract is dissolved. Ideally, no solvent exchange and/or concentration step is necessary and final extracts are injected as they are, preferably using a large volume injection (LVI) technique to compensate for a lower analyte concentration. Due to added expense and complications of LVI, however, many methods employ solvent exchange before GC analysis; toluene, isooctane, and hexane being the most popular exchange solvents.

With respect to pesticide stability in organic solvents, Nemoto et al. [12] investigated stability of 89 pesticides in methanol (MeOH), ethanol (EtOH), 2-propanol, EtAc, hexane and acetone for 6 h at room temperature in dark vials. Dicofol degraded rapidly in acetone. All other tested pesticides were stable in the given solvents with the exception of captan, phosmet, and chinomethionat in MeOH and captafol in MeOH and EtOH. Other authors [13,14] observed degradation of certain organophosphorus pesticides stored for a longer period of time (4–8 weeks) in EtAc solutions at elevated temperatures (40 or 60 °C).

In this study, we evaluated 6 organic solvents commonly featured in either sample preparation (MeCN, acetone, and EtAc) or solvent exchange (toluene, isooctane, and hexane) in pesticide multiresidue analysis. Our aim was to answer key questions such as: What is the most suitable solvent for sample introduction in GC analysis of pesticide residues? What solvent(s) should be avoided and why? Is it necessary to perform solvent exchange after extraction and, if yes, what is the best exchange solvent? The presented discussion covers the most important aspects of the issues related to the compatibility of the tested solvents with the analytes, sample preparation step and the GC analysis, with emphasis placed on the stability of the selected types of pesticides in the investigated solvents.

2. Experimental

2.1. Chemicals and materials

Pesticide reference standards, all 95% or higher purity, were obtained from the National Pesticide Standard Repository of the US Environmental Protection Agency (Fort Meade, MD, USA), Dr. Ehrenstorfer GmbH (Augsburg, Germany), Ultra Scientific (North Kingstown, RI, USA), and Chemservice (West Chester, PA, USA). Composite stock standard solutions (50 μg/mL) of 31 pesticides (acephate, azinphos-methyl, captan, carbaryl, chlorothalonil, chlorpyrifos, coumaphos, cyprodinil, deltamethrin, diazinon, dichlofluanid, dichlorvos, dicofol, dimethoate, endosulfan sulfate, fenthion, folpet, imazalil, iprodione, lindane, metalaxyl, methamidophos, methiocarb, mevinphos, omethoate, permethrins, pirimiphos-methyl, *o*-phenylphenol, phosalone, thiabendazole and vinclozolin) were prepared in MeCN and toluene. For further investigation of the stability of *N*-trihalomethylthio pesticides in different lots of MeCN, captafol and tolylfluanid were added (at 50 μg/mL) to the composite stock standard solution of 31 pesticides in toluene that was used for spiking purposes. For the stability study of pesticides with a thioether group, a 50 μg/mL composite standard solution of seven pesticides (disulfoton, fenamiphos, fenthion, methiocarb, phorate, terbufos and an internal standard diazinon) was prepared in toluene.

Acetone, MeCN (lots A–H), EtAc, hexane, isooctane, and toluene were high purity grade solvents for pesticide residue analysis from Burdick & Jackson (Muskegon, MI, USA), and EtOH was obtained from Merck (Darmstadt, Germany). For comparison purposes, MeCN (lots I–L) was also obtained from Fisher Scientific (Fair Lawn, NJ, USA). Glacial acetic acid (HAc) was HPLC grade from Fisher Scientific. Anhydrous $MgSO_4$ and NaCl were obtained from Aldrich (Milwaukee, WI, USA) and PSA (primary secondary amine) sorbent was from Varian (Harbor City, CA, USA). The $MgSO_4$ was heated for 5 h at 500 °C in a muffle furnace to remove phthalates. Zucchini, tomato and grape samples were purchased in local organic food stores.

Matrix extracts were prepared by the QuEChERS method [1], according to which 10 g of a homogenized sample were extracted with 10 ml of MeCN in a 40 ml fluorinated ethylene propylene (FEP) centrifuge tube for 1 min using a vortex mixer. Then, 4 g anhydrous $MgSO_4$ and 1 g NaCl were added, and the tube was vortexed immediately for 1 min and then centrifuged for ≈3 min at 5000 rpm. For spiking of the extract at 0.5 μg/mL, 10 μL of the 50 μg/mL solution of the 31 pesticides in MeCN were added to a 990 μL aliquot of the upper layer. For the dispersive-SPE clean-up, a 1.6 ml aliquot of the upper layer was transferred into a 2 ml centrifuge vial containing 40 mg PSA sorbent and 240 mg $MgSO_4$. After vortexing for 30 s and centrifuging for ≈1 min at 5000 rpm, a 990 μL aliquot of the extract after PSA clean-up was spiked at 0.5 μg/mL in the same way as the extract without undergoing clean-up.

2.2. Stability study experiments

For the stability study experiments, test solutions of the 31 pesticides were prepared in the investigated solvents at 0.5 μg/mL using the composite stock solutions in MeCN (Experiment 1) or toluene (Experiments 2 and 3). The 31 pesticides were chosen carefully based on their diverse properties (e.g. volatility, thermolability, polarity, pH sensitivity) and quality of their GC analysis, including those prone to degradation and/or adsorption in the GC system and also non-problematic pesticides for comparison/normalization purposes.

The solutions were injected immediately after spiking ($t = 0$ h) and stored in different conditions: in dark and clear vials at room temperature and in dark vials in the refrigerator (+2 °C) and freezer (−22 °C). The solutions were analyzed by GC–mass spectrometry (MS) in regular intervals with the following injection order of the tested solvents: (1) toluene—"Tol"; (2) isooctane—"Iso"; (3) MeCN; (4) acidified MeCN (0.1% HAc, v/v)—"MeCN/HAc"; (5) EtAc; (6) acetone—"Ace"; (7) acidified acetone (0.1% HAc, v/v)—"Ace/HAc"; and (8) hexane—"Hex". Thus, the selected pesticides were injected periodically in different solvents in one sequence in order to eliminate the potential influence of different GC system conditions on the results. In the figures and throughout the discussion, the pesticide stability is mostly expressed as a pesticide residue (in %) providing information about how much of the tested pesticide was detected at the given time versus $t = 0$ h.

The stability of the pesticides with a thioether group was monitored in the above-mentioned solvents and also in EtOH and EtAc with addition of EtOH (0.1 and 1%, v/v) or HAc (0.1%, v/v). For further investigation of the potential degradation of *N*-trihalomethylthio fungicides (captafol, captan, folpet, dichlofluanid, and tolylfluanid) in MeCN, eight different MeCN lots (E–H from one MeCN manufacturer and lots I–L from another one) were spiked at 0.5 μg/mL using the composite stock standard solution of 31 pesticides in toluene (fortified with captafol and tolylfluanid) and the prepared solutions were repeatedly analyzed in one GC sequence.

2.3. GC–MS conditions

Most of the analyses were conducted with a Hewlett-Packard (Agilent, Little Falls, DE, USA) Model 5890 Series II Plus GC coupled to a 5972 mass-selective detector. The system was equipped with a split/splitless injection inlet, electronic pressure control, and a 7673A autosampler; Chemstation software was used for instrument control and data analysis. Samples were injected into a double taper liner with internal volume of 800 μL (No. 5181-3315, Agilent). The chromatographic conditions were: a Rtx-5ms column (Restek, Bellefonte, PA, USA) or a DB-5ms column (Agilent) capillary column of 30 m × 0.25 mm i.d., 0.25 μm film thickness, He constant flow of 1 mL/min, inlet temperature $t_{inj} = 250$ °C, injection volume 1 μL (splitless), MS transfer line temperature 290 °C, temperature program: initial temperature (t_i) held for 1.5 min, then a 20 °C/min ramp to 180 °C followed by a 5 °C/min ramp to 230 °C and a 25 °C/min ramp to 290 °C (held for 10 min). Table 1 gives optimum t_i for analysis of the 31 pesticides using the six tested organic solvents along with some other parameters and solvent properties. The quadrupole was operated in selected ion monitoring (SIM) mode detecting two to three ions for each analyte (see [18] for chosen ions and retention times). When a mixture of 31 pesticides was analyzed, responses (peak areas) of all analytes were normalized to the average peak areas of stable pesticides diazinon (*m*/*z* 179), pirimiphos-methyl (*m*/*z* 290) and chlorpyrifos (*m*/*z* 197). In the case of pesticides with a thioether group, diazinon (*m*/*z* 179) served as an internal standard (IS) for the peak area normalization.

The analyses of zucchini, tomato and grape extracts were performed using a Saturn 2000 (Varian, Walnut Creek, CA, USA) GC–MS instrument equipped with a Model 3800 gas chromatograph, a Model 1079 temperature programmable inlet and a CombiPal autosampler (CTC Analytics, Switzerland). Samples were injected into a 3.4 mm i.d. deactivated liner packed with a 7 mm piece of Carbofrit (Restek). The GC–MS conditions were: a DB-5 ms column (Agilent) capillary column of 30 m × 0.25 mm i.d., 0.25 μm film thickness connected to a 3 m × 0.25 mm i.d. deactivated

Table 1
Properties of the tested solvents [15–17]

Solvent	M_r (g/mol)	ρ (g/mL)	bp (°C)	p_v (kPa)	t_i (°C)	p_i (psi)	V_{vapor} (μL)	V_{inj}(max) (μL)	P'
MeCN	41	0.78	82	9.6	80	9.4	499	1.2	6.2
Acetone	58	0.79	56	24.6	60	8.2	375	1.6	5.4
EtAc	88	0.90	77	9.7	70	8.8	275	2.1	4.3
Hexane	86	0.66	69	16.3	50	7.7	215	2.7	0.0
Toluene	92	0.87	111	2.9	95	10.3	238	2.5	2.3
Isooctane	114	0.69	99	5.1	80	9.4	159	3.7	−0.4

M_r: molecular mass, ρ: solvent (liquid) density (at 20 °C, p_{atm}); bp: boiling point (at p_{atm}); p_v: vapor pressure (at 20 °C); t_i: initial oven temperature determined for the analysis of the 31 pesticides; p_i: column head pressure at t_i, helium flow rate 1 mL/min, vacuum outlet operation, using a 30 m × 0.25 mm i.d. column; V_{vapor}: vapor volume generated by 1 μL injection ($V_{inj} = 1$ μL) of the given solvent at p_i and injection temperature $t_{inj} = 250$ °C; calculated from the following equation: $V_{vapor} = 22.4 \times 10^3(\rho/M_r)[(t_{inj} + 273)/273][p_{atm}/(p_i + p_a)]V_{inj}$, where $p_{atm} = 14.7$ psi (101 kPa) and p_a is ambient pressure, usually taken as p_{atm}; V_{inj}(max): maximum safe injection volume for the 800 μL liner used at p_i and $t_{inj} = 250$ °C, i.e. injection volume that generates 600 μL of vapors (75% of the liner volume); P': polarity index.

retention gap; MS transfer line temperature 290 °C; MS ion trap temperature 200 °C; MS manifold temperature 50 °C; data acquisition in full scan mode (*m*/*z* 70–420); injection volume 5 μL; inlet temperature: 50 °C held for 0.15 min, then a 200 °C/min ramp to 280 °C (held till the end of the analysis); split vent open for the initial 0.15 min (split ratio 50:1), then closed for 2.85 min and then open again (split ratio 50:1 for 7 min, then 15:1 for the rest of the analysis); temperature program: 50 °C held for 3 min, then a 25 °C/min ramp to 180 °C followed by a 5 °C/min ramp to 230 °C and a 25 °C/min ramp to 290 °C (held for 10 min); and pressure was programmed to provide a 40 psi pressure pulse (1 psi = 6894.76 Pa) during the splitless period and a constant He flow of 1.2 mL/min for the rest of the GC run.

3. Results and discussion

3.1. Suitability of organic solvents for sample preparation in pesticide multiresidue analysis

The main aspects that should be considered in the selection of an extraction solvent have been mentioned in Introduction. MeCN, acetone and EtAc are three extraction solvents most commonly used for isolation of multiple pesticide residues from produce and each of them has been demonstrated to give acceptably high recoveries of a wide range of pesticides [1–11]. In contrast to MeCN and acetone, EtAc is practically immiscible with water (only 7.94%, w/w, of water is soluble in EtAc at 20 °C [15]) which can easily be removed from EtAc extracts by a drying agent (usually anhydrous Na_2SO_4). To induce a distinct separation from the water phase, acetone needs an addition of a non-polar solvent, which leads to dilution and possibly to lower recoveries of more polar analytes. In the case of MeCN, the use of a proper combination of salts (such as anhydrous $MgSO_4$ and NaCl in the QuEChERS method [1]) can provide a well-defined phase separation without dilution and high recoveries including rather polar pesticides. Moreover, anhydrous $MgSO_4$ removes residual water remaining in organic phase after the partitioning step more efficiently from MeCN than acetone [19,20]. Also, a simple experiment showed that $MgSO_4$ is practically insoluble in MeCN, whereas about 13 mg of $MgSO_4$ can be dissolved in 1 ml of acetone at room temperature. This is an important consideration because salts must be avoided in GC analysis.

In terms of extraction selectivity, MeCN isolates much less lipophilic compounds from samples in comparison with acetone and EtAc (amount of lipophilic co-extractives decreases in the order: EtAc > acetone > MeCN [19]). EtAc is more effective in avoiding sugar co-extractives, mainly comparing to acetone (amount of sugar co-extractives decreases in the order: acetone > MeCN > EtAc). With regard to clean-up, EtAc is more compatible with gel permeation chromatographic (GPC) methods for removal of some larger lipophilic and pigment molecules. Acetone and mainly acetonitrile are suitable for various SPE clean-up applications, which is generally preferred over solvent-consuming GPC. For example, dispersive-SPE clean-up used in the QuEChERS method [1] for removing of co-extractives from MeCN extracts makes the clean-up step relatively fast, inexpensive, and convenient. An additional important advantage of MeCN versus EtAc and acetone is its compatibility with reversed-phase liquid chromatography (LC), which offers a possibility to analyze both GC- and LC-amenable analytes in the same extract.

Acetone has a lower boiling point than both EtAc and MeCN (56 °C versus 77 and 88 °C at p_{atm}, respectively) and its volatility at room temperature is also significantly higher (vapor pressure of 24.6 kPa versus 9.7 and 9.6 kPa at 20 °C, respectively) [16]. In some respects, a higher solvent volatility represents a disadvantage in the extraction process because increased evaporation during sample handling may lead to changes in the extract volume and to a higher exposure of the analyst to the solvent vapors. However, if a solvent exchange and/or extract concentration is performed prior to the GC analysis, a higher volatility becomes advantageous (but it is still preferable to avoid these steps if possible).

To summarize, the three extraction solvents can be ordered according to their suitability for sample preparation in analysis of pesticide residues in produce as follows: MeCN > EtAc ≫ acetone. In terms of cost, MeCN is approx. 1.4 and 1.7 times more expensive than a similar grade EtAc and acetone, respectively. MeCN is also more toxic, but its negative impact on human health and the environment is much lower than in the case of chlorinated solvents (such as dichloromethane), which are still used in routine practice. According to the US Environmental Protection Agency (EPA), MeCN is not classified as a human carcinogen and is not persistent in the environment. Moreover, the lower volatility of MeCN reduces analyst exposure, which can be further minimized by following proper handling procedures [1].

3.2. Pesticide stability in common organic solvents

The QuEChERS method takes advantage of several features of MeCN to provide a rather selective isolation of pesticide residues over a wide polarity range. However, during the further optimization of this method, we encountered a decreased stability of certain pesticides (dicofol, chlorothalonil, captan, folpet, and dichlofluanid) in a standard mixture prepared in MeCN. Generally, the compounds that were unstable in the tested MeCN are prone to degradation under basic conditions [21]. Therefore, we attempted to stabilize them by adding HAc to the standard solutions.

Fig. 1 shows the stability of dicofol, chlorothalonil, captan, folpet, and dichlofluanid after addition of 0, 0.001, 0.01, 0.02, 0.05 and 0.1% (v/v) HAc to a 0.5 μg/mL solution of 31 selected pesticides in MeCN that were placed in dark vials in the GC autosampler tray for 24 h (a period for which a

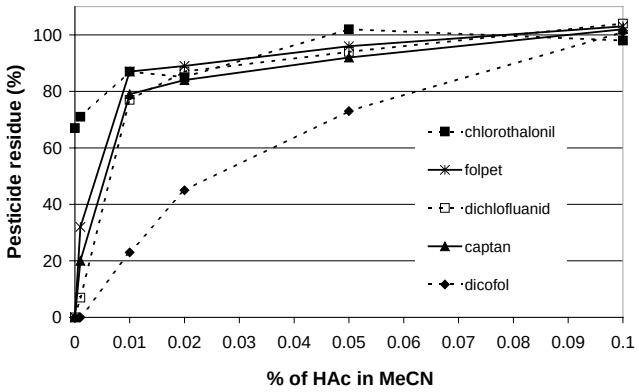


Fig. 1. Stability of chlorothalonil, folpet, dichlofluanid, captan and dicofol at 0.5 μg/mL in MeCN (lot A) with 0–0.1% (v/v) HAc in dark vials placed in the GC autosampler tray for 24 h (100% = analyte normalized response in the respective solution at time $t = 0$ h).

sample is typically placed in a GC autosampler tray when a sequence of samples is being analyzed in practice). When no HAc was added to the tested MeCN, dicofol, captan, folpet, and dichlofluanid were not detected in the solutions analyzed after 24 h and chlorothalonil concentration decreased by 30%. As Fig. 1 indicates, 0.1% HAc was necessary to stabilize dicofol in the tested MeCN for 24 h and 0.05% appeared to be sufficient for the other four pesticides. The addition of HAc did not negatively affect the stability of the other 26 pesticides. Also, we did not observe deterioration of the GC system performance that could be associated with long-term injections of MeCN containing 0.1% HAc.

In order to compare the stabilities of the selected pesticides in different solvents, we performed experiments as described in Section 2.2. The results of the first experiment (Experiment 1) agreed with the previous observations for dicofol, chlorothalonil, captan, folpet, and dichlofluanid in MeCN and acidified MeCN. Other pesticides that gave >15% decrease in normalized responses after 24 h in dark vials in the GC autosampler tray were dicofol and chlorothalonil in acetone and fenthion in EtAc. In the case of deltamethrin, the formation of its diastereoisomer was recorded in the chromatograms from MeCN and acetone solutions (see Section 3.2.4 for further details).

No significant pesticide concentration decreases were encountered in toluene, isooctane, or hexane, even when the solutions were kept for 5 days in the GC autosampler tray in dark or clear vials. Therefore, toluene instead of MeCN was used for the preparation of the spiking solution in Experiments 2 and 3 (toluene was preferred over isooctane and hexane because of its good miscibility with the other tested solvents). These experiments confirmed the results from Experiment 1 with the exception of captan, folpet, dichlofluanid, and chlorothalonil, which were stable in MeCN solutions even after 5 days in the GC autosampler tray in dark vials (chlorothalonil degraded in clear vials as discussed in Section 3.2.2). These contradictory findings led us to further experiments, which helped associate the degradation problem of *N*-trihalomethylthio pesticides (captan, folpet, captafol, dichlofluanid and tolylfluanid) and chlorothalonil with particular lots of MeCN (see Section 3.2.1 for a detailed discussion). Also, we further investigated the stability issues of fenthion and some other pesticides with a thioether group in EtAc as described in Section 3.2.3.

3.2.1. *N*-Trihalomethylthio fungicides

The group of *N*-trihalomethylthio fungicides consists of dichlofluanid, tolylfluanid, folpet, captan and structurally similar captafol. Fig. 2 gives the structures of these pesticides and also outlines the formation of their main degradation products (*N*′,*N*′-dimethyl-*N*-phenylsulfonyldiamide from dichlofluanid; *N*′,*N*′-dimethyl-*N*-*p*-tolylsulfonyldiamide from tolylfluanid; phthalimide from folpet; and 1,2,3,6- tetrahydrophthalimide from captan and captafol). The degradation (loss of the $-SCX_3$ moiety) is rather rapid in the presence of basic compounds [21,22] and can occur not only in the environment and living organisms, but also during sample processing and analysis. It should be noted that the degradation products are not included in the definitions of the corresponding pesticide residues [23], thus analyte losses during sample handling and analysis should be eliminated and/or minimized using appropriate procedures, such as

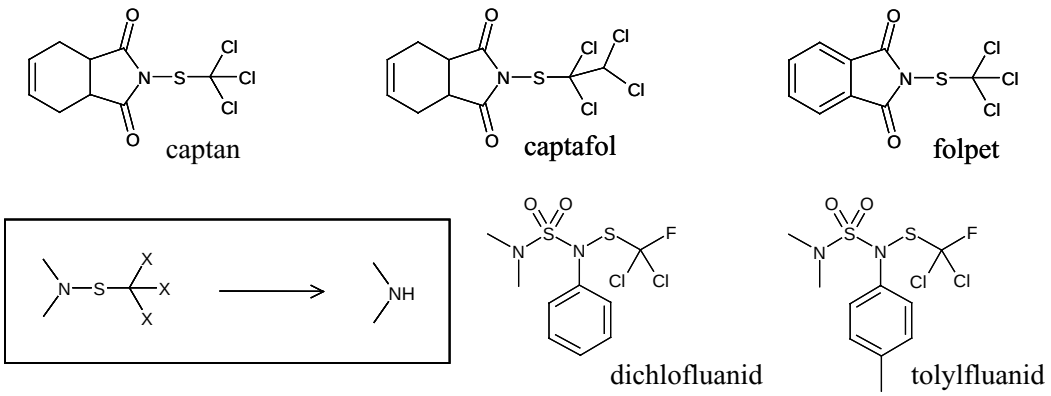


Fig. 2. Structures of *N*-trihalomethylthio fungicides and the scheme of formation of their main degradation products.

comminution of samples at low temperatures (cryogenic milling) [24,25].

The GC analysis of *N*-trihalomethylthio pesticides itself is rather problematic because the degradation may occur both in the injection port and on the column. The extent depends on the injection and GC system conditions, mainly on the injection temperature and speed, sample composition, and degree of the GC system contamination [26,27]. Thus in practice, the samples and calibration standards should be analyzed under as similar conditions as possible. This was also essential in our solvent evaluation studies; therefore, the tested solutions were always injected on a rotating basis in one GC sequence as described in Section 2.

As already discussed, captan, folpet and dichlofluanid gave stable responses when injected in all tested solvents except for MeCN in our preliminary experiments and in Experiment 1 (MeCN lots A and B). The observed degradation in MeCN lot A was relatively fast, resulting in undetectable amounts of these analytes (<0.005 μg/mL) in solutions originally spiked at 0.5 μg/mL after 24 h spent at room temperature. In the case of lot B, 13 and 6% of the initial folpet and captan concentrations, respectively, were determined after 24 h, whereas no dichlofluanid was detected (after 4 h, folpet, captan and dichlofluanid concentrations decreased to 35, 26 and 4%, respectively). Acidifying the tested MeCN with 0.1% HAc helped stabilized these analytes and no degradation was observed even after 5 days at room temperature. Interestingly, no degradation of the tested *N*-trihalomethylthio compounds also occurred when the solutions were prepared in MeCN lots C and D from the same manufacturer and monitored for 5 days at room temperature (in Experiments 2 and 3). We performed additional experiments with eight other MeCN lots from two different manufacturers and found that the use of some MeCN lots led to faster degradation rates of *N*-trihalomethylthio fungicides than others.

Fig. 3A shows the stability of all five pesticides from this group (tolylfluanid and captafol were also monitored this time) in 0.5 μg/mL solutions prepared in MeCN lots E–H from manufacturer 1 and in lots I–L from manufacturer 2 and stored for 24 h in dark vials at room temperature (the lots were ordered according to the pesticide stability). Except for lot E, the monitored analytes degraded in MeCN from all other lots tested in this experiment. Lot E was used in many other studies in our laboratory, in which no degradation of these fungicides was observed. In terms of degradation rate, the fastest decrease of the analyte concentrations was recorded in MeCN from lot H, in which dichlofluanid and tolylfluanid were not detected already after 30 min and the concentration of the other three analytes dropped below 20% after 30 min, whereas only 7, 4 and 3% of folpet, captan and captafol, respectively remained after 24 h at room temperature. Under the same conditions, the slowest degradation occurred in MeCN lot I, resulting in ≈50% decrease in the concentrations of folpet, captan and, captafol and in a drop to 19 and 27% of the initial dichlofluanid and tolylfluanid content after 24 h, respectively.

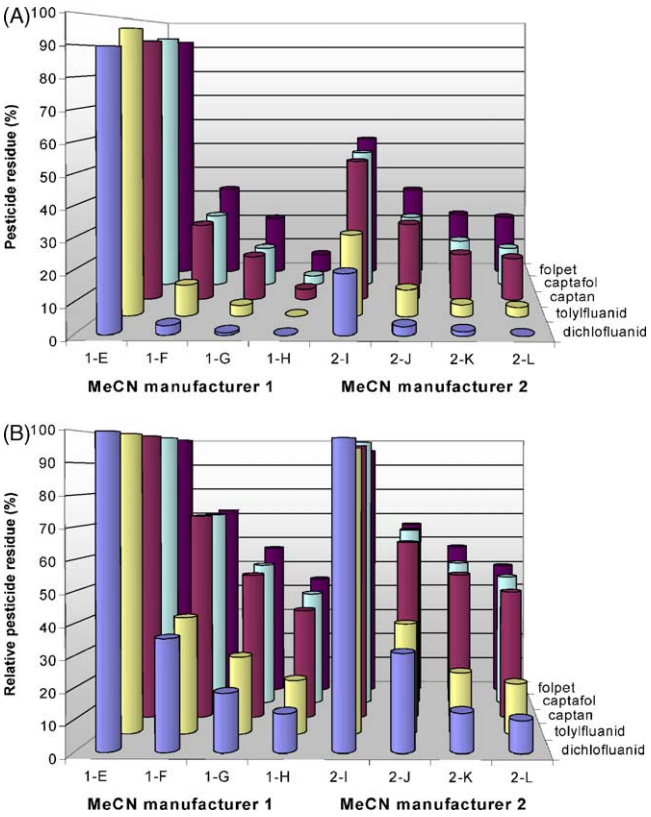


Fig. 3. Stability of *N*-trihalomethylthio fungicides in 0.5 μg/mL solutions prepared in MeCN from eight different lots (lots E–H from manufacturer 1 and lots I–L from manufacturer 2) and stored: (A) for 24 h in dark vials placed in the GC autosampler tray (100% = analyte normalized response in the respective MeCN solution at time $t = 0$ h) and (B) for 5 days in dark vials in the freezer (100% = analyte normalized response in MeCN lot E stored for 5 days in the freezer), $n = 2$.

In terms of the variable stability of the monitored pesticides in the tested MeCN lots, similar degradation trends were observed when the solutions were stored in the freezer as shown in Fig. 3B (normalized to lot E results). The lower temperature significantly reduced the degradation rates (e.g. no degradation occurred in lot I at −22 °C over 5 days).

Generally, *N*-trihalomethylthio pesticides can be ordered according to their susceptibility to potential degradation in MeCN as follows: dichlofluanid > tolylfluanid > captan ≈ captafol > folpet. The decreased stability of these analytes in particular MeCN lots is presumably caused by a variable residual content of amines or some other basic compounds in these lots. No significant difference or trend were observed in pH values of MeCN-water (50:50, v/v) solutions. It should be also noted that manufacturer 2 includes titratable acid and base tests in MeCN specifications, but the "good" lots were obtained from manufacturer 1, which does not perform these tests. We recommend acidifying the MeCN solutions preventively, even if the

discussed fungicides have been demonstrated to be stable in a particular lot of MeCN. Also, the original QuEChERS method has been modified to address this problem and to further extend the range of analytes independent of the food matrix pH using a sodium acetate–HAc buffer [28].

3.2.2. Dicofol and chlorothalonil

Dicofol and chlorothalonil count among the most challenging pesticides included in MRMs with GC as a determinative step. Dicofol readily degrades to 4,4′-dichlorobenzophenone when exposed to a higher pH, light or a higher temperature [21], but only the parent compound is included in the pesticide residue definition [23]. It is known to degrade in the GC system [12], thus the same precautions as in the case of *N*-trihalomethylthio fungicides must be taken. Addition of acid (e.g. orthophosphoric or acetic acid) at extraction with EtAc was reported to improve recoveries of dicofol from weakly acidic vegetable samples, such as lettuce, and prevent degradation of this analyte in solutions [24].

Unlike dicofol, chlorothalonil is thermally stable in GC, but it is susceptible to tailing and matrix effects [18,27]. Losses of chlorothalonil were observed during sample processing of lettuce and onions [24] and long-term storage of cabbage extracts in EtAc [13]. Comminution of lettuce samples in the presence of dry ice can prevent degradation of chlorothalonil, but this procedure is not effective with onions, in which case the presence of a strong acid (e.g. orthophosphoric added at 1–2 M) is necessary to avoid chlorothalonil losses [24]. Fungicidal activity of chlorothalonil is based on conjugation with thiols [21], thus the high content of sulfur-containing compounds in onions is most likely responsible for rapid degradation during comminution and in the extracts in this case.

In this study, we observed degradation of dicofol and chlorothalonil in acetone and MeCN solutions. It should be noted that 4,4′-dichlorobenzophenone was recorded in the chromatograms from solutions of all tested solvents, but the concentration of dicofol was rapidly degreasing only in acetone and MeCN over the tested period of time. Dicofol was unstable in all tested MeCN lots, whereas chlorothalonil only in some of them. Fig. 4 shows stability of these pesticides in solutions prepared in acetone and MeCN from lots, in which *N*-trihalomethylthio fungicides were stable. This figure compares the situation observed at room temperature over the period of 5 days in dark versus clear vials and in acidified versus non-acidified solutions. The following conclusions can be made: (i) clear vials should be avoided because their use generally resulted in faster degradation rates; (ii) acidification helped improve stability in both MeCN and acetone; and (iii) addition of 0.1% HAc to MeCN solutions could prevent degradation of dicofol in dark vials and chlorothalonil in clear vials, but it was not sufficient in the case of the tested acetone.

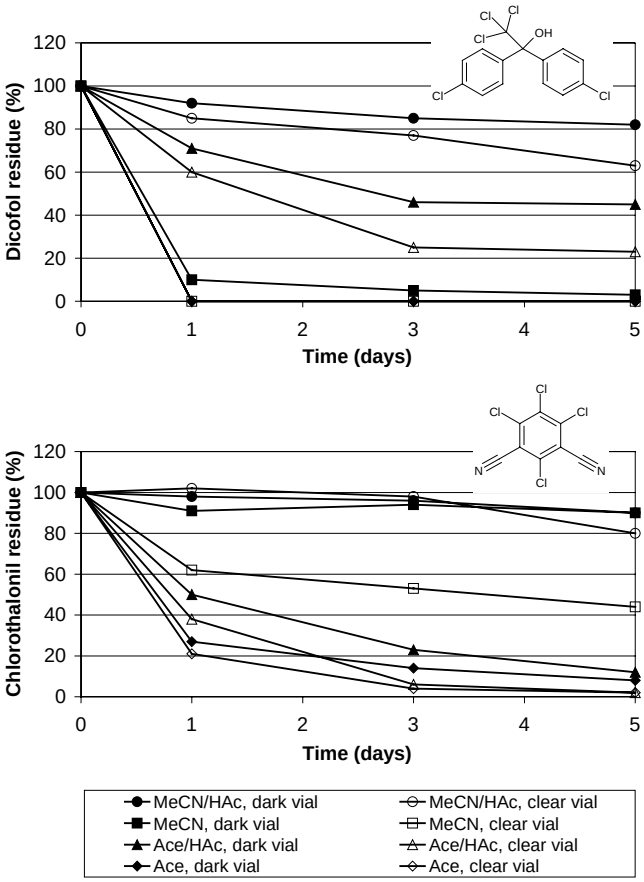


Fig. 4. Stability of dicofol and chlorothalonil in 0.5 μg/mL solutions prepared in MeCN (lots C and D) and acetone (both without and with 0.1% (v/v) HAc) and placed in dark and clear vials in the GC autosampler tray (Experiment 2 and 3, $n = 2$).

3.2.3. Pesticides with a thioether group

Pesticides containing a thioether (sulfide, –S–) group are prone to oxidation. In the environment and living organisms, they usually degrade to sulfoxides and sulfones [21] (as shown in Fig. 5), both of which possess insecticial properties and therefore are included in the definition of the corresponding pesticide residue [23]. Thionophosphate (P=S) sulfides may also be oxidized to biologically active oxons (P=O), thus their residue definitions include 6 compounds (two sets of sulfides, sulfoxides and sulfones). Fenthion and methiocarb represent the group of pesticides with a thioether group in our test mixture of 31 analytes, and other important pesticides of this nature include aldicarb, demeton-*S*-methyl, disulfoton, ethiofencarb, fenamiphos, phorate, propaphos, sulprofos, temephos, terbufos, thiometon and vamidothion. Fig. 5 gives structures of those compounds discussed and further investigated in this study.

In our experiments, methiocarb and fenthion were stable in all tested solvents for 24 h in dark vials placed in the GC autosampler tray, the only exception being fenthion in EtAc (decreased to ≈20% of the initial fenthion concentration). Fig. 6A shows the stability of fenthion and methiocarb in the tested EtAc over the course of 3 days (solutions in dark vials

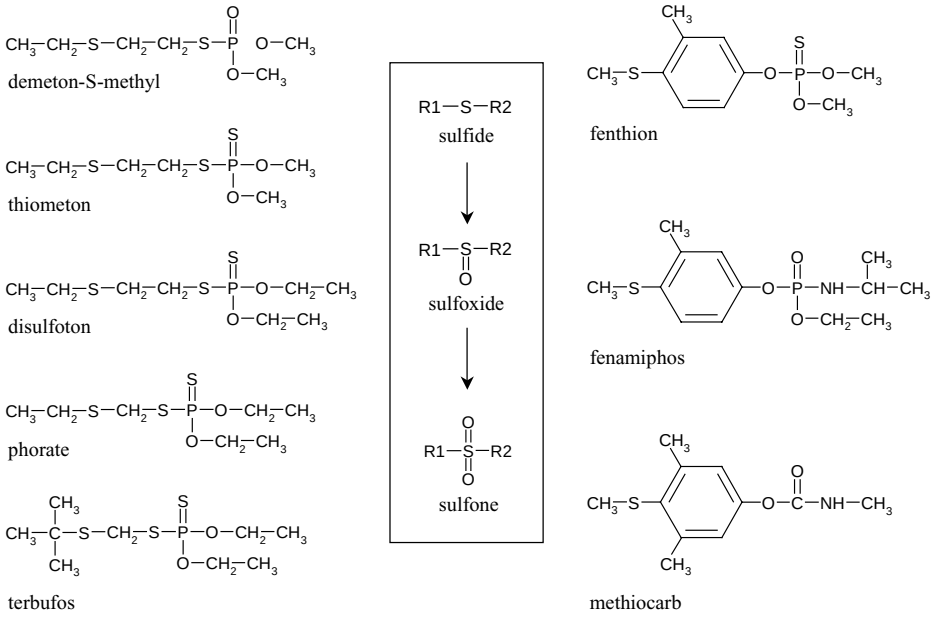


Fig. 5. The main degradation pathway of pesticides with a thioether (sulfide) group in the environment and living organisms together with structures of the compounds investigated or discussed in this study.

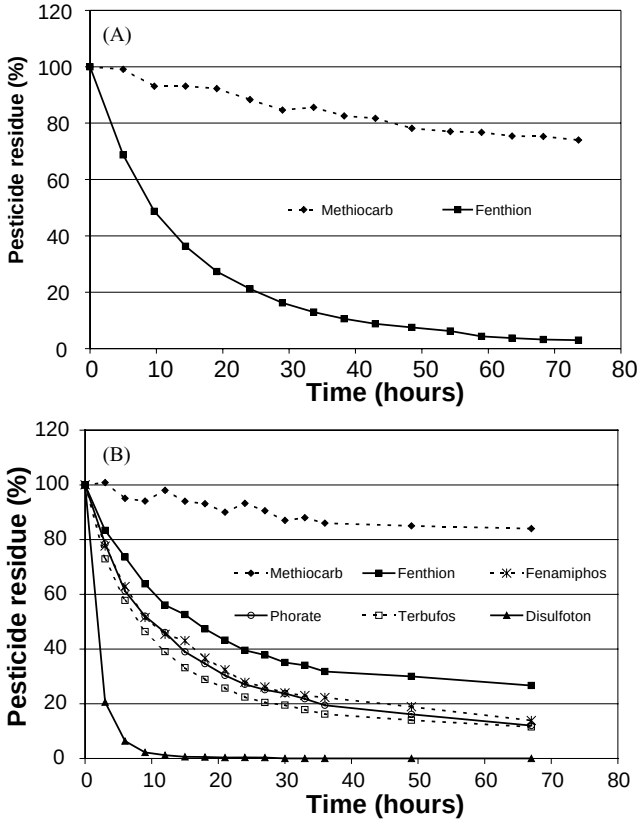


Fig. 6. Stability of pesticides with a thioether group in the tested EtAc: (A) methiocarb and fenthion in the mixture of 31 pesticides in EtAc ($n = 2$) and (B) disulfoton, fenamiphos, fenthion, methiocarb, phorate and terbufos in the mixture of these thioether pesticides in EtAc ($n = 2$); both solutions were prepared at 0.5 μg/mL and placed in dark vials in the GC autosampler tray.

placed in the GC autosampler tray). Methiocarb degraded slowly (to 74% after 3 days), whereas a rapid degradation was observed in the case of fenthion (down to 3% after 3 days). Moreover, fenthion content in EtAc also decreased during the storage in the refrigerator and freezer, and after 10 days, only 5 and 45% of the initial fenthion content was found in the EtAc solutions stored at 2 and −22 °C, respectively. Stability of selected pesticides in EtAc was tested previously [13,14], but none of these studies included pesticides with a thioether group.

In order to further investigate the stability of this group of pesticides, a mixture of disulfoton, fenamiphos, fenthion, methiocarb, phorate, terbufos and diazinon (an internal standard) was prepared in all tested organic solvents and also in EtOH and EtAc with addition of EtOH (0.1 and 1%, v/v) and HAc (0.1%, v/v) to determine the potential influence of EtAc hydrolysis products. All tested analytes were stable for at least 24 h in both dark and clear vials at room temperature in toluene, hexane, isooctane, MeCN (without and with 0.1% HAc addition) and EtOH. Disulfoton degraded in acetone, with a faster degradation rate observed in clear vials (decrease to 3% in 24 h) as compared to dark vials (to 28%). Similarly, Hirahara et al. [29] reported a relatively rapid photooxidation of disulfoton, aldicarb and ethiofencarb in acetone solutions exposed to sunlight and a slower degradation in the case of fenthion, vamidothion and fensulfothion.

Fig. 6B gives the results obtained in the tested EtAc. All investigated pesticides with a thioether group degraded except for methiocarb. No significant difference was observed between the degradation rates in dark and clear vials, and furthermore, the addition of EtOH and HAc had no significant influence on analyte stability. Disulfoton content in the EtAc solution decreased very quickly and it was hardly de-

tected after 12 h. In this experiment, the stability of fenthion and methiocarb was slightly improved from before due to the presence of other susceptible analytes acting as degradation retardants (antioxidants) for each other. We confirmed the presence of fenthion and fenamiphos sulfoxides (relative retention times, t_R, of 1.27 and 1.17 versus the corresponding parent compounds, respectively) in the degraded mixture (and in the degraded solutions of individual pesticides in EtAc) by the injection of the respective standards and GC–MS analysis in full scan mode, which also indicated high probability of the presence of phorate sulfoxide (relative t_R 1.33 versus phorate).

In the case of disulfoton, a degradation product peak appeared with a shorter t_R than the parent compound (relative t_R 0.61 versus disulfoton) whereas the corresponding sulfoxide would have been expected to elute later than disulfoton. Some authors, such as Hirahara et al. in their study [29], actually label a similarly eluting peak as disulfoton sulfoxide but it is more likely its thermodegradation product [30]. The GC analysis of some sulfoxides is rather difficult because they are prone to decomposition at elevated temperatures [30,31]. Greenhalgh et al. [31] reported thermodegradation of demeton-*S*-methyl sulfoxide (oxydemeton-methyl) to a compound characterized by a relatively short retention time in GC and a GC–electron impact ioniozation (EI) MS spectrum with *m/z* 168 as a (potential) molecular ion to which they attributed the structure $(CH_3O)_2P(O)SCH{=}CH_2$. Similarly, Satoh et al. [32] observed degradation of thiometon in EtAc to a compound eluting sooner than the parent analyte and, based on a high-resolution GC–MS analysis giving *m/z* 183.9797 as the highest ion in the spectrum, they suggested that the most probable structure of the degradation product was $(CH_3O)_2P(S)SCH{=}CH_2$. Fig. 7 shows the GC–EI-MS spectrum we obtained for the disulfoton degradation product along with its proposed structure $(CH_3CH_2O)_2P(S)SCH{=}CH_2$ corresponding to *m/z* 212 as a probable molecular ion. Thus, disulfoton, demeton-*S*-methyl and thiomethon (or their sulfoxides in GC) give analogical degradation products, which can be explained by a similar structure of these pesticides containing a $–CH_2–CH_2–$ group bound to the thioether group (see Fig. 5). Phorate and disulfoton have almost identical structures, but phorate molecule contains only one $–CH_2–$ group in the proximity of the thioether group, presumably accounting for the dissimilar behavior of these two compounds in our experiment.

In the case of methiocarb, the thioether group is probably less accessible due to the steric constraints caused by two methyl groups in *ortho* positions on the benzene ring (see Fig. 5), which can explain much lower susceptibility of methiocarb to oxidative degradation as compared to other tested pesticides with a thioether group. Nevertheless, as with other carbamate pesticides, methiocarb is prone to thermodegradation and a corresponding phenol formed by the break-down of the labile carbamate group (presumably in the injection port) was present in chromatograms of all tested solvents, however no gradual increase in its response was observed over time in the tested solutions.

In the past, we also observed variability in recoveries of pesticides with a thioether group included in our studies (unpublished data and [33]) that employed EtAc for the extraction and GPC clean-up. The GPC separation process was presumably a critical point in terms of the degradation of these analytes due to their relatively long exposure to the mobile phase containing EtAc. In this study, we did not test different lots of EtAc as in the case of MeCN because we were less interested in the performance of EtAc due to the use of MeCN in the QuEChERS method. We should also note that the degradation of sulfides in EtAc may depend

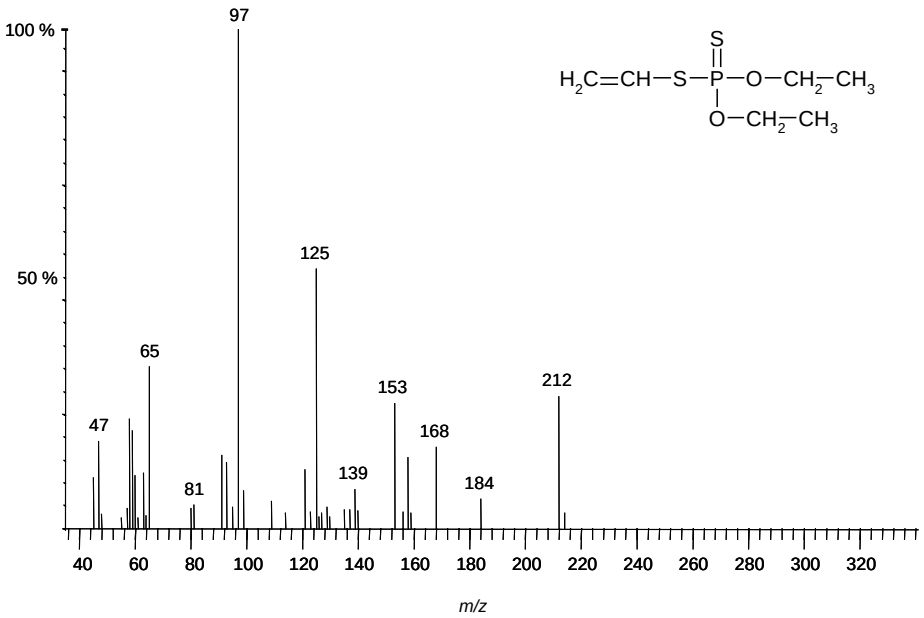


Fig. 7. GC–EI-MS spectrum of a disulfoton degradation product along with its proposed structure.

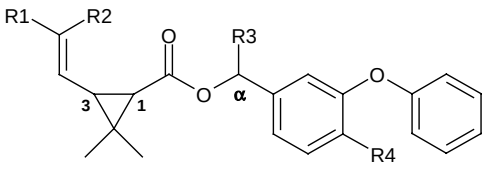
on several factors, such as solvent lot and age (presumably related to the content of acetaldehyde [32]), and pesticide mixture composition and concentration.

Generally, the degradation of sulfides to corresponding sulfoxides (and sulfones) during sample preparation should not present a problem in compliance and enforcement applications because these degradation products are part of the respective pesticide residue definitions and should be monitored together with the parent compounds. However, in many instances, such as testing of newly developed analytical procedures (simplified validation studies) and/or screening of potential pesticide residues, the target group of analytes often includes only parent compounds (application forms of pesticides). Other problematic cases are studies in which the pesticide form is important (metabolic studies, monitoring of pesticide fate in the environment, etc.) and thus analyte degradation during sample preparation and analysis is not acceptable. In any event, accurate quantitation becomes rather difficult or even impossible if degradation occurs in standard (calibration) solutions.

A practical approach to analysis of pesticides with a thioether group for enforcement purposes is to quantitatively oxidize both sulfides and sulfoxides to corresponding sulfones by potassium permanganate, 3-chloroperbenzoic acid or other strong oxidant after extraction of residues [30,34]. This procedure can solve the potential degradation problems, provide an effective clean-up and generally simplify the analysis of this group of pesticides because it mostly leads to the conversion of all toxicologically important residues into one analytical form. However, this approach is unsuitable for multiclass pesticide residue analysis because some other analytes will be destroyed using such strong oxidation agents.

3.2.4. α-Cyano substituted pyrethroids

The α-cyano substituted pyrethroid pesticides with a cyclopropane ring, such as cyfluthrin, cypermethrin, λ-cyhalothrin, and deltamethrin, contain three asymmetric carbon atoms (chiral centers) in their molecules (as shown in Fig. 8) and, therefore, may potentially exist as eight stereoisomers, consisting of four diastereoisomeric pairs of enantiomers [21]. Unlike enantiomers (mirror images),



Pyrethroid	R1	R2	R3	R4
Cyfluthrin	Cl	Cl	CN	F
Cypermethrin	Cl	Cl	CN	H
Cyhalothrin	Cl	CF_3	CN	H
Deltamethrin	Br	Br	CN	H
Permethrin	Cl	Cl	H	H

Fig. 8. Structures of selected synthetic pyrethroid pesticides.

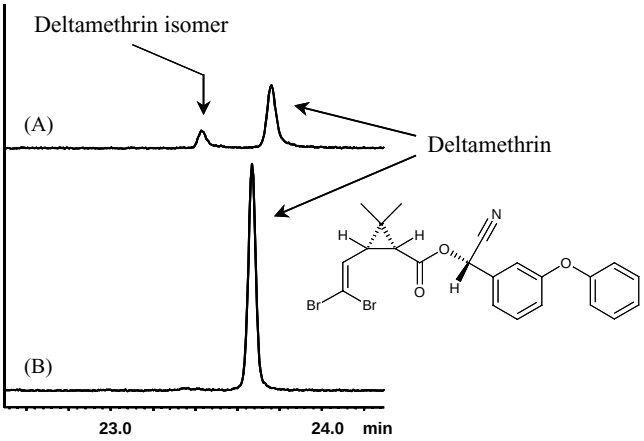


Fig. 9. Chromatograms of deltamethrin [(*S*)-α-cyano-3-phenoxybenzyl (1*R*)-*cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate] injected in the same MeCN 1 μg/mL solution (from the same vial): (A) before and (B) after the GC system maintenance.

diastereoisomers can be separated based on their different physico-chemical properties using conventional GC columns. The application forms of cyfluthrin and cypermethrin usually comprise all four diastereoisomers, thus giving four peaks in a conventional GC analysis [35]. On the other hand, only one peak is expected in the case of λ-cyhalothrin that consists of two *cis* entantiomers: (*S*)-α-, (1*R*)-*cis*- and (*R*)-α-, (1*S*)-*cis*-isomers; and deltamethrin, which is a single enantiomer, the most effective (*S*)-α-, (1*R*)-*cis*-isomer (see the structure in Fig. 9). During our experiments, however, when deltamethrin was injected in MeCN or acetone, a second peak appeared with the same MS spectrum (presumably a deltamethrin diastereoisomer), eluting with a relative $t_R = 0.988$ versus the original deltamethrin peak. An analogical effect was also observed in the case of λ-cyhalothrin, with a relative t_R of the second peak being 0.992 at the same GC conditions.

The extent of the conversion of deltamethrin to its isomer was rather variable. In some cases, the second peak was not detected at the beginning of the sequence and its concentration increased over time (generally with a peak area ratio up to ≈30:70 for new versus original deltamethrin peaks). This may suggest that the formation occurred in solutions, but in other cases, the isomer was present even in the chromatograms of freshly prepared pesticide solutions, which indicates that the conversion more likely occurred during the GC injection process. We hypothesized that the formation of the second deltamethrin peak depended on the activity of the GC system (usually increasing with the number of the GC injections), and we proved this by injecting the same MeCN solution containing deltamethrin before and immediately after GC system maintenance (the liner was replaced and the front part of the GC column cut; no MS tuning was performed). As Fig. 9 shows, about a 20:80 peak ratio was recorded prior to the system maintenance, whereas practically no isomer was detected in the "clean" system (also

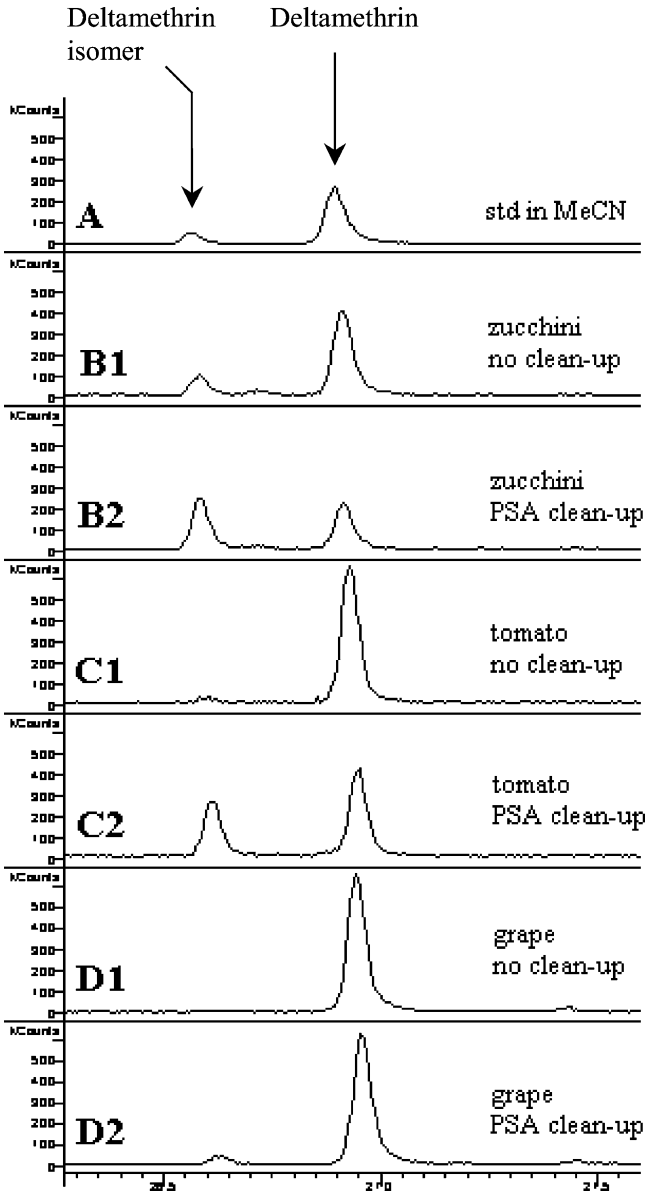


Fig. 10. Chromatograms of deltamethrin and its isomer injected in: (A) MeCN standard solution (0.5 μg/mL) and immediately after spiking (B) zucchini, (C) tomato, and (D) grape extracts: (1) without clean-up and (2) after PSA clean-up at the same concentration level.

note the significant improvement in deltamethrin response). After several injections, the situation returned to the one before system maintenance, even though no matrix injections were made.

The acidification of both MeCN and acetone solutions with HAc helped reduce (or sometimes eliminate) the formation of the deltamethrin isomer. The protective effect of acidic compounds (co-extractives) is demonstrated in Fig. 10. This figure shows chromatograms of deltamethrin and its isomer recorded immediately after spiking blank zucchini, tomato and grape extracts in MeCN, which were obtained by the QuEChERS sample preparation procedure with and without the dispersive-SPE clean-up using PSA. Unlike previous experiments, the ion trap GC–MS LVI with Carbofrit were used in this case as described in Section 2. The tomato and grape extracts were more acidic than the zucchini extract due to the naturally high content of organic acids, which are removed to a great extent by the PSA sorbent [1]. No isomer peak was observed in the spiked grape extract, and only a minute amount (5:95 peak ratio) was recorded in the chromatogram of the same extract treated by PSA. Similarly, virtually no isomer appeared in the chromatogram of the tomato extract without PSA clean-up, however, ≈38% of deltamethrin was converted into its isomer in the case of the cleaned-up sample. The same situation was also observed in the chromatograms of the same samples analyzed after 24 h at room temperature. The injection of deltamethrin in the less acidic zucchini extracts resulted in formation of the second peak with peak ratios of 19:81 and 53:47 without and with PSA clean-up, respectively. The following conclusions from these experiments were made: (i) practically no conversion was observed when deltamethrin was injected in highly acidic matrices; and (ii) the PSA clean-up removed components that suppressed the isomerization process.

In the cases of cyfluthrin and cypermethrin, the peak areas of all four diastereoisomers are usually summed for quantitation purposes [35], thus potential isomerization (a change of peak ratios) is not critical. We summed isomer peak areas for deltamethrin and λ-cyhalothrin and achieved good linearity.

The α-cyano substituted pyrethroids can be converted to their diastereoisomers either by isomerization (racemization) at the α-position or by *cis–trans* isomerization (recombination on the cyclopropane ring), which is the main photodegradation pathway for deltamethrin exposed to ultraviolet or sunlight radiation [21,36]. During our experiments, we did not observe changes in the ratio of *cis* and *trans* isomers of permethrin (a pyrethroid without the cyano substituent on α-C, see Fig. 8), which would suggest the former process resulting in the formation of the (*R*)-α-, (1*R*)-*cis*-isomer. However, we also cannot rule out the *cis–trans* isomerization (formation of *trans*-diastereoisomers), because permethrin undergoes the *cis–trans* isomerization less readily than deltamethrin (or at least when irradiated [36]), and vapor pressure information for cyfluthrin diastereoisomers [21] indicates that all three potentially formed deltamethrin and λ-cyhalothrin diastereoisomers should be more volatile than the (*S*)-α-, (1*R*)-*cis*- and (*R*)-α-, (1*S*)-*cis*-enantiomers (corresponding to shorter t_R in GC), which is the case that we observed.

3.3. Suitability of organic solvents for GC multiresidue analysis

As outlined in Section 1, an ideal solvent serving as a medium for the GC injection should allow optimum sample introduction and not adversely affect separation and detection of the analytes. Considering that pesticide residues

are usually less volatile than the discussed solvents, direct interferences in the GC separation and/or detection are less likely to occur (although the sample introduction in MeCN in combination with a nitrogen-phosphorus detector may be problematic). Also, the use of bonded, cross-linked stationary phases does not restrict the solvent choice, enabling injections in more polar solvents (even water) without the risk of the phase damage [37]. Thus, the sample introduction step becomes the main critical point in our evaluation.

In splitless injection, solvent liquid-gas expansion (vapor) volume dictates the maximum injection volume at any given set of conditions (temperature, pressure and liner volume). Therefore, the solvent expansion volume should be as small as possible to allow high injection volumes without a risk of liner overflow, which provides high sensitivity without a potential for inlet contamination, sample discrimination and/or a reduction of reproducibility. Table 1 gives a comparison of vapor volumes generated by 1 μL injections of the discussed solvents at the optimized conditions used for GC analyses in this study. For the given conditions, this table also provides maximum volumes of the solvents that can be safely injected into the 800 μL liner, with the values decreasing in the following order: isooctane > hexane > toluene > EtAc > acetone > MeCN. The injection volumes can be increased by applying a pressure pulse during the injection to reduce the expansion volume (and analyte residence time in the injection port), thereby suppressing adsorption and/or degradation of susceptible analytes [38,39].

In LVI with a programmed temperature vaporizing (PTV) injector, it is desired to trap the solvent in the liner at acceptable high temperatures (to avoid a need for excessive cooling), but more importantly, to be able to eliminate the majority of the solvent by venting without losing the most volatile analytes. Therefore, the boiling point of the solvent should be sufficiently low and the suitability of the discussed solvents for this kind of injection decreases in the following order: acetone > hexane > EtAc > MeCN > isooctane > toluene (see Table 1).

In non-splitting injection techniques, effective focusing is essential to minimize band broadening and peak distortion. In this respect, t_i plays an important role in both solvent and cold trapping mechanisms. In terms of the sample throughput, t_i should be as high as possible to minimize cool-down times between the GC runs. In this study, we experimentally determined optimal t_i for the analysis of the 31 selected pesticides (representing the volatility range of GC-amenable pesticides from dichlorvos to deltamethrin) by monitoring analyte peak heights and shapes in each tested solvent at different t_i (all other conditions remained the same). Table 1 lists the determined optimal values that decrease in the following order: toluene > isooctane ≈ MeCN > EtAc >

Table 2
Average relative responses (100% = normalized peak area in toluene) of stable analytes obtained during 24 h in Experiment 2 ($n = 6$, R.S.D.s < 10%); values ≥115% are in bold and values <85% are also underlined

Pesticide	*m/z*	Relative normalized response vs. normalized response in toluene (%)							
		Tol	Hex	Iso	EtAc	Ace	Ace/HAc	MeCN	MeCN/HAc
Methamidophos	141	100	**56**	**51**	**129**	**121**	**129**	101	**129**
Dichlorvos	185	100	98	87	100	103	104	96	98
Meviphos	192	100	106	89	106	107	110	99	106
Acephate	136	100	**31**	**44**	113	**121**	**117**	**116**	**137**
o-Phenylphenol	170	100	95	91	99	103	93	99	101
Omethoate	156	100	**56**	**60**	**123**	111	**115**	108	**117**
Dimethoate	87	100	97	87	104	104	103	94	105
Lindane	181	100	99	94	96	102	102	96	95
Diazinon	179	100	100	98	99	100	100	99	99
Vinclozolin	285	100	107	113	101	103	102	99	99
Carbaryl	144	100	95	103	112	105	**120**	100	**118**
Metalaxyl	206	100	104	104	102	102	104	99	102
Pirimiphos-methyl	290	100	101	103	101	99	99	101	101
Methiocarb	168	100	97	101	100	103	**116**	102	**115**
Dichlofluanid	224	100	102	98	98	101	100	92	96
Chlorpyrifos	197	100	99	100	100	100	101	100	99
Cyprodinil	224	100	104	103	103	101	101	102	101
Thiabendazole	201	100	**69**	**77**	105	100	103	107	108
Captan	149	100	108	95	96	91	96	88	92
Folpet	260	100	103	90	93	91	94	86	93
Imazalil	215	100	**70**	**75**	110	109	111	113	**115**
Endosulfan sulfate	272	100	107	99	101	102	106	102	102
Iprodione	316	100	101	104	103	97	104	105	102
Phosalone	367	100	101	103	101	95	102	102	98
Azinphos-methyl	132	100	95	100	95	86	94	96	94
Permethrins	183	100	104	**131**	99	95	101	111	98
Coumaphos	362	100	94	103	95	88	92	99	93

acetone > hexane. Thus, the use of toluene can significantly increase the sample throughput in this case.

The above discussion indicates that the selection of an optimal solvent for the GC introduction depends on several factors, one of them being the employed GC injection technique. For instance, isooctane is superior to other discussed solvents in terms of its small expansion volume, but it is not the best choice for a PTV injection with solvent venting. Also, a higher injection volume (higher sensitivity) does not necessary translate into lower detection limits in the analysis of real-world samples because co-extracted matrix components may represent the limiting source of noise [40].

Another important factor is the actual analyte response obtained in different solvents. Table 2 gives normalized responses of stable pesticides in the tested solvents expressed relatively versus the normalized responses in toluene. Significantly lower normalized responses of acephate, methamidophos, omethoate, thiabendazole and imazalil were obtained in hexane and isooctane compared to the other solvents. These relatively polar pesticides are notorious for interactions with the active sites in the GC system resulting in their losses and peak tailing [18]; therefore, they usually constitute the weakest links in multiresidue GC analysis. Although the solubility of these problematic pesticides is lower in hexane and isooctane than in more polar solvents (see Table 1 for polarity indexes), it should not be an issue at 0.5 μg/mL (e.g. solubility in hexane at 20 °C is 0.1 g/L for acephate, 0.1–1 g/L for methamidophos, and 19 g/L for imazalil [21]).

Even though solubility per se may not be the factor, the solvent polarity still plays a significant role in the phenomenon documented in Table 2 because adsorption of some relatively polar pesticides in the syringe may occur when a less polar solvent is used as an injection medium [41]. To confirm this possibility, 1 μL of the mixture of 31 pesticides (at 1 μg/mL) was injected in hexane. Then, the syringe was flushed three times with hexane and 1 μL aliquot of acetone was drawn into the syringe and injected manually using air gap injection technique. As a result of this injection, peaks representing the missing amounts (as compared to the 1 μL injection of the mixture of 31 pesticides at 1 μg/mL in acetone) of methamidophos, acephate, omethoate, thiabendazole, and imazalil were recorded in the chromatogram. To prevent a potential carry-over of these analytes in all our experiments, the syringe was rinsed four times with acetone between the injections performed by the autosampler.

Slightly improved responses of carbaryl and methiocarb (carbamate pesticides susceptible to thermodegradation in the injection port) and certain other problematic pesticides in the presence of HAc (comparison of normalized responses in acetone and MeCN solutions with and without HAc in Table 2) may also be explained by a protecting effect of HAc, which can interact with the active sites in the injection port and front part of the column *via* hydrogen bonding [18].

4. Conclusions

In this study, we evaluated the suitability of three extraction and three exchange solvents for GC analysis of multiclass pesticide residues with respect to comprehensive needs. It is preferable to avoid a solvent exchange step, thus MeCN, acetone, and EtAc have strong benefits in this regard. Among the three extraction solvents, acetone is the least suitable for isolation of multiclass pesticide residues from produce samples, and MeCN offers advantages in extraction selectivity and compatibility with more diverse analytical techniques compared to EtAc. Selection of an optimal solvent for GC analysis depends on several factors including the type of GC injection technique and the range of analytes. The exchange solvents generally enable higher injection volumes in the splitless mode, but, due to the adsorption in the syringe, the injection of more polar pesticides in hexane and isooctane resulted in lower relative responses of these analytes as compared to other solvents.

In terms of stability of the tested pesticides, the exchange solvents proved to be superior to the more polar extraction solvents. Table 3 summarizes the stability issues identified in this study along with the possible sources of the problems in the respective solvents. Degradation of *N*-trihalomethylthio fungicides in MeCN was associated only with certain lots of the tested MeCN, but even if it occurred, the stability of these analytes as well as of dicofol and chlorothalonil was dramatically improved by addition of 0.1% HAc (v/v) to MeCN. Dicofol and chlorothalonil were also unstable in acetone, but the addition of 0.1% HAc

Table 3
Problematic pesticide-solvent combinations identified in the presented study

Pesticide(s)	Solvent(s)	Factor(s)
N-Trihalomethylthio pesticides (dichlofluanid, tolylfluanid, folpet, captan, and captafol)	MeCN	MeCN lot, pH
Dicofol	Acetone, MeCN	pH, light
Chlorothalonil	Acetone	pH, light
	MeCN	MeCN lot, pH, light
Pesticides with a thioether group (fenthion, phorate, disulfoton, etc.)	EtAc	Content of acetaldehyde?
	Acetone	Light
α-Cyano substituted pyrethroids (deltamethrin, λ-cyhalothrin)	Acetone, MeCN	Activity of the GC system, pH

did not sufficiently suppress degradation in this case. Pesticides with a thioether group degraded in the tested EtAc as did disulfoton in acetone. Formation of deltamethrin and λ-cyhalothrin isomers was recorded in the chromatograms from MeCN and acetone solutions, but this was shown to occur during the GC injection process rather than in the solutions.

To conclude, MeCN is the most suitable solvent for extraction of a wide polarity range of pesticide residues from produce. After acidification, the stability of problematic pesticides in MeCN is acceptable, and MeCN can also serve as an adequate medium for the GC injection, therefore solvent exchange is necessarily not required prior to GC analysis. If sensitivity is an issue in splitless injection, then toluene is judged to be the best exchange solvent due to its miscibility with MeCN and good responses of troublesome pesticides. In addition to these factors, excellent stability of dissolved pesticides, medium polarity (good solubility of wide range of pesticides), and very low volatility of toluene make this solvent also highly suitable for preparation and long-term storage of pesticide stock solutions.

Acknowledgements

This work was supported in part by Research Grant Award No. US-3500-03 from BARD, the United States—Israel Binational Agricultural Research and Development Fund. The authors thank Michelangelo Anastassiades (Chemisches und Veterinäruntersuchungsamt Stuttgart, Germany) and Darinka Štajnbaher (Public Health Institute, Slovenia) for fruitful discussions.

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