ELSEVIER

Journal of Chromatography A, 800 (1998) 283–295

JOURNAL OF CHROMATOGRAPHY A

# Matrix-induced effects: a critical point in the gas chromatographic analysis of pesticide residues

J. Hajšlová[a,*], K. Holadová[a], V. Kocourek[a], J. Poustka[a], M. Godula[a], Petr Cuhra[b], Milan Kempný[b]

[a]*Institute of Chemical Technology, Department of Food Chemistry and Analysis, Technická 3, 166 28 Prague 6, Czech Republic*
[b]*Czech Agriculture and Food Inspection, Pobřežní 10, 186 00 Prague 8, Czech Republic*

Received 29 August 1997; received in revised form 7 November 1997; accepted 11 November 1997

**Abstract**

The influence of several experimental factors related to the enhanced gas chromatographic responses yielding apparent recoveries of pesticide residues greater than 100% was investigated. Optimisation of a gel permeation chromatographic clean-up step with respect to the trueness and precision of generated data was performed. An increase of relative detector response (100%=response of analyte in pure solvent solution) was evidenced to be dependent both on the concentration of the analyte and the character of the matrix: pronounced matrix-induced effects were observed particularly in orange and wheat extracts at low concentration levels of analytes (especially for GC–electron-capture detection analysis of certain pesticides). As soon as the splitless injector became contaminated after injection of large series of matrix-containing samples, a decrease of relative responses of pesticides, largely below 100%, was experienced. Although troublesome compounds tending to give matrix-induced effects can be identified, and increased recoveries may be tentatively predicted, poor accuracy of generated data can be presumed as long as quantitation is not based on a standard prepared in blank matrix extract to compensate for matrix-induced effects. © 1998 Elsevier Science B.V.

*Keywords:* Matrix effects; Fruits; Vegetables; Food analysis; Pesticides

## 1. Introduction

Similarly to other areas in organic trace analysis, results reported for pesticide residues in biotic matrices may be significantly influenced by the manner in which the samples are processed prior to the determinative (mostly chromatographic) step. Multiresidue methods represent an effective way to screen a large number of samples for multiple pesticides in a relatively short period. However, due to the broad range of physicochemical properties of target analytes, obtaining optimum recoveries for all of them is practically impossible.

Extensive clean-up of extracts may result in the partial loss of some compounds, as well as increased labour and cost demands, but inadequate clean-up can lead to adverse effects related to the quality of generated data, such as: (i) the masking of residue peaks by coeluted matrix components, (ii) occurrence of false positives and (iii) inaccurate quantitation [1]. Problems due to the presence of impurities in analysed sample can be encountered both at the detector and injector site. In the latter case, increased transfer of sample components to the gas chromato-

*Corresponding author.

0021-9673/98/$19.00 
*PII* S0021-9673(97)01145-X

graphic column may occur due to the blocking of active sites within the injector by sample matrix, thus preventing thermal degradation/adsorption of analytes [2]. These phenomena known as ''matrix-induced chromatographic response enhancement'' allow for explanation of recoveries largely exceeding 100% which are reported for some pesticides in studies utilising calibration standards dissolved in neat solvent. Based on the literature survey, pesticides giving particularly high recoveries can be identified, see Table 1. The extent of these matrix effects is related to both the chemical structure and the type of matrix.

Although the existence of matrix effects is well recognised by experienced analysts, only a limited amount of papers are concerned with this topic from a theoretical point of view. One of the most thorough studies in this field was conducted by Erney et al. [2] who investigated the influence of injection conditions on flame photometric detection (FPD) response of organophosphorus pesticides. In another report [3], minimisation of matrix effects by addition of single compound additives was explored by these authors. In their most recent study [4] concerned with matrix-induced chromatographic enhancement, general guidelines for using matrix-standard calibrations solutions in pesticide residue analysis are presented. Improved accuracy of results achieved for a range of pesticides by the use of matrix matched standards was documented by Johnson et al. [5].

This study was intended to further investigate the matrix-induced effects in relation to diverse parameters of a multiresidue procedure. In our experiments, 37 common pesticides frequently reported in monitoring studies were analysed in wheat, orange and cabbage using gas chromatography (GC) with electron-capture detection (ECD) and GC with nitrogen–phosphorous detection (NPD) in a series of experiments to investigate the effects of matrix in quantitation.

## 2. Experimental

### 2.1. Chemicals and materials

Pesticide standards, purity >95%, were obtained from Dr. Ehrenstorfer (Germany). Stock solutions were prepared in toluene. Concentrations of analytes in ''ECD'' mixture (Sa) ranged from 10.8 to 101.0 μg/ml and in ''NPD'' mixture (Sb) from 4.4 to 95.5 μg/ml, respectively; differences in detector response of particular analytes were considered what is reflected in their relatively wide concentration range. The list of investigated pesticides along with some of their physicochemical characteristics [15] is shown in Table 2. All solvents used were of pesticide grade (Merck, Germany).

Oranges, wheat grains, cabbage and other fruits/vegetables were obtained at retail market. The absence of examined residues was checked by GC–mass spectrometry (MS) screening of respective extracts.

### 2.2. Apparatus

An ASPEC XL system (Gilson, France) equipped with PL gel (600×7.5 mm; 50 Å) high-performance

Table 1
Pesticides tending to give high recoveries – literature survey of recent studies (1988–1997) employing capillary GC with splitless injection

| Pesticide | Maximum reported value of recovery in %[a] | Studies reporting recoveries >100%/studies involving respective analyte | Refs. (list of studies reporting recoveries >100%) |
|---|---|---|---|
| Acephate | 183 | 6/6 | [3,4,6–8] |
| Methamidophos | 194 | 6/6 | [4,6–8] |
| Omethoate | 178 | 3/3 | [2,6,8] |
| Iprodione | 230 | 5/5 | [8–12] |
| Dimethoate | 244 | 8/14 | [3,4,7,8,10–14] |
| Malathion | 169 | 6/11 | [3,8–14] |
| Methidation | 165 | 5/10 | [7,8,11–13] |

[a] No correction for matrix effects.

Table 2
Investigated pesticides

| "ECD" mixture (Sa) | *c* (μg/ml) | w.s. (mg/l) | log $K_{ow}$ | "NPD" mixture (Sb) | *c* (μg/ml) | w.s. (mg/l) | log $K_{ow}$ |
|---|---|---|---|---|---|---|---|
| Brompropylate | 28.3 | 0.5 | 5.4 | Bupirimate | 79.6 | 22 | 3.9 |
| Captan | 52.9 | 3.3 | 2.78 | Chlorfenvinphos | 12.2 | 145 | 3.85 |
| Chlorothalonil | 10.8 | 0.9 | 6.8 | Chlorpropham | 92.6 | 89 | 3.06 |
| λ-Cyhalotrin | 59.0 | 0.004 | 6.6 | Chlorpyrifos | 5.4 | 1.4 | 4.69 |
| Cypermethrin | 91.7 | 0.004 | 4.6 | Chlorpyrifos-methyl | 6.3 | 4 | 4.23 |
| Deltamethrin | 50.4 | 0.0002 | 3.69 | Diazinon | 5.4 | 60 | 3.3 |
| Dichlofluanid | 57.8 | 1.3 | 4.74 | Dichlorvos | 6.2 | 8000 | 1.9 |
| α-Endosulfan | 29.2 | 0.32 | 4.79 | Ethion | 5.8 | 2 | 5.07 |
| β-Endosulfan | 20.7 | 0.32 | 4.79 | Fenitrothion | 4.6 | 21 | 3.43 |
| Endosulfane $SO_4$ | 23.0 | 0.01 | 5.01 | Heptenophos | 3.8 | 2200 | 2.32 |
| Fenvalerate | 82.0 | 0.0009 | 2.89 | Imazalil | 95.5 | 160 | 3.82 |
| Iprodione | 34.2 | 13.0 | 2.89 | Metalaxyl | 82.0 | 8400 | 1.75 |
| Lindane | 14.1 | 7.3 | 3.72 | Methamidophos | 49.4 | 200 000 | −0.8 |
| Permethrin | 101.0 | 0.2 | 6.1 | Methidathion | 4.4 | 200 | 2.2 |
| Procymidone | 19.5 | 4.5 | 3.14 | *cis*-Mevinphos | 6.7 | x | 1.34 |
| Tolylfluanid | 24.9 | 0.9 | 3.95 | *trans*-Mevinphos | 5.2 | x | 1.34 |
| Vinclozolin | 16.5 | 3.4 | 3 | Parathion | 4.9 | 11 | 3.83 |
| | | | | Parathion-methyl | 6.5 | 55 | 3.0 |
| | | | | Phosalone | 23.9 | 1.7 | 4.3 |
| | | | | Pirimicarb | 30.2 | 3000 | 1.7 |
| | | | | Pirimiphos-methyl | 6.2 | 9.9 | 4.2 |

*c*=Concentration of stock solutions (standards in toluene); w.s.=water solubility (20°C); log $K_{ow}$=log of *n*-octanol–water partition coefficient; x=not specified, good water solubility declared.

column (Pl Labs., UK) was utilised for automated clean-up by high-performance gel permeation chromatography (HPGPC). A Büchi rotary evaporator was used whenever evaporation of bulk solvents was needed. A HP 6890 gas chromatograph (Hewlett-Packard, USA) equipped with NPD/ECD, split/splitless injector, electronic pressure control (EPC) and autosampler was employed for GC analyses. The separation of sample components was performed using a DB-5 MS (60 m×0.25 mm, 0.25 μm) capillary column. An Ultra Turrax homogenizer was used for sample disintegration.

### *2.3. Analytical procedure*

#### *2.3.1. Crude extract preparation*

Fifty g of representative sample (homogenate) were blended for 5 min (10 000 rpm) with 250 ml ethyl acetate and 100 g anhydrous sodium sulphate. The suspension was filtered through the layer of sodium sulphate (20 g) and the filtrate was concentrated by rotary evaporation to ca. 50 ml. The volume of sample was then adjusted by cyclohexane to 100 ml (in volumetric flask).

#### *2.3.2. Clean-up*

A 2-ml aliquot of crude extract (i.e., equivalent of 1 g of original matrix) was loaded onto HPGPC column. The flow-rate of mobile phase (cyclohexane–ethyl acetate, 1:1, v/v) was 1 ml/min, dump time 16 min, collect time 14 min (i.e., elution volume of "pesticide fraction" was in the range 16–30 ml). Eluate (14 ml) was evaporated next to the dryness using a rotary evaporator, remaining solvent was blown down by a gentle stream of nitrogen and the remainder was redissolved in 1 ml of toluene prior to GC analysis. For determination of elution profiles of plant pigments, on-line diode array detection (DAD) was used, refractive index (RI) detection was applied for recording of waxes elution. Elution profiles of pesticides (1 ml of stock solutions Sa or Sb loaded onto column) were measured by GC analysis of particular 1-ml fractions after solvent exchange to toluene.

*2.3.3. GC identification/quantitation*

One μl of purified extract in toluene (corresponding to 1 mg of original matrix) was analysed under the following conditions: injection: splitless, purge off 120 s; injector temperature: 250°C; oven temperature: (i) for ''NPD'' compounds: 90°C (2 min), 3°C/min to 270°C, held 8 min, (ii) for ''ECD'' compounds: 90°C (2 min), 10°C/min to 200°C, 2.5°C/min to 280°C, held 20 min; carrier gas: helium, constant flow: 0.8 ml/min.

*2.4. Sample spike preparation*

The ''pesticide fraction'' obtained by HPGPC clean-up of crude extract (obtained from respective residue-free commodity), was evaporated to dryness using a mild stream of nitrogen. The residue was then dissolved in 10 ml ethyl acetate. To prepare ''spiked sample'', a 5-ml aliquot was evaporated carefully and the remainder was dissolved in a 0.5-ml toluene solution of pesticides (working solutions). Stock solutions shown in Table 2 were diluted for this purpose as follows: ''ECD'' mixture (Sa): 1000× (=$Sa_1$) and 100× (=$Sa_2$), ''NPD'' mixture (Sb): 250× (=$Sb_1$) and 25× (=$Sb_2$). Blank sample was prepared in a similar way: 4 ml of purified ethyl acetate extract were evaporated to dryness by rotary evaporation and the residue was dissolved in 0.4 ml of toluene.

## 3. Results and discussion

To assess the performance of an analytical method, several criteria have to be considered before the method is employed in routine practice. At concentrations 5-times the limit of determination (LOD), pesticide recoveries should be 70–110% range with relative standard deviations (R.S.D.s) <20% [16].

Besides extraction efficiency of residues from matrix, the performance characteristics of clean-up step are closely related to the quality of generated data. The absence of matrix components in purified sample and thus elimination of matrix-induced effects is a theoretical solution that can be hardly achieved in practice as long as recoveries of many analytes of interest are not significantly reduced as a result of multistep (and commonly very laborious) purification procedure. HPGPC applied in our experiments as a single clean-up technique provided good separation of bulk plant coextracts (represented namely by pigments and cuticular waxes) from most of examined pesticides with exception of synthetic pyrethroids (these compounds have relatively high molecular masses – over 400 g/mol), elution bands some of which were rather overlapped with matrix components, see Table 3. To avoid insufficient extent of sample clean-up, a compromise was accepted consisting in starting to collect narrower ''pesticide fraction'' i.e., from 16 ml instead of 15 ml when the first analyte – λ-cyhalothrin appears in the eluate. Under such conditions (experiments employing pure standards), the recovery of this pyrethroid was only 62%, reduced recoveries 91% and 95% were also obtained for cypermethrin and fenvalerate, respectively. HPGPC recoveries for all other pesticides were in 92–103% range. Comparison of chromatograms obtained by GC analysis of real, matrix containing samples clearly documented justification of our approach. Many interfering coextracts in broader ''pesticide fraction'' were detected, especially when ECD was used for detection. Consequences of less thorough clean-up are illustrated in Fig. 1 showing GC–ECD chromatograms of orange extracts spiked at lower concentration level ($Sa_1$). Coextracts were eluted over the whole range of retention times often obscuring peaks of analytes (see for instance early eluting pesticides and/or pyrethroids at high retention times). A new splitless injection liner as well as new DB-5 MS capillary were installed prior to recording these chromatograms. Captan was not detected initially, probably due to its decomposition or interaction with active sites in the injection liner, but as soon as the injection port became more contaminated after repeated injections, peak of this pesticide was recorded, although, the repeatability of analyses was poor.

Figs. 2 and 3 display plotted ratios of peak areas corresponding to pesticides used for spiking of both narrower (i.e., cleaner) and broader (dirtier) HPGPC ''pesticide fraction''. Values lower than 100% may suggest occurrence of matrix-induced effects. As regards ''ECD'' pesticides, these phenomena were most evident for captan and procymidone in orange extract and for iprodione in all extracts. Among

Table 3
HPGPC elution profiles of typical coextracts and pesticides (expressed as % of pesticide determined in particular fraction related to the total amount loaded onto column) involved in experiments

| Components | Elution volume (ml) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Coextracts | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 |
| Carotenoids | | B | D | G | E | D | B | | | | | | |
| Chlorophylls | | B | C | F | F | C | A | | | | | | |
| Waxes | | B | C | E | H | D | B | A | | | | | |
| Pesticides | | | | | | | | | | | | | |
| λ-Cyhalotrin | | | | | 38 | 40 | 19 | 3 | | | | | |
| Cypermethrin | | | | | 9 | 16 | 35 | 34 | 6 | | | | |
| Fenvalerate | | | | | 5 | 11 | 46 | 33 | 5 | | | | |
| Deltamethrin | | | | | | | 48 | 48 | 4 | | | | |
| Brompropylate | | | | | | | 33 | 45 | 20 | 2 | | | |
| Permethrin | | | | | | | 33 | 45 | 21 | 1 | | | |
| Diazinon | | | | | | | 24 | 47 | 29 | | | | |
| Vinclozolin | | | | | | | 24 | 45 | 28 | 3 | | | |
| Bupirimate | | | | | | | 23 | 47 | 28 | 2 | | | |
| Ethion | | | | | | | 17 | 45 | 34 | 4 | | | |
| Endosulfan-$SO_4$ | | | | | | | 17 | 46 | 33 | 5 | | | |
| Iprodione | | | | | | | 15 | 42 | 36 | 6 | | | |
| Chlorpropham | | | | | | | 12 | 46 | 38 | 5 | | | |
| Tolylfluanid | | | | | | | 10 | 40 | 41 | 9 | | | |
| Procymidone | | | | | | | 6 | 40 | 40 | 14 | | | |
| Dichlofluanid | | | | | | | 4 | 35 | 41 | 17 | 2 | | |
| Chlorpyrifos | | | | | | | | 34 | 51 | 15 | | | |
| Parathion | | | | | | | | 32 | 46 | 22 | | | |
| β-Endosulfan | | | | | | | | 29 | 45 | 24 | 3 | | |
| Pirimiphos-methyl | | | | | | | | 26 | 39 | 14 | 22 | | |
| Phosalone | | | | | | | | 24 | 48 | 27 | | | |
| Chlorfenvinphos | | | | | | | | 17 | 48 | 35 | | | |
| α-Endosulfan | | | | | | | | 12 | 43 | 38 | 7 | | |
| Lindane | | | | | | | | 12 | 43 | 39 | 6 | | |
| Metalaxyl | | | | | | | | 11 | 44 | 39 | 6 | | |
| *trans*-Mevinphos | | | | | | | | | 93 | 7 | | | |
| *cis*-Mevinphos | | | | | | | | | 75 | 25 | | | |
| Fenitrothion | | | | | | | | | 43 | 39 | 17 | | |
| Heptenophos | | | | | | | | | 36 | 46 | 18 | | |
| Parathion-methyl | | | | | | | | | 34 | 41 | 25 | | |
| Chlorpyrifos-methyl | | | | | | | | | 13 | 41 | 39 | 6 | |
| Dichlorvos | | | | | | | | | 8 | 74 | 18 | | |
| Methamidophos | | | | | | | | | 5 | 14 | 32 | 48 | |
| Pirimicarb | | | | | | | | | 4 | 57 | 33 | 5 | |
| Methidathion | | | | | | | | | | 36 | 54 | 10 | |
| Chlorothalonil | | | | | | | | | | | 41 | 45 | 14 |
| Imazalil | | | | | | | | | | | 26 | 51 | 23 |

A=<1%; B=1–5%; C=6–10%; D=11–15%; E=16–20%; F=21–25%; G=26–30%; H=31–35%.

''NPD'' pesticides the lowest ratio was observed for heptenophos and imazalil in cabbage extracts. Values exceeding 100%, see for instance imazalil in orange extract, can also be attributed, besides some analytical error, to a decomposition of analytes in less thoroughly cleaned extract counting more matrix components. The consequences of abundant coextracts in terms of matrix effects were documented by Andersson et al. [17] who used very similar method to that applied in our study (i.e., ethyl acetate

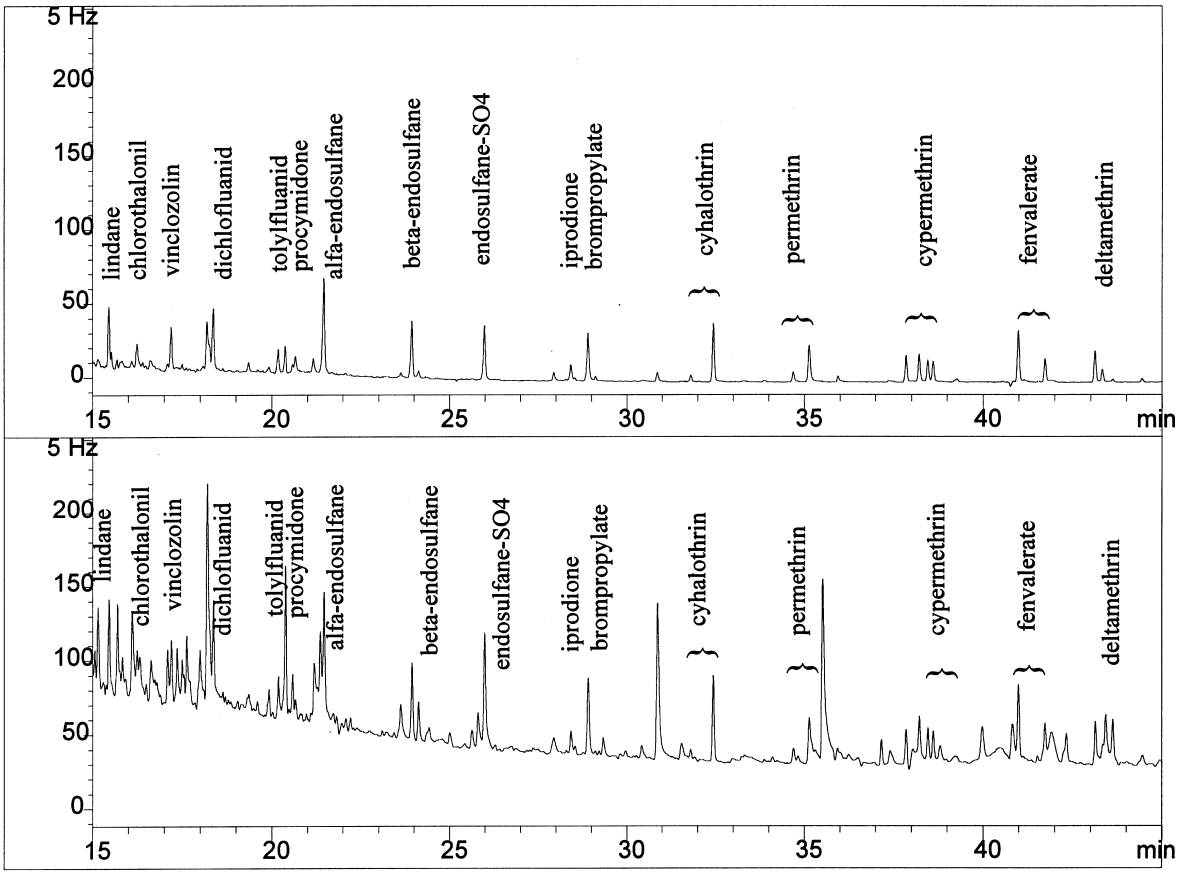


Fig. 1. GC–ECD chromatograms illustrating the effect of crude extract clean-up (orange, spiking level Sa1); upper: narrower ''pesticide fraction'' collected from 16 to 30 ml, bottom trace: broader ''pesticide fraction'' (collected from 15 to 30 ml).

extraction followed by GPC clean-up and GC–FPD/NPD/ECD for quantitation). Because of the need to extend the list of pesticides embraced in their multiresidue method, a change in the volume of collected GPC fraction (insisting in setting earlier ''start collect point'' to avoid the loss of early eluted analytes) was adopted. In ''normal'' matrix concentrations, 46% of the pesticides tested gave more than 110% enhanced response; propiconazol, iprodione, captan and acephate were amongst the worst. Contrary to our experience, matrix effects were mostly independent of commodity in quoted study.

In our following experiments, purified extracts of tested crops (narrower ''pesticide fractions'') spiked by respective pesticides at two concentration levels ($Sa_1$, $Sa_2$; $Sb_1$ and $Sb_2$, see Section 2.4) were analysed by GC to assess the dependence of matrix-induced effects on various experimental factors. Table 4 illustrates relative ECD responses (100%= response of standard in pure solvent) of pesticide residues in three different matrix solutions. Distinct matrix-induced effects could be seen especially in case of wheat and orange extracts at lower ($Sa_1$) spiking levels; apparent recovery of chlorothalonil in orange extract exceeded even 200% (this compound was identified as troublesome also by Lee at al. [7] who encountered poor precision of chlorothalonil determination in various fruits and vegetables). Less pronounced increase of relative responses was observed in cabbage extract, probably because of fewer coextracts contained in purified sample. More accurate results obtained for ''NPD'' pesticides, see Table 5, may be attributed to higher concentration levels of

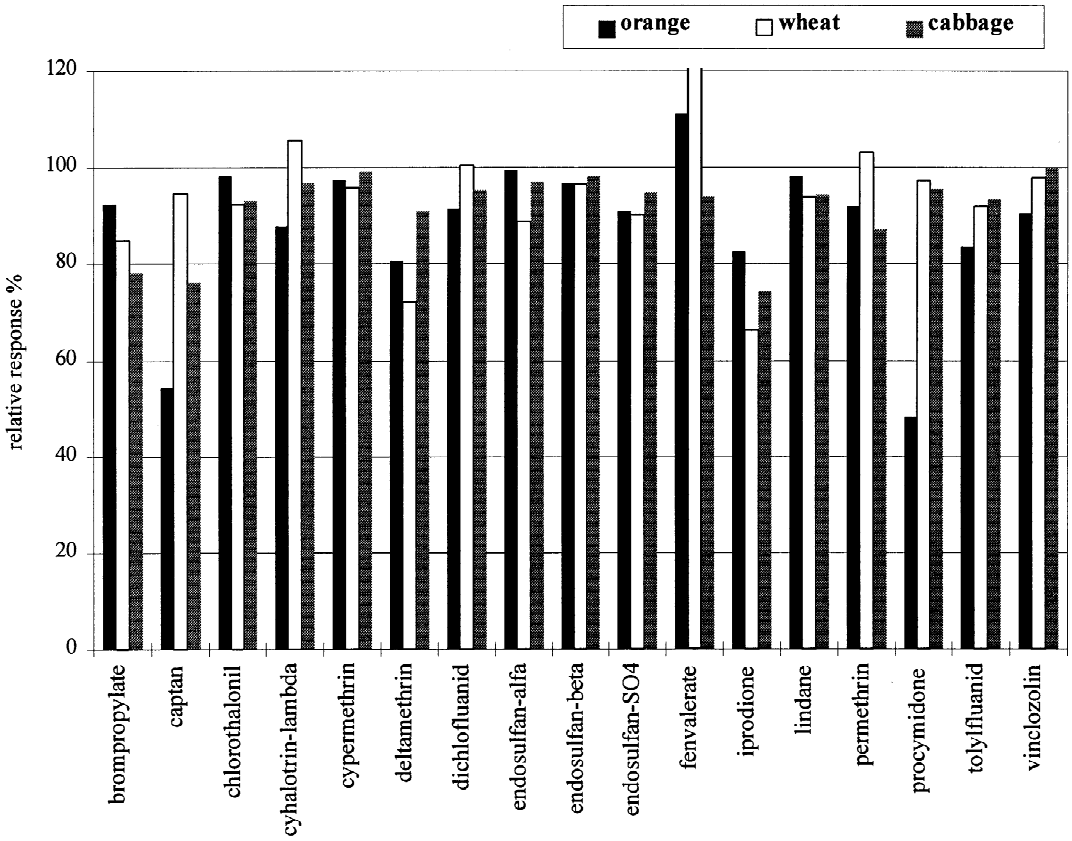


Fig. 2. Effect of the extent of sample clean-up expressed as relative ratio of ''ECD'' pesticides responses (peak areas) (A1/A2)·100; A1=''pesticide fraction'' collected from 16 to 30 ml, A2=''pesticide fraction'' collected from 15 to 30 ml (A1 and A2 used for calculations are averages of values obtained in two repeated injections of respective fraction).

spikes which had to be adopted in this case due to a higher detection limits achievable by NPD for most of target pesticides. Reduced detector response due to the adsorption losses of analyte (their extent is limited by the amount of active sites) injected in pure solvent was thus relatively less significant. It should be emphasised that comparable conditions in two sets of measurements employing alternatively NPD and ECD were ensured by thorough cleaning injection liner prior to starting the respective GC sequence (three injections of matrix containing samples were followed by one injection of pure solvent and two injections of standards in pure solvent). As aforementioned, low relative responses of imazalil observed in orange extract can be attributed to some decomposition of this compound in the presence of specific matrix components (similarly, low recoveries for imazalil and pirimicarb were observed in our previous experiments with strawberries). The stability of detector responses of pesticide standards in pure solution was recorded during repeated injections realised within GC sequence. In Tables 6 and 7 the repeatabilities (expressed as R.S.D., $n=5$) of these measurements are shown. Relatively poor precision observed in this experiment for some compounds is assumed to be influenced by matrix components progressively deposited in the injection port (significantly better R.S.D.s, not exceeding 5% even for most diluted standard mixtures $Sa_1$ and $Sb_1$, respectively, were recorded when repeated injections were carried out prior to injections of matrix extracts, i.e., when the liner was clean). For many pesticides, the lowest response was observed in the first injection (i.e., into a relatively clean injector). The worst

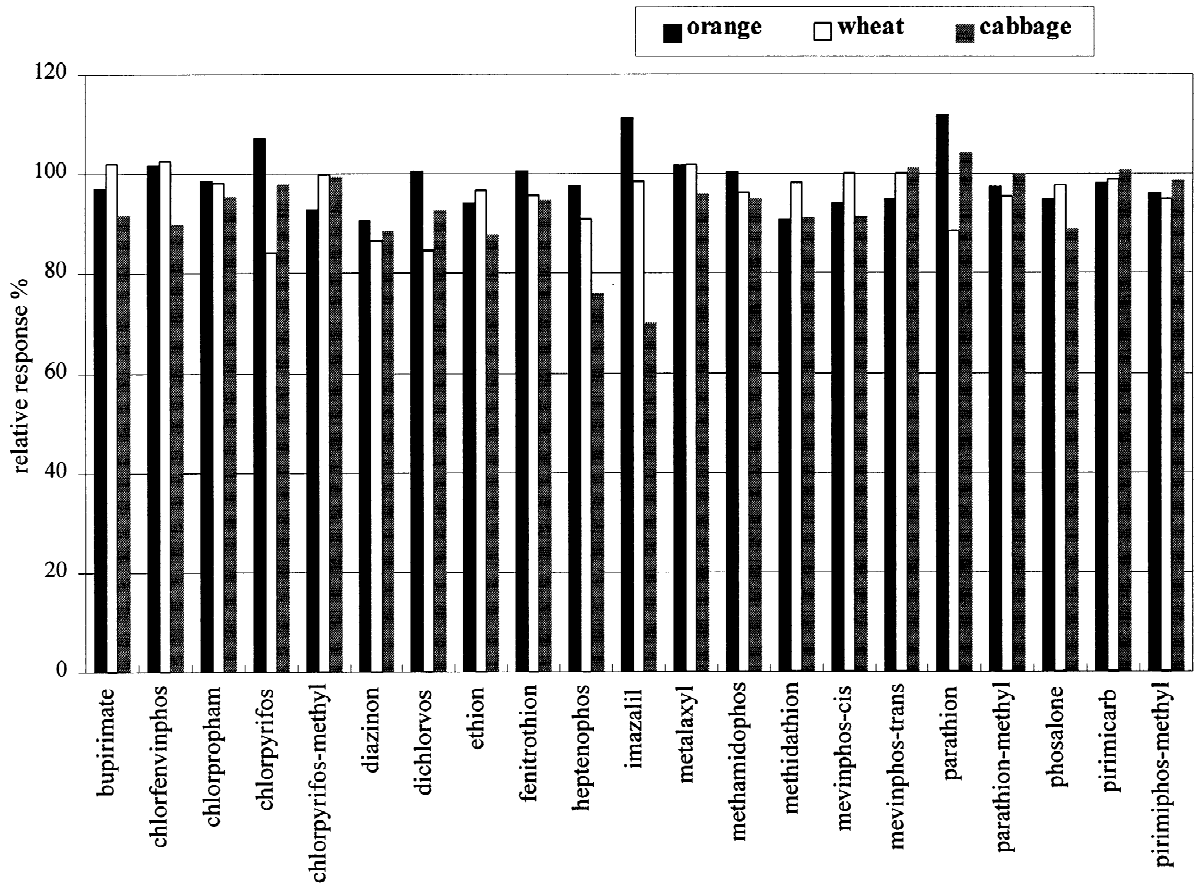


Fig. 3. The effect of the extent of sample clean-up expressed as relative ratio of ''NPD'' pesticides responses (peak areas) (A1/A2)·100; A1=''pesticide fraction'' collected from 16 to 30 ml, A2=''pesticide fraction'' collected from 15 to 30 ml.

fluctuation of detector response was recorded for methamidophos – a troublesome compound with a strong tendency for giving matrix effects. Successive increase of detector response within five injections carried out was experienced for dichlofluanid and tolylfluanid at both concentration levels. These pesticides are known to be easily thermodegraded as long as the masking of active sites in injector port is not sufficient. In any case, instability of some standards responses applies for careful calibration.

To generalise our results, the relationship between some physicochemical properties of pesticides (summarised in Table 2) and observed matrix-induced effects was tested by statistical methods. The highest coefficient for linear correlation, $r=0.647$, was found for relative responses of ''ECD'' pesticides (spiking level $Sa_1$) and their water solubility, see Fig. 4. Weaker linear correlation $r=-0.491$ was calculated for relative responses and log $K_{ow}$ ($n$-octanol–water partition coefficient). It can be speculated that the probability of the matrix effects occurrence is higher for more polar pesticides. These results are in agreement with the literature survey shown in Table 1 as well as with the study by Erney et al. [2] concerned with matrix-induced chromatographic response enhancement of organophosphates. It was noticed, that changes in apparent recovery vary with the chemical structure of the pesticide and those compounds containing P=O bonds such as acephate, methamidophos and/or azodrine were identified as tending to give particularly high recoveries. It should be noted that most of commonly used organophos-

Table 4
Relative responses (R1, R2, %) of ''ECD'' pesticides (100%=the response of corresponding standard in pure solvent) in extracts from several matrices; spiking levels $Sa_1$: 0.011–0.101 mg/kg, $Sa_2$: 0.108–1.01 mg/kg; precision (repeatibility, $n=2$) as relative standard deviation (R.S.D., %)

| Pesticide | Wheat | | | | Orange | | | | Cabbage | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | R1 ($Sa_1$) | R.S.D. | R2 ($Sa_2$) | R.S.D. | R1 ($Sa_1$) | R.S.D. | R2 ($Sa_2$) | R.S.D. | R1 ($Sa_1$) | R.S.D. | R2 ($Sa_2$) | R.S.D. |
| Bromopropylate | 107 | 4 | 99 | 1 | *135* | 7 | 107 | 1 | 100 | 9 | 103 | 2 |
| λ-Cyhalotrin | 107 | 6 | 100 | 0 | *131* | 3 | 104 | 6 | 103 | 11 | 105 | 3 |
| Cypermethrin | *138* | 13 | 108 | 1 | *112* | 2 | 105 | 5 | 107 | 10 | 106 | 2 |
| Deltamethrin | 68 | 4 | 76 | 8 | 0 | ns | *126* | 12 | 0 | ns | *112* | 11 |
| Dichlofluanid | *149* | 6 | *111* | 1 | *131* | 3 | 107 | 4 | 103 | 7 | 101 | 2 |
| α-Endosulfan | *113* | 3 | 102 | 0 | 105 | 3 | 106 | 2 | 92 | 3 | 108 | 1 |
| β-Endosulfan | 100 | 2 | 97 | 0 | 106 | 4 | 106 | 3 | 94 | 4 | 107 | 1 |
| Endosulfan-$SO_4$ | *110* | 5 | 105 | 1 | *138* | 3 | *119* | 3 | 106 | 5 | 112 | 1 |
| Fenvalerate | 75 | 8 | 96 | 1 | 105 | 0 | 106 | 5 | *110* | 12 | 105 | 2 |
| Chlorothalonil | *124* | 5 | 103 | 2 | *224* | 2 | *114* | 5 | *134* | 5 | *110* | 3 |
| Iprodione | *130* | 4 | 92 | 2 | *114* | 3 | *114* | 6 | *131* | 14 | 108 | 2 |
| Lindane | *112* | 3 | 104 | 0 | 103 | 4 | *110* | 2 | 92 | 6 | 104 | 1 |
| Permethrin | 100 | 8 | 95 | 0 | *110* | 2 | 105 | 6 | 98 | 9 | 101 | 2 |
| Procymidone | *144* | 4 | 99 | 1 | *134* | 8 | 97 | 9 | 99 | 8 | 100 | 1 |
| Tolylfluanid | *171* | 8 | *114* | 1 | *122* | 3 | 106 | 4 | 103 | 7 | 101 | 2 |
| Vinclozolin | *131* | 3 | 107 | 1 | 105 | 2 | 104 | 2 | 101 | 6 | 106 | 1 |

Italicized values indicate recoveries exceeding acceptable levels (110%).

Table 5
Relative responses (R1, R2, %) of ''NPD'' pesticides (100%=response of corresponding standard in pure solvent) in extracts from several matrices; spiking levels $Sb_1$: 0.0176–0.382 mg/kg, $Sb_2$: 0.176–3.820 mg/kg; precision (repeatibility, $n=2$) as relative standard deviation (R.S.D., %)

| Pesticide | Wheat | | | | Orange | | | | Cabbage | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | R1 ($Sa_1$) | R.S.D. | R2 ($Sa_2$) | R.S.D. | R1 ($Sa_1$) | R.S.D. | R2 ($Sa_2$) | R.S.D. | R1 ($Sa_1$) | R.S.D. | R2 ($Sa_2$) | R.S.D. |
| Bupirimate | 107 | 1 | 103 | 1 | 100 | 1 | 99 | 2 | 98 | 0 | 94 | 2 |
| Diazinon | 94 | 2 | 103 | 3 | 102 | 0 | 100 | 2 | 100 | 0 | 101 | 2 |
| Dichlorvos | 97 | 10 | 102 | 3 | 104 | 1 | 104 | 2 | 103 | 24 | *136* | 3 |
| Ethion | 96 | 1 | 105 | 0 | 104 | 1 | 98 | 2 | 99 | 1 | 100 | 0 |
| Fenitrothion | 91 | 3 | 106 | 5 | 102 | 3 | 101 | 2 | 111 | 4 | 101 | 2 |
| Heptenophos | 107 | 2 | 104 | 0 | 105 | 3 | 100 | 1 | 100 | 1 | 102 | 2 |
| Chlorfenvinphos | 98 | 2 | 103 | 2 | 102 | 1 | 100 | 2 | *125* | 4 | 101 | 1 |
| Chlorpropham | *112* | 1 | 103 | 2 | 103 | 2 | 99 | 2 | 100 | 0 | 101 | 2 |
| Chlorpyrifos | 100 | 1 | 104 | 2 | 106 | 2 | 99 | 2 | 101 | 1 | 101 | 1 |
| Chlorpyrifos-methyl | 101 | 2 | 104 | 3 | 103 | 4 | 101 | 2 | 102 | 1 | 95 | 2 |
| Imazalil | *111* | 1 | 107 | 1 | 45 | 0 | 36 | 2 | 88 | 0 | 92 | 1 |
| Metalaxyl | 102 | 2 | 104 | 2 | 101 | 2 | 99 | 2 | 98 | 1 | 92 | 1 |
| Methamidophos | 107 | 2 | 105 | 1 | 74 | 1 | 84 | 3 | 112 | 4 | 102 | 3 |
| Methidathion | 95 | 2 | 108 | 1 | *111* | 3 | 103 | 2 | 107 | 2 | 100 | 1 |
| *cis*-Mevinphos | 75 | 2 | 103 | 2 | 100 | 2 | 99 | 2 | 103 | 1 | 95 | 2 |
| *trans*-Mevinphos | 77 | 1 | 103 | 1 | 102 | 2 | 100 | 2 | 107 | 0 | 102 | 3 |
| Parathion | 93 | 1 | 104 | 2 | 107 | 1 | 100 | 2 | 106 | 0 | 99 | 2 |
| Parathion-methyl | 93 | 4 | 105 | 1 | 110 | 6 | 102 | 2 | 103 | 4 | 85 | 1 |
| Phosalone | 104 | 1 | 105 | 1 | 105 | 2 | 102 | 1 | 105 | 1 | 100 | 1 |
| Pirimicarb | 107 | 1 | 104 | 2 | 82 | 0 | 98 | 2 | 99 | 1 | 100 | 2 |
| Pirimiphos-methyl | 100 | 1 | 104 | 2 | 100 | 1 | 99 | 2 | 99 | 0 | 100 | 2 |

Italicized values indicate recoveries exceeding acceptable levels (110%).

Table 6
''ECD'' pesticides in pure solvent: average of normalised responses (expressed as signal per ng) obtained by five repeated injections within the GC sequence – each injection of standard solution was always followed by approx. four matrix extracts

| Pesticide | Standard mixture $Sa_1$ | | Standard mixture $Sa_2$ | |
|---|---|---|---|---|
| | ECD response | R.S.D. (%) | ECD response | R.S.D. (%) |
| Brompropylate | 3.00 | 11.2 | 2.29 | 5.7 |
| λ-Cyhalothrin | 1.17 | 15.7 | 1.08 | 5.0 |
| Cypermethrin | 0.32 | 33.1 | 0.32 | 8.2 |
| Deltamethrin | 0.08 | 34.8 | 0.20 | 16.3 |
| Dichlofluanid | 1.63 | 22.7 | 1.46 | 10.9 |
| α-Endosulfan | 6.92 | 4.3 | 6.50 | 2.7 |
| β-Endosulfan | 5.75 | 4.8 | 5.37 | 3.1 |
| Endosulfan-$SO_4$ | 3.74 | 11.6 | 3.85 | 7.8 |
| Fenvalerate | 0.33 | 20.2 | 0.63 | 4.3 |
| Chlorothalonil | 2.69 | 21.0 | 3.09 | 7.9 |
| Iprodione | 0.64 | 23.5 | 0.60 | 7.8 |
| Lindane | 6.60 | 8.6 | 7.62 | 4.2 |
| Permethrin | 0.49 | 6.6 | 0.39 | 5.6 |
| Procymidone | 1.49 | 7.8 | 1.27 | 3.7 |
| Tolylfluanid | 1.29 | 35.7 | 1.39 | 19.9 |
| Vinclozolin | 3.82 | 17.4 | 3.21 | 5.9 |

Table 7
''NPD'' pesticides in pure solvent: average of normalised responses (expressed as signal per ng) obtained by five repeated injections within the GC sequence – each injection of standard solution was always followed by approx. four matrix extracts

| Pesticide | Standard mixture $Sb_1$ | | Standard mixture $Sb_2$ | |
|---|---|---|---|---|
| | NPD response | R.S.D. (%) | NPD response | R.S.D. (%) |
| Bupirimate | 0.116 | 6.5 | 0.118 | 2.1 |
| Diazinon | 0.278 | 13.2 | 0.329 | 7.6 |
| Dichlorvos | 0.282 | 16.3 | 0.371 | 14.3 |
| Ethion | 0.388 | 19.6 | 0.431 | 9.0 |
| Fenitrothion | 0.272 | 6.8 | 0.310 | 9.0 |
| Heptenophos | 0.263 | 16.8 | 0.276 | 10.8 |
| Chlorfenvinphos | 0.102 | 13.4 | 0.113 | 7.0 |
| Chlorpropham | 0.030 | 6.8 | 0.033 | 3.0 |
| Chlorpyrifos | 0.370 | 9.2 | 0.440 | 7.0 |
| Chlorpyrifos-methyl | 0.198 | 13.6 | 0.258 | 8.0 |
| Imazalil | 0.055 | 16.2 | 0.071 | 4.0 |
| Metalaxyl | 0.027 | 25.4 | 0.031 | 4.0 |
| Methamidophos | 0.207 | 30.2 | 0.343 | 10.7 |
| Methidathion | 0.170 | 28.4 | 0.244 | 8.0 |
| *cis*-Mevinphos | 0.299 | 17.7 | 0.396 | 10.3 |
| *trans*-Mevinphos | 0.192 | 27.0 | 0.231 | 8.4 |
| Parathion | 0.255 | 16.0 | 0.281 | 4.8 |
| Parathion-methyl | 0.269 | 15.8 | 0.304 | 11.2 |
| Phosalone | 0.167 | 20.1 | 0.194 | 24.0 |
| Pirimicarb | 0.166 | 3.5 | 0.173 | 2.0 |
| Pirimiphos-methyl | 0.282 | 6.0 | 0.302 | 4.8 |

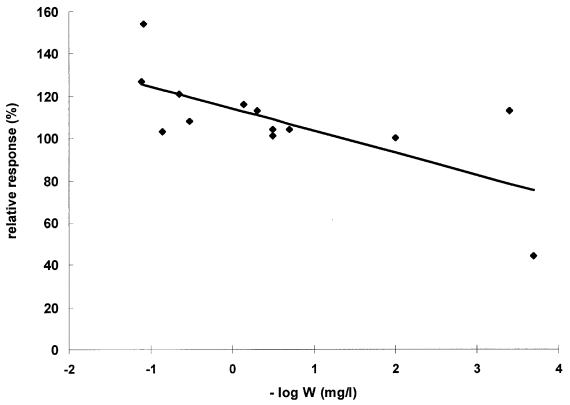


Fig. 4. Matrix enhancement effects vs. water solubility (W) of tested ''ECD'' pesticides (linear regression model).

phorous pesticides typically contain the less polar P=S bond and, accordingly, an incidence of matrix-induced effects is less extensive. Considering the contribution of functional groups contained in particular pesticides to interactions with active sites in liner, amino groups may play an important role in this respect. This assumption complies with the observed behaviour of methamidophos which contains a primary amino group (no other pesticide with this group was involved in our study, a NH group is contained in bupirimate and chlorpropham).

To increase laboratory throughput, multiple injections of great series of samples into the gas chromatograph without any instrument maintenance are commonly carried out in routine practice. Non-volatile sample components traces of which are unavoidably left in extracts even after thorough clean-up are thus gradually deposited around the injector liner as well as at the front part of the chromatographic column. To explore the effect of such contamination on relative pesticide responses over the longer time period, analysis of freshly prepared set of spiked matrix extracts (orange and wheat) together with pesticide standards in pure solvent (the value of response set 100%) was performed just after injector clean-up and then repeated after approx. 80 injections of routine, matrix-containing samples. Besides some loss of column resolution accompanied by increased baseline noise, changes in relative pesticides responses occurred. With exception of methamidophos, pirimicarb and imazalil, an apparent decrease of relative responses was recorded for all ''NPD'' pesticides in orange extract in second set of experiments, see Table 8. These differences were less pronounced in wheat extract. In Table 9 the results obtained for ''ECD'' pesticides are shown. As can be seen, lower relative responses were recorded in the latter (second) experiment. Similarly to our results, decreased responses of some pesticide standards as the effect of injector contamination were reported by Hsu et al. [18] who directed their study at the quantitation problems experienced with multiresidue screen for pesticides in produce (preventive maintenance schedule for GC system was set-up). Not only distinct diminution of matrix-induced effects was recorded in our study, moreover for many analytes the response of standard in pure solution was higher than that in extract (relative responses were below 100%). Elucidation of these differences may insist in potential degradation due to a reaction with non-vaporising matrix components accumulated in this part of GC system [12,19]. This process seems to be more intensive in case of injection of analytes together with coextracts contained in real samples. Similar conclusions were stated in comparative study by Stan and Müller [20] who evaluated various GC injection techniques with respect to the relative peak areas of several organophosphorous pesticides. Polar active sites originating from matrix deposits were identified to be responsible for adsorption as well as thermal stress posed on analytes in vaporising injector. On the other hand, significantly higher GC responses from a ''sample conditioned'' column than from a relatively new one were reported by Gillespie and Walters [21]. Such rather discrepant reports only document the limited comparability of laboratory data when presented uncorrected for matrix-induced effects.

## 4. Conclusions

The occurrence of matrix-induced effects and their extent are simultaneously influenced by many factors characterised below. The measured analyte response then reflects their contribution in actual case.

(i) Pesticide character: higher apparent recoveries together with poorer precision of repeated injections

Table 8
Comparison of relative responses of ''NPD'' pesticides (100%=response of standard in pure solvent analysed in respective set of experiments) in extracts from wheat and oranges after repeated injections

| ''NPD'' pesticides | Wheat | | | | | | Oranges | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | R2 ($Sb_1$) | % R1 | R.S.D.2 | R2 ($Sb_2$) | % R1 | R.S.D.2 | R2 ($Sb_1$) | % R1 | R.S.D.2 | R2 ($Sb_2$) | % R1 | R.S.D.2 |
| Bupirimate | 93 | 87 | 0 | 103 | 100 | 1 | 94 | 94 | 2 | 100 | 102 | 0 |
| Diazinon | 96 | 102 | 0 | 103 | 99 | 1 | 91 | 89 | 2 | 100 | 100 | 2 |
| Dichlorvos | 91 | 94 | 0 | 99 | 97 | 2 | 66 | 63 | 0 | 103 | 99 | 0 |
| Ethion | 99 | 103 | 1 | 104 | 100 | 1 | 87 | 83 | 2 | 100 | 102 | 2 |
| Fenitrothion | 95 | 105 | 2 | 104 | 99 | 3 | 80 | 79 | 2 | 103 | 101 | 2 |
| Heptenophos | 96 | 90 | 1 | 104 | 101 | 1 | 92 | 88 | 1 | 100 | 100 | 1 |
| Chlorfenvinphos | 98 | 100 | 2 | 104 | 100 | 1 | 92 | 91 | 2 | 102 | 102 | 2 |
| Chlorpropham | 94 | 84 | 1 | 104 | 101 | 2 | 98 | 95 | 1 | 101 | 102 | 0 |
| Chlorpyrifos | 94 | 95 | 4 | 103 | 99 | 3 | 94 | 89 | 5 | 101 | 102 | 3 |
| Chlorpyrifos-methyl | 96 | 95 | 1 | 105 | 101 | 2 | 85 | 83 | 1 | 100 | 99 | 0 |
| Imazalil | 102 | 92 | 2 | 105 | 98 | 0 | 62 | 138 | 4 | 47 | 133 | 3 |
| Metalaxyl | 94 | 92 | 1 | 101 | 97 | 1 | 93 | 92 | 1 | 100 | 101 | 1 |
| Methamidophos | 102 | 95 | 0 | 96 | 91 | 1 | 83 | 113 | 5 | 97 | 114 | 1 |
| Methidathion | 99 | 104 | 3 | 107 | 99 | 1 | 98 | 89 | 3 | 100 | 98 | 0 |
| *cis*-Mevinphos | 99 | 133 | 1 | 104 | 101 | 1 | 84 | 83 | 1 | 100 | 100 | 0 |
| *trans*-Mevinphos | 104 | 135 | 1 | 104 | 101 | 1 | 103 | 101 | 2 | 100 | 100 | 1 |
| Parathion | 97 | 104 | 0 | 105 | 101 | 1 | 94 | 89 | 1 | 102 | 102 | 0 |
| Parathion-methyl | 99 | 107 | 1 | 105 | 99 | 1 | 90 | 82 | 2 | 100 | 98 | 2 |
| Phosalone | 99 | 95 | 3 | 103 | 99 | 0 | 91 | 87 | 3 | 104 | 101 | 0 |
| Pirimicarb | 94 | 88 | 0 | 99 | 96 | 1 | 93 | 113 | 1 | 99 | 102 | 1 |
| Pirimiphos-methyl | 95 | 95 | 0 | 103 | 99 | 2 | 93 | 93 | 1 | 100 | 101 | 2 |

R1: relative response in the first set of experiments shown for spiking levels $Sb_1$ and $Sb_2$ in Table 5; R2: relative response (%) in the second set of experiments; R.S.D.2 ($n=2$): relative standard deviation (%) in the second set of experiments (R.S.D. in first set, see Table 5).

Table 9
Comparison of relative responses of ''ECD'' pesticides (100%=response of standard in pure solvent analysed in respective set of experiments) in extracts from wheat and oranges after repeated injections

| ''ECD'' pesticides | Wheat | | | | | | Oranges | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | R2 ($Sa_1$) | % R1 | R.S.D.2 | R2 ($Sa_2$) | % R1 | R.S.D.2 | R2 ($Sa_1$) | % R1 | R.S.D.2 | R2 ($Sa_2$) | % R1 | R.S.D.2 |
| Brompropylate | 97 | 91 | 2 | 116 | 118 | 1 | 94 | 69 | 5 | 109 | 102 | 1 |
| λ-Cyhalotrin | 93 | 87 | 3 | 107 | 107 | 2 | 86 | 66 | 5 | 101 | 97 | 1 |
| Cypermethrin | 77 | 56 | 2 | 113 | 104 | 1 | 83 | 74 | 5 | 106 | 101 | 1 |
| Deltamethrin | 86 | 126 | 1 | 99 | 130 | 1 | 60 | ns | ns | 110 | 87 | 1 |
| Dichlofluanid | 78 | 52 | 0 | 105 | 94 | 1 | 77 | 59 | 4 | 99 | 92 | 1 |
| α-Endosulfan | 82 | 72 | 0 | 99 | 97 | 0 | 100 | 95 | 2 | 101 | 95 | 0 |
| β-Endosulfan | 92 | 93 | 1 | 104 | 108 | 1 | 85 | 80 | 1 | 100 | 94 | 2 |
| Endosulfan-$SO_4$ | 99 | 90 | 1 | 109 | 104 | 1 | 96 | 70 | 6 | 108 | 91 | 1 |
| Fenvalerate | 115 | 154 | 3 | 109 | 114 | 3 | 92 | 88 | 4 | 105 | 99 | 1 |
| Chlorothalonil | 100 | 81 | 0 | 105 | 101 | 1 | 95 | 42 | 3 | 102 | 89 | 2 |
| Iprodione | 90 | 69 | 2 | 112 | 121 | 1 | 84 | 74 | 2 | 112 | 99 | 2 |
| Lindane | 93 | 83 | 0 | 100 | 96 | 0 | 88 | 86 | 9 | 97 | 88 | 1 |
| Permethrin | 98 | 97 | 2 | 111 | 116 | 0 | 98 | 89 | 2 | 108 | 103 | 0 |
| Procymidone | 88 | 61 | 2 | 101 | 102 | 0 | 77 | 58 | 3 | 101 | 104 | 3 |
| Tolylfluanid | 95 | 55 | 0 | 109 | 95 | 1 | 83 | 68 | 1 | 107 | 101 | 1 |
| Vinclozolin | 87 | 67 | 0 | 104 | 97 | 1 | 91 | 86 | 3 | 96 | 92 | 1 |

R1: Relative response in the first set of experiments shown for spiking levels $Sa_1$ and $Sa_2$ in Table 5; R2: relative response (%) in the second set of experiments; R.S.D.2 ($n=2$): relative standard deviation (%) in second set of experiments (R.S.D. in first set, see Table 5).

was determined for more polar pesticides (the degree of ''polarity'' classified according to water solubility and/or $K_{ow}$).

(ii) Matrix type: matrix-induced effects recorded for particular pesticides were clearly proved to depend on matrix type (i.e., type of coextracts in sample), most distinct detector response enhancement occurred for commodities with high content of essential oils (orange) and waxes (grains) which cannot be completely removed by HPGPC.

(iii) Analyte/matrix concentration: unacceptable accuracy of measurements was encountered especially at lower concentration levels of analytes and/or at higher matrix concentration in sample.

(iv) The state (history) of the GC system: relative GC responses (the response of standard in pure solvent=100%) of most pesticides were gradually diminished over the time when these analytes were injected in matrix-containing solutions; this is obviously a consequence of increasing contamination of the GC inlet (deterioration of chromatographic conditions when more samples were injected was another accompanying symptom).

It should be emphasised, that although our results are in relatively good agreement with existing literature data (studies referring to ''pure'' matrix-induced effects, i.e., focused on phenomena related to enhanced analyte transfer from injector to column, are very limited), controversial results may be generated by laboratories employing either different methods of extract preparation (and thus yielding another amount and character of coextracts in sample) or running GC analysis under completely different conditions. Considering GC hardware, the type as well as geometry of injector seem to be most important in relation to matrix-induced effects. The influence of the GC column and/or detector on analyte response is minute as long as good separation of analyte peak from sample components is obtained.

In conclusion, once analytical procedure has been developed and further minimisation of matrix burden is not perspective solution, quantitation based on a standard prepared in blank matrix extract to compensate for the matrix-induced effects and to obtain more accurate results should be accepted as a compromise. The concentration and character of solutions used for calibration should correspond to extract as closely as possible.

## Acknowledgements

This study was conducted within the Copernicus project (CIPA-CT94-0144). We also express our thanks to Ministry of Education and Youth of Czech Republic for providing additional funds to support research in this field (projects OK 208 and OK 191).

## References

[1] Anon., in J.H. Gibbons (Editor), Pesticide Analytical Methods in Pesticide Residues in Food, Technologies for Detection, Technomic Publ. Co., Lancaster, Basel, 1990.
[2] D.R. Erney, A.M. Gillespie, D.M. Gilvydis, C.F. Poole, J. Chromatogr. 638 (1993) 57–63.
[3] D.R. Erney, C.F. Poole, J. High Resolut. Chromatogr. 16 (1993) 501–503.
[4] D.R. Erney, T. Pawlowski, C.F. Poole, J. High Resolut. Chromatogr. 20 (1997) 375.
[5] P.D. Johnson, D.A. Rimmer, R.H. Brown, J. Chromatogr. A 765 (1997) 3–11.
[6] A. Andersson, H. Palsheden, Fresenius J. Anal. Chem. 339 (1988) 365–367.
[7] S.M. Lee, M.L. Papathakis, H.M.C. Feng, G.F. Hunter, J.E. Carr, Fresenius J. Anal. Chem. 339 (1991) 376–383.
[8] L. Kadenczki, Z. Arpad, I. Gardi, J. Assoc. Off. Anal. Chem. 75 (1992) 53–61.
[9] H.M. Pylypiw, J. Assoc. Off. Anal. Chem. 76 (1993) 1369–1373.
[10] S.J. Lehotay, K.I. Eller, J. Assoc. Off. Anal. Chem. 78 (1995) 821–830.
[11] M. de Kroon, G. Ubbels and H.A. van der Schee, presented at the 1st European Pesticide Residue Workshop, Alkmaar, 10–12 June 1996.
[12] W. Liao, T. Joe, W.G. Cusick, J. Assoc. Off. Anal. Chem. 74 (1991) 554–564.
[13] F.H. Hernádez, J.M. Grases, J. Beltrán, V. Sancho, Chromatographia 29 (1990) 459–465.
[14] M. Miyahara, Y. Okada, H. Takeda, G. Aoki, A. Kobayashi, Y. Saito, J. Agric. Food Chem. 42 (1994) 2795–2802.
[15] C. Tomlin (Editor), The Pesticide Manual, RSC/BCPC, Cambridge, 10th ed., 1994.
[16] Council Directive 94/43/EC. Off. J. Eur. Com. L227 (1994) 31-55
[17] A. Andersson, H. Pålsheden and B. Arén, poster presented at 1st European Pesticide Residue Workshop, Alkmaar, 10–12 June 1996.
[18] J.P. Hsu, H.J. Schattenberg, M.M. Garza, J. Assoc. Off. Anal. Chem. 74 (1991) 886–892.
[19] J. Fillion, R. Hindle, M. Lacroix, J. Selwyn, J. Assoc. Off. Anal. Chem. 78 (1995) 1252–1266.
[20] H.-J. Stan, H.-M. Müller, J. High Resolut. Chromatogr., Chromatogr. Commun. 11 (1988) 141–143.
[21] M. Gillespie, S. Walters, Anal. Chim. Acta 245 (1991) 259–265.