

Applying Supersaturated Experimental Designs to the Study of Composite Sampling for Monitoring Pesticide Residues in Water



The authors propose the use of supersaturated experimental designs for composite sampling and demonstrate their efficiency and economy in the environmental screening of pesticide residues. Because supersaturated designs are factorial designs in which the number of factors examined exceeds the number of experiments performed, they provide a direct economy in the analytical effort expended in screening analytical campaigns. This characteristic is advantageously applied in strategic sample composition. Results are compared for two different design matrices: 30 sample specimens in 12 composite samples and 24 sample specimens in 12 composite samples. Both matrices allow chemists to identify clearly the contaminated sample specimens and have produced satisfactory estimations of their concentration levels.

In the past few decades, increasing attention has been devoted to the detection and quantification of a wide variety of organic microcontaminants, mainly because of their adverse (that is, toxic) effects on environmental compartments, food, and human health. Thus, the number of published papers concerning the development and application of multiresidue environmental analysis on pesticides has increased enormously and has resulted in an extensive bibliography (1–6).

One major field for monitoring water pollutants in the environment is the determination of pesticide compounds. If not biodegraded within the soil, these pesticides can penetrate through the soil profile and cause pollution in ground water. These agents can persist for years, thus representing a possible risk where ground water is the source of drinking water (7). Legislation or control regulations (8,9) concerning pesticides in drinkable water samples accepts 0.5 mg/L for total pesticides and 0.1 mg/L for individual pesticides (0.03 mg/L for heptachlor, aldrin, and dieldrin). The American Food Quality Protection Act (10) would ensure that all pesticides meet new safety standards. More than 9000 pesticide uses must be assessed by 2006. Extensive monitoring analytical campaigns currently are performed in most developed countries and must be con-

tinued in the future. Advances in the analytical methodology that can decrease the time, effort, and costs in these campaigns will contribute to a safe environment and sustainable development.

Improvement of the selectivity and development of reliable confirmatory techniques are adequate approaches for eliminating false positive results. Pesticides always contain several heteroatoms in their molecules. Therefore, from the early days of pesticide residue analysis, selective detection methods were applied successfully in gas chromatography (GC) analysis. Inexpensive electron-capture detection (ECD) made it possible to detect the chlorinated hydrocarbon pesticides (11,12) at very low levels. Additionally, major advances have been made in the development of techniques and methods, leading to minimization of the analysis time by simplification of sample preparation procedures and increasing the sample throughput by automation. Efforts also have been focused on reducing the consumption of hazardous solvents as this is essential for environmental safety. Trace enrichment via nonselective solid-phase extraction (SPE) currently is the preferred approach for its easy automation and the decreased need for organic solvents (6,13). Among others, SPE adsorbents using graphitized carbon black (14,15) and divinylbenzene plus *N*-vinylpyrrolidone

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Figure 2: Device for the automatic preparation of liquid composite samples following strategic sample composition principles.

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remaining composite samples formed. Firstly, C_{S_n} is a conventional composite sample in the sense that all the original specimens have been mixed in one composite sample. Secondly, C_{S_n} will represent the maximum dilution in the sample composition process. Because of this characteristic, this composite sample must be analyzed first. If the analysis of this composite sample produced negative (background) results we can derive that no original sample specimen in this set was polluted and the remaining composite samples can be discarded without being analyzed. On the contrary, if the C_{S_n} sample appears positive, the remaining $C_{S_1, n_2, \dots}$ samples must be processed. The second characteristic of the C_{S_n} sample, the dilution, must be considered at the time of selecting the composition matrix (that is, the number of columns) to avoid false negatives due to excessive dilution. For the same reason, it is advisable to carry out the analysis of this C_{S_n} sample at least twice, while the remaining samples need to be analyzed only once due to robustness characteristics derived from the design matrix (24–26).

When all the composite samples have been processed, we will have a vector of n analytical responses (R_1, R_2, \dots, R_n) that gives direct information about the composite samples but not about the original sample specimens. To estimate the concentration level for analytes in the original sample specimens, this vector of results must be submitted to regression. Supersaturated matrices need special regression procedures

(23,32), and evolutionary driven regression (23) has produced excellent results in strategic sample composition practical situations. The information produced in the regression process corresponds to concentration levels

supersaturated design matrices are factorial designs in N observations, in which the number of factors is more than $N/2 - 1$, the number of total analyses carried out is significantly lower than the number of sample

Sample name	Draw vol	Total vol	Proportion	Sample name
S_1	1.0	18.0	3.00	S_1
S_2	1.0	24.0	4.00	S_2
S_3	1.0	24.0	4.00	S_3
S_4(S)	1.0	12.0	2.00	S_4(S)
S_5	1.0	12.0	2.00	S_5
S_6	1.0	24.0	4.00	S_6
S_7	1.0	24.0	4.00	S_7
S_8	1.0	24.0	4.00	S_8
S_9	1.0	24.0	4.00	S_9
S_10(M)	1.0	24.0	4.00	S_10(M)
S_11	1.0	6.0	1.00	S_11
S_12	1.0	7.0	1.00	S_12
S_13	1.0	28.0	6.00	S_13
S_14	1.0	28.0	4.00	S_14
S_15	1.0	28.0	4.00	S_15
S_16	1.0	28.0	4.00	S_16

Figure 3: Input form in the Gamich software for designing the automatic sample composition process.

for the analytes in the original samples with the obvious advantage that these original samples actually have not been analyzed on an individual basis. Moreover, because

specimens. This is the enormous advantage of strategic sample composition compared to conventional sample composition: strategic sample composition using supersatu-

Table II: 12-24 Sample composition matrix used in the analysis of pesticide residues in water samples belonging to Sample Set 2

	S ₁	S ₂	S ₃	S ₄	S ₅	S ₆	S ₇	S ₈	S ₉	S ₁₀	S ₁₁	S ₁₂	S ₁₃	S ₁₄	S ₁₅	S ₁₆	S ₁₇	S ₁₈	S ₁₉	S ₂₀	S ₂₁	S ₂₂	S ₂₃	S ₂₄
C _{S1}	0	0	0	1	0	1	1	1	0	0	0	1	0	0	0	1	1	1	0	1	1	1	1	0
C _{S2}	0	0	0	1	1	0	0	0	1	1	1	0	1	0	0	1	0	1	0	1	0	1	1	1
C _{S3}	0	1	0	1	0	0	0	0	1	1	0	1	0	1	0	0	1	0	1	0	1	0	0	1
C _{S4}	0	1	1	0	1	0	1	0	0	0	0	0	0	1	1	0	0	1	0	0	1	1	1	1
C _{S5}	1	0	0	0	0	1	0	1	1	0	0	0	1	1	1	0	1	0	0	1	0	1	0	1
C _{S6}	0	0	1	0	0	1	0	1	0	1	1	0	0	0	1	1	0	1	1	0	0	0	0	1
C _{S7}	1	0	0	0	1	0	1	0	1	0	1	0	1	0	1	1	1	0	1	0	1	0	1	0
C _{S8}	0	0	1	0	0	0	1	1	1	0	1	1	1	1	0	0	1	1	0	0	1	0	0	1
C _{S9}	1	1	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0	1	1	1	1	0	0
C _{S10}	1	1	1	1	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	0	0	0	0	0
C _{S11}	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C _{S12}	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

rated matrices can be applied under the assumption that the number of polluted original sample specimens is low compared with the total of sample specimens that need to be analyzed (effect sparsity or Pareto principle). This type of situation is common in many environmental screening campaigns (21). Otherwise, the use of conventional screening matrices (for example, Plackett-Burman design matrices [33]) is advisable (26).

In this paper, supersaturated experimental designs were applied to the study of composite sampling for the analysis of organochlorine pesticides in water samples. In this study, two batches of 30 and 24 tap-water sample specimens were screened for the presence of nine organochlorine pesticides (*a*-BHC, *g*-BHC, heptachlor, aldrin, dieldrin, 4,4'-DDT, endrin ketone, *a*-endosulfan, and endrin). Also evaluated was the concentration level of each pesticide in the original discrete water samples. The main advantages of strategic sample composition are well appreciated when the sample composition is run under full automation conditions and the strategic sample composition technique is combined with sample preparation procedures, allowing the easy and rapid handling of reasonable sample volumes. Because of this, the work here compares two SPE materials (graphitized carbon black and divinylbenzene-vinylpyrrolidone copolymer), followed by a GC-ECD procedure for determination of pesticide residues in the composite water samples prepared in a specially developed fully automatic device.

Experimental

Materials: The organochlorine compounds (*a*-BHC, *g*-BHC, heptachlor, aldrin, dieldrin, 4,4'-DDT, endrin ketone) were obtained from Supelco (Bellefonte, Pennsylvania), and *a*-endosulfan and endrin were

obtained from Riedel-de-Haën (Seelze-Hannover, Germany).

Stock standard solutions of 100 mg/L of each compound were prepared in methanol, except endrin ketone, which was prepared in 1:1 (v/v) toluene-hexane. Working standard solutions were prepared by dilution with 9:1 (v/v) methylene chloride-methanol and with ethyl acetate according to the SPE sorbent to be applied in the sample preparation process. All solutions were stored at 2 ± 18 °C.

Methanol, acetonitrile and ethyl acetate (HPLC grade), acetic acid (100%), acetone and *n*-hexane (for trace organic analysis), and ascorbic acid were from Merck (Darmstadt, Germany). Methylene chloride (for pesticide residue analysis) was supplied by Scharlau (Barcelona, Spain) and toluene (super purity solvent) was supplied by Romil (Cambridge, United Kingdom).

Sample preparation: Two water-sample batches were prepared by taking a sufficient amount of water and analyzing it for the considered pesticides. Once we verified that the water did not give appreciable signals of any of the pesticides, a number of identical 1-L sample specimens were taken from this analyzed water. Individual sample specimens were numbered and some of them randomly chosen and spiked with variable levels of some of the considered organochlorine pesticides.

Sample Set 1: Sample Set 1 was formed by 30 sample specimens with pesticides at concentrations as high as 1000 ng/L. Spiking was performed to produce three levels of pollution in samples, one of them below the maximum residue limit accepted by the European Norms (8). To prepare the corresponding composite samples, the composition matrix shown in Table I was used. Due to volume limitations, composite samples were prepared by manually mixing equal volume aliquots (50 mL) of the original

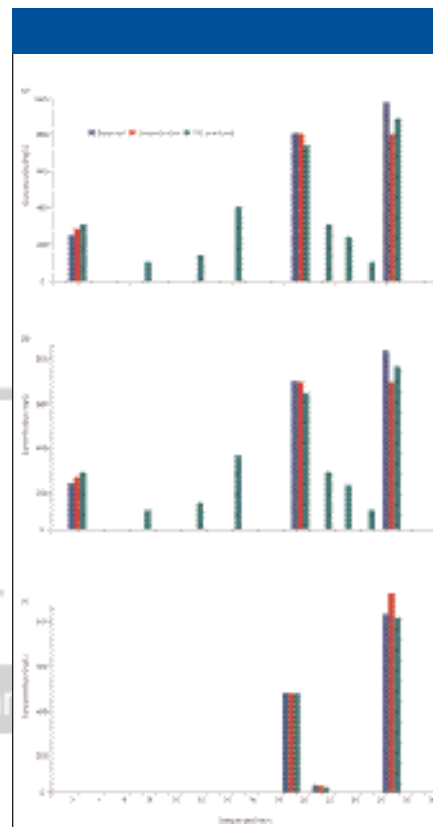


Figure 4: Comparative results for (a) *a*-BHC, (b) *a*-endosulfan, and (c) dieldrin in Sample Set 1. Expected 5 concentration expected in sample specimens on the basis of the spiked amount of pesticide; Found (individ) 5 actual concentrations of spiked sample specimens measured individually; Predicted 5 results predicted by the strategic sample composition method using the design matrix in Table I.

sample specimens. Finally, all the composite samples were diluted to the same volume (1.5 L).

To have comparative results, not only were the composite samples prepared, but the individual sample specimens spiked were processed and analyzed as well. Sample Set 1 processing was performed with graphitized carbon black (ENVI-Carb, Supelco) cartridges. Cartridges were conditioned by washing with 5 mL of 9:1 (v/v) methylene chloride-methanol, followed by 2 mL of methanol and 15 mL of a 10-g/L ascorbic acid solution. The SPE method involves passing water samples through the cartridge under vacuum for adsorption of the pesticides. After the total 1.5-L sample was passed through the cartridge, the pump was disconnected and residual water was removed from the cartridge by letting nitrogen pass through it for 15 min. After the cartridge was turned upside down, nitrogen was passed through it for a further 20 min. Then the analytes were back-eluted by pass-

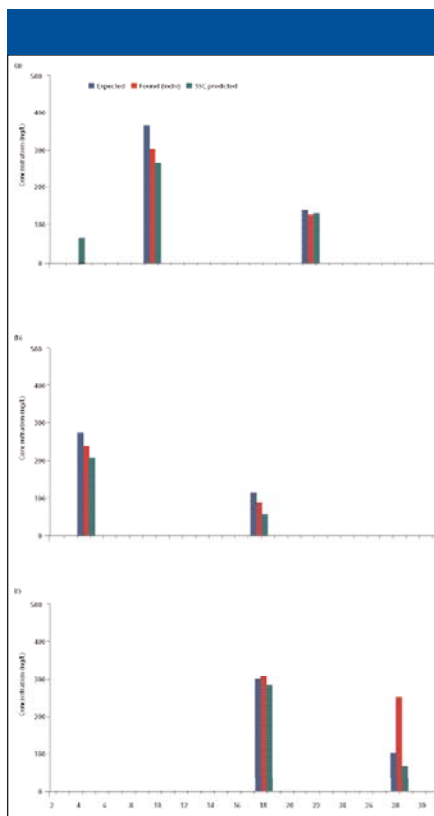


Figure 5: Comparative results for (a) α -BHC, (b) α -endosulfan, and (c) dieldrin in Sample Set 2. Expected 5 concentration expected in sample specimens on the basis of the spiked amount of pesticide; Found (indiv) 5 actual concentrations of spiked sample specimens measured individually; Predicted 5 results predicted by the strategic sample composition method using the design matrix in Table II.

ing 9:1 (v/v) methylene chloride–methanol solution through the cartridge, and the eluate was concentrated to approximately 0.5 mL in a TurboVap II Station (Zymark, Hopkinton, Massachusetts) and exactly diluted to 1 mL with the eluent phase.

Sample Set 2: Sample Set 2 was formed by 24 specimens. In this case, spiking was made to obtain concentration levels of up to 350 ng/L in sample specimens. Some samples resulted below the European maximum residue limits (100 ng/L for individual pesticides), while others were above these limits. Sample Set 2 was processed using the automatic device shown in Figure 2, supplied by Crison Instruments (Barcelona, Spain). Equal volume aliquots of 12.5 mL were taken from the original sample specimens and composed according to the design matrix of Table II. Composite samples were not made to any particular final volume. Composition software accounted for the composite sample volumes, although the possibility of diluting these samples exists.

Composite samples of 200 mL (as well as the individual spiked specimens for comparative purposes) were extracted with divinylbenzene–vinylpyrrolidone copolymer cartridges (Oasis-HLB, Waters Corp., Milford, Massachusetts). Before processing water samples, the pH of the sample was adjusted to 3.5 with concentrated acetic acid. The cartridges were conditioned with 2 mL of methanol, followed by 2 mL of ultrapure water (produced by a Milli-Q system from Millipore Co., Bedford, Massachusetts). Cartridges were dried with a nitrogen stream for 30 min. Analytes were eluted with 3 mL of ethyl acetate. The solvent was evaporated to dryness by nitrogen stream, and then the dry extract was taken off by 40 mL of the 9:1 (v/v) methylene chloride–methanol mixture.

GC-ECD conditions: A model 5890 Series II Plus gas chromatograph (Hewlett-Packard, Palo Alto, California) was used. It was equipped with a split-splitless capillary injection port, an electron-capture detector, and an HP 7673 automatic injector.

In all experiments, the analytical column was a Supelco 30 m \times 0.32 mm capillary column coated with a 0.25- μ m layer of SPB-5.

The injector was set at a temperature of 250 °C and was used in the splitless mode (purge time 60 s and splitting ratio 1:9). The detector temperature was 285 °C. The injection volume was 3 mL. The carrier and makeup gas was nitrogen (Carbueros Metálicos, La Coruña, Spain) at flow rates of 3.6 and 31 mL/min, respectively. The GC oven temperature program was set as follows: initial temperature 100 °C for 2 min, increased 3 °C/min to 230 °C (no initial time), then increased 10 °C/min to 280 °C, then 280 °C for 10 min.

Software: Supersaturated matrices applied in this study were produced with the a programs package (Superga) specifically devised for building supersaturated matrices adapted to strategic sample composition (22). A large number of supersaturated matrices and methods for its construction can be found in the statistical literature (29,30,34), although most of them are not suited for strategic sample composition. For evolutionary regression of strategic sample composition experiments, a dedicated package (Gamich) was used (23). These software packages were developed and implemented in the laboratory using CA-Realizer 3.0A (Computer Associates, Islandia, New York) programming language.

Results and Discussion

Figures of merit of the determination procedure:

A very important aspect in the practical application of strategic sample composition is the selection of the design matrix to be used for sample composition. As described in the introduction, the number of columns in the design matrix is equivalent to the number of sample specimens to be accounted, while the number of rows in the matrix indicates the number of composite samples to prepare and analyze. Because the maximum dilution is produced in the C_{S_n} composite sample, it is quite easy to derive the maximum allowed dilution by considering the number of sample specimens and the proportions ascribed to each sample specimen in the composite samples. Thus, the maximum dilution in the composition experiment will be

$$\sum_{i=1}^M S_i \text{ (proportions)} \quad [1]$$

Assuming only one sample specimen was contaminated just above the maximum residue limit for a given analyte, the applied determination procedure must be able to accurately detect the presence of this analyte in the C_{S_n} composite sample. In our case, the analytical procedures were tested for detection and quantification limits, linearity, and precision (% relative standard deviation). The results corresponding to both SPE alternatives are shown in Tables III and IV. The calibration plots in the measurement of extracts were drawn in the range of 5–100 ng/mL when method 1 was used and 20–500 ng/mL for method 2. Linear regression of chromatographic peak area on analyte concentration afforded equations that show good linearity over the concentration ranges studied, passing linearity and lack of fit tests (95% probability) (35). Acceptable values of recovery (67–102% for Sample Set 1 and 93–117% for Sample Set 2) were obtained. Oasis HLB cartridges provide better recovery values and reduce sample and solvent volumes consumption, reducing the overall process time needed. This reduction in sample volumes allows the use of automatic sample-composition devices, which is a very important practical aspect.

Similar precision was obtained in both procedures (4.6–9.8% for Set 1 and 2.8–16.4% for Set 2). Detection limits were calculated as 3 S/N and quantification limits as 10 S/N. These limits are low enough to ensure that contaminated sample specimens would be detected in spite of the dilution (1:30 for Set 1 and 1:24 for Set 2) of

the original samples due to the sample composition.

Automation of the Sample Composition Process

Although strategic sample composition can be applied without having special software or automation tools, the automation of the sample-composition process is of utmost importance. In the Gamich software, an option exists to guide composition processes developed in manual mode. This option gives detailed messages to users about the sample specimen to be handled at any moment, the sample volume that must be taken, and the composite sample where the aliquot must be placed. However, the risk of human mistake is high following a design matrix when handling a large number of sample specimens and composite samples, variable proportions, and volumes. Although strategic sample composition has shown impressive robustness (24–26) versus accidental errors in the analytical measurement of the composite samples, mistakes in preparing the composite samples often are critical. Thus, automation of this cornerstone step warrants the absence of composition errors while at the same time freeing the analyst from that tedious task.

The inexpensive device in Figure 2 allows the easy preparation of composite samples. It is formed by two identical turntable samplers adapted from automatic titration devices. Only arms adaptors need small modifications to prepare composite samples. The third element in this device is a standard automatic dispenser fitted with a 10-mL syringe. These elements are controlled by the Gamich software and the process runs unattended once the analyst has decided the composition options. Figure 3 shows the form used to define these options. The volume of each sample specimen to be added in the corresponding composite samples is defined by a fixed base volume and a proportion value. In this way, proportional composite samples can be designed easily. Each sample is assigned a fixed name that cannot be changed in the process and another name to easily associate the sample specimen with the particular sampling campaign under development. Additionally, two sample specimens can be flagged along the process. A flagged sample means analysts must substitute a sample specimen with a known blank or a standard. Obviously, the insertion of known sample specimens in the process serves as a validation mechanism. The software knows that these flagged samples must have special

Table III: Performance of the whole analytical process applied for Sample Set 1 (graphitized carbon black cartridge, using 90:10 [v/v] methylene chloride–methanol as eluent)

Compound	Retention time (min)	Correlation coefficient (Range 5–100 ng/mL)	Recovery (%) <i>n</i> = 6	RSD (%) <i>n</i> = 5	Detection limit (ng/L)	Quantification limit (ng/L)
a-BHC	18.8	0.9994	88	6.2	2.1	7.0
g-BHC	21.0	0.9994	102	9.4	1.9	6.5
Heptachlor	25.6	0.9990	85	8.4	1.7	5.8
Aldrin	27.8	0.9996	83	9.5	1.8	6.1
a-Endosulfan	32.6	0.9997	87	4.6	1.7	5.7
Dieldrin	34.3	0.9994	72	8.2	1.4	4.7
Endrin	35.5	0.9988	75	6.8	3.1	10.1
4,4'-DDT	39.5	0.9985	80	9.8	1.7	5.9
Endrin ketone	41.4	0.9976	67	9.4	2.9	9.7

characteristics, but the known concentration values are not actually entered in the system. So with regard to the software concerns, these samples are not different from any other in the set. Also, analysts can decide if all the composite samples must be diluted to the same volume or not. Other practical aspects, such as the rinsing sequence on sample specimen change and the cleaning sequence after a sample specimen is fully processed, can be defined in this moment.

The device in Figure 2 allows the handling of 19 sample specimens (the tube in position 20 is filled with solvent used to clean the sampler tip, thus reducing sample carry over) and 19 composite samples (position 20 in sampler 2 is occupied by a special tube having a bottom hole so this position serves as the collector of rinsing liquids and sample excess). It is unusual to prepare more than 19 composite samples, but frequently it is required to handle more than that. In this case, the sample composition process runs in blocks of 19 sample specimens and the system stops and alerts analysts that they must substitute the sample specimens tubes with the new ones before further processing.

The obvious limitation of this automatic device is the total volume of the composite samples and the original sample-specimen

holders. Tubes of 200 mL maximum capacity can be handled. Thus, if the final volumes of the composite samples need to be larger than this value, the manual process must be applied. Of course, other automatic devices can be designed to enable the handling of larger volumes using more expensive commercial liquid handlers, but in many cases, the adequate selection of the sample preparation technique allows the utilization of the simple and inexpensive device shown in Figure 2.

Screening of Pesticide Residues in Water Samples by Strategic Sample Composition

As described in the experimental section, to achieve comparative results, all spiked sample specimens were analyzed individually before the composite samples were prepared. Then composite samples were prepared and analyzed following the design matrix in Tables I and II.

Table V and VI summarize the results of the composite samples corresponding to the Sample Sets 1 and 2. These results were processed using the Gamich software to obtain the estimated concentration of each of the nine pesticides in the individual specimens that formed the sample sets.

Predicted versus expected and individu-

Table IV: Performance of the whole analytical process applied for Sample Set 2 (Oasis-HLB using ethyl acetate as eluent)

Compound	Retention time (min)	Correlation coefficient (Range 20–500 ng/mL)	Recovery (%) <i>n</i> = 8	RSD (%) <i>n</i> = 8	Detection limit (ng/L)	Quantification limit (ng/L)
a-BHC	18.8	0.9999	95	5.2	0.5	1.5
g-BHC	21.0	0.9999	117	2.8	0.6	2.0
Heptachlor	25.7	0.9995	109	4.2	1.0	3.3
Aldrin	27.8	0.9999	95	16.4	0.7	2.4
a-Endosulfan	32.6	0.9999	97	8.4	0.2	0.8
Dieldrin	34.3	0.9999	100	4.9	0.2	0.7
Endrin	35.5	0.9989	110	10.8	1.0	3.3
4,4'-DDT	39.6	0.9982	93	6.5	3.8	12.8
Endrin ketone	41.5	0.9979	109	7.3	0.5	1.8

Table V: Results obtained in the analysis of the composite samples prepared for Sample Set 1 (all concentrations are expressed in ng/L, nd = nondetected)

	α -BHC	γ -BHC	Heptachlor	Aldrin	α -Endosulfan	Dieldrin	Endrin	4,4' DDT	Endrin Ketone
C _{S1}	42.4	8.80	nd	15.7	12.5	nd	30.0	38.4	34.5
C _{S2}	23.8	24.3	5.4	nd	34.3	28.1	40.6	nd	31.0
C _{S3}	27.4	11.3	3.2	8.2	18.6	12.9	31.5	66.1	15.4
C _{S4}	37.6	28.8	15.0	15.9	22.1	27.5	28.1	38.7	7.9
C _{S5}	121.9	39.2	15.4	8.9	526.8	31.6	nd	4.2	11.3
C _{S6}	41.9	4.0	6.1	8.5	9.1	12.6	14.1	57.4	46.5
C _{S7}	32.9	151.3	3.1	13.5	31.3	42.8	3.8	68.4	5.3
C _{S8}	9.2	3.9	19.9	nd	11.4	47.2	50.1	22.3	17.7
C _{S9}	49.1	nd	20.4	16.2	12.5	nd	12.4	23.8	31.4
C _{S10}	51.4	11.0	19.7	7.8	10.9	13.3	15.6	nd	29.7
C _{S11}	9.3	60.2	3.4	13.7	44.9	nd	4.4	22.8	12.0
C _{S12}	143.6	66.1	21.8	22.7	60.7	49.6	58.6	75.5	59.7

ally found concentrations in the original sample specimens have been compared in the graphs of Figures 4 and 5 for α -BHC, α -endosulfan, and dieldrin, which are representative of the less favorable results obtained. In general, spiked sample specimens are actually detected as contaminated sample specimens by the strategic sample composition technique, although small false positives are present. Also, good estimates of the concentration values for the really polluted sample specimens are shown. The classification of a particular predicted concentration value as false positive or false negative requires additional consideration. We can establish criteria based on limits of detection or quantification. Logically, strategic sample composition gives numerical outputs in the form of predicted concentrations for the individual sample specimens that can be judged in terms of the practically attainable detection or quantification limits. Values above these limits must be considered as positives, whereas below these limits, noise and sample specimens must be classified as negatives. Of course, if

any sample specimen contains the analytes at a higher level than limits of detection or quantification, and the strategic sample composition prediction is zero or negligible (less than the limit of detection or quantification), a false negative is produced. On the contrary, if a sample specimen did not contain the analytes and strategic sample composition gives a numerical output higher than the limit of detection and quantification, a false positive is present.

However, from a practical point of view, the only way to evaluate the presence of false positives or false negatives in a strategic sample composition experiment is to analyze each individual sample specimen so that any attempt to evaluate these figures will disappoint the use of strategic sample composition. Instead, for practical purposes, the allowable maximum residue limits define the borderline of false positives and negatives. For example, in the case of pesticides considered in this study, the European limit is 100 ng/L for each individual pesticide. Thus, strategic sample composition outputs around this value will

mark sample specimens suspected of being polluted. Of course, significantly higher values will mark clearly polluted samples and values below this limit will mark unpolluted samples. Thus, we can consider a band around the legal limit that will define the course of action with samples. Samples predicted within this band probably should be reanalyzed individually to confirm pollution, while samples predicted outside this band do not require any additional confirmation.

Using these criteria, the proportions of false positives and false negatives, as well as full success proportions in prediction, have been calculated and summarized in Table VII. In the case of using maximum residue limits, a band of 30% around the limit was established in calculations.

From a practical point of view, it is clear that results for Sample Set 1 appear worse than for Sample Set 2. To understand the differences shown in Table VII for both sample sets, it should be noticed that design matrices were selected in both cases with an eye toward making sure that dilution in the

Table VI: Results obtained in the analysis of the composite samples prepared for Sample Set 2 (all concentrations are expressed in ng/L, nd = nondetected)

	α -BHC	γ -BHC	Heptachlor	Aldrin	α -Endosulfan	Dieldrin	Endrin	4,4' DDT	Endrin Ketone
C _{S1}	nd	28.7	10.2	6.2	nd	5.6	13.7	13.9	18.3
C _{S2}	18.8	8.9	nd	4.8	4.9	14.5	12.8	nd	18.6
C _{S3}	nd	nd	nd	nd	14.8	nd	nd	nd	6.4
C _{S4}	34.9	nd	nd	nd	15.6	12.9	8.6	21.9	25.3
C _{S5}	13.2	30.6	16.9	4.2	5.9	34.6	13.5	23.8	19.9
C _{S6}	14.7	35.8	15.4	5.3	nd	nd	nd	25.3	nd
C _{S7}	37.6	10.9	nd	4.5	5.7	26.7	8.6	20.5	nd
C _{S8}	nd	nd	18.2	4	6.5	32.4	13	16.6	nd
C _{S9}	30.4	32.1	11.4	4.4	17	11	21.9	nd	33.1
C _{S10}	12.7	10.6	nd	4.2	21	32.3	nd	18	6.1
C _{S11}	33.9	33.7	23.4	nd	19.2	nd	9.7	18.9	8.2
C _{S12}	17.8	15.4	4.7	2.5	10.7	15.5	9.9	11.6	13.8

Table VII: Evaluation of efficiency of SSC predictions for Sample Sets 1 and 2 in terms of false positives and false negatives percentage

	Sample Set 1			Sample Set 2		
	Criteria for definition of false positive and false negatives					
	LODs (Table III)	LOQs (Table III)	MRLs (reference 8)	LODs (Table IV)	LODs (Table IV)	MRLs (reference 8)
Full success (%)	79.3	83.0	95.6	95.8	95.8	99.1
False positives (%)	20.0	16.3	4.1	4.2	4.2	0.0
False negatives (%)	0.7	0.7	0.3	0.0	0.0	0.9

composition process would not compromise the detection and quantification of analytes in the composite samples. If we look at the Table III figures, we can see that for Sample Set 1, the dilution applied as a consequence of the 12:30 design matrix used allows the detection of all the pesticides considered. However, all of them fall below the quantification limits. On the other side, Table IV shows that in the 12:24 matrix, all pesticides (except 4,49DDT) are above quantification limits. This means that in Sample Set 1, experimental errors in the analysis of composite samples seriously compromises the strategic sample composition predictions for sample specimens with spiked levels close to maximum residue limits, and this influence is shown in the form of a small percentage of false negatives and false positives. On the contrary, if the dilution factor does not compromise the determination in composite samples, as in Set 2, strategic sample composition results clearly are advantageous compared with the individual analysis of the sample specimens. This is because very good estimations of the concentration levels in the samples is provided while reducing the cost and the analytical effort by a factor of 2.

Conclusions

In screening studies, where minimum cost and analysis time for large series of sample specimens are critical, strategic sample composition is a valuable tool that reduces the number of analyses to be carried out, thus producing direct savings in time, cost, and analytical effort. Depending upon the sample preprocessing and the allowable quantification limits, smaller or larger design matrices can be applied to build the composite samples. The presented results clearly show the importance of the selection of the sample preprocessing technique. The risk of false positives and negatives must be balanced with dilution in sample composition and the reduction of the number of analyses to be carried out. In general, design matri-

ces with column-to-row ratios of approximately 2:1 provide excellent performance, although larger ratios can be advantageously applied provided low enough limits of quantification. The use of automatic devices for unattended composite sample preparation derives the maximum from strategic sample composition because it avoids human errors and tedious working procedures, thus providing increased sample throughput in practical situations.

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