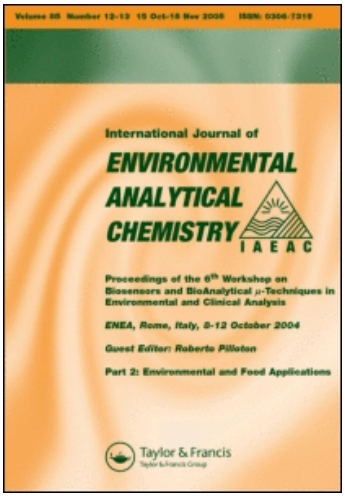

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EVALUATION OF AN IMMUNOCHEMICAL TEST KIT FOR POLYCHLORINATED BIPHENYLS IN SOILS AND COMPARISON WITH GAS CHROMATOGRAPHIC ANALYSIS

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The commercial immunochemical test kit Dräger EnviCheck PCB for the determination of polychlorinated biphenyl concentrations in soil was evaluated, and the results were validated by GC-ECD measurement. Four different types of soil were spiked with different concentrations of the PCB mixture Clophen A40 and analyzed by either of the methods. The test was carried out as a direct competitive enzyme immunoassay in test tubes. The test kit classified three different groups of PCB concentrations in soil: 'less than 1 mg/kg soil', 'between 1 and 10 mg/kg soil', and 'at least 10 mg/kg soil'. The results produced by the test kit showed a good intra- and inter-assay reproducibility and corresponded mainly with the nominal values of the fortification. Soils with a high content of organic matter produced a slight overestimation. False-negative determinations did not occur. GC-ECD measurement showed a good correspondence with the results of the test kit and the spiked PCB values.

KEY WORDS: Enzyme immunoassay, evaluation, GC-ECD, polychlorinated biphenyls, PCB, soil analysis.

INTRODUCTION

Polychlorinated biphenyls (PCB) have favourable physical properties that promoted their use in electrical equipment such as transformers or condensers until their ban in 1986. The most important properties are low thermal conductivity, low inflamability and chemical inertness. They are highly lipophilic and have a poor biodegradation rate which results in a wide spread distribution in the global ecosystem[1–3]. PCB have 209 possible isomers with different toxic and biologic responses[4,5]. The increase of chlorine atoms renders them more lipophilic and stable which leads to an accumulation in the food chain[6,7]. This hazardous potential needs a careful monitoring system. For a rapid analysis of many samples, cheap and simple methods are requested that are able to detect critical concentrations. Immunochemical methods are of broad application in medical diagnosis and have gained importance for the determination of environmental pollutants[8–12]. They are based upon the reaction between an antibody and its corresponding antigen.

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Environmental analysis can be easily carried out by immunoassays at low costs. Gas chromatographic analysis on the other hand affords a well equipped laboratory and time-consuming sample clean-up, especially when a high number of samples needs to be analyzed. Several immunochemical test kits for the detection of PCB in soil are commercially available:

- Dräger EnviCheck PCB, Dräger, Lübeck, FRG
- PCB RISC Soil & Wipe Test, Ensys, Research Triangle Park, NC, USA[13]
- Dtech PCB-Test, Merck, Darmstadt, FRG[14]
- Envirogard PCB, Immunosystems/Millipore, Scarborough, ME, USA

In this study the commercial test kit Dräger EnviCheck PCB has been evaluated. Soil samples can be analyzed in 30 minutes without sample clean-up. Up to 5 samples can be handled at the same time. Soils comprise a very inhomogeneous matrix containing substances that might cause interferences in immunochemical analysis. To establish analytical methods for certain substances, the validation of the results requires an alternating analytical method. For this purpose we determined the PCB concentrations of different soil samples by the test kit Dräger EnviCheck PCB and compared the results with analysis by gas chromatography-electron capture detection (GC-ECD). Several aspects of the test kit were tested: Intra-assay and inter-assay reproducibility were evaluated. The efficiency and reproducibility of the extraction were examined. Furthermore, the influence of different soil types and organic matter contents were evaluated.

EXPERIMENTAL

Test kit Dräger EnviCheck PCB

The PCB concentrations of different soil samples were analyzed by the test kit Dräger EnviCheck PCB according to the following procedure: 10 g of a soil sample were weighed into an extraction vial and 20 mL of methanol were added. The vial was vigorously shaken for one minute with 5 stainless steel beads for extraction. After one minute of sedimentation the supernatant layer was filtered through a plastic filter syringe to remove soil particles. 60 µL of sample were diluted in 540 µL of methanol, for a 1 : 10 dilution 60 µL of this mixture were diluted with 540 µL of methanol. 30 µL of the sample or the standard solution were buffered in assay buffer before immunoanalysis. The test was performed as a direct competitive enzyme immunoassay in test tubes which were coated with PCB specific antibodies. PCB standard solutions and samples were incubated in different antibody coated test tubes for 10 min, afterwards 3 drops of enzyme tracer solution were added. After 5 min a washing step followed to remove all unbound reagents. For the substrate-chromogen reaction 5 drops of H_2O_2 solution and the same amount of tetramethylbenzidine solution were incubated for 2.5 min. The reaction was stopped by 5 drops of H_2SO_4 and the colour intensity was measured at 450 nm. The intensity of the resulting yellow colour was inversely proportional to the PCB concentration in the soil. The determination of unknown PCB concentrations in soil was carried out by comparing the absorption values of standards and samples. The standard represented the PCB concentration 1 mg/kg soil. Each sample was measured in the

dilutions 1 : 1 and 1 : 10 and compared to the absorption value of the standard. The samples were classified into one of three concentration ranges:

'less than 1 mg/kg soil'
'between 1 and 10 mg/kg soil'
'at least 10 mg/kg soil'

A sample with a lower absorption value than the standard was positive. A positive 1 : 1 dilution meant a PCB concentration of at least 1 mg/kg soil, while a positive 1 : 10 dilution contained at least 10 mg/kg soil. When the sample showed a higher absorption value than the standard, the concentration was below 1 mg/kg soil. Twelve test kits served for the evaluation of the immunochemical test Dräger EnviCheck PCB. Each kit comprised 4 single tests for the determination of PCB concentrations in soils. Due to the manufacturer's information cross reactivities occur with several PCB mixtures. The detection limits are presented in Table 1.

Spiking procedure

Four different soils were chosen in order to determine the influence of different soil types on the test kit analysis:

I sandy loam with a low content of organic matter
II sand with a low content of organic matter
III loamy sand with a high content of organic matter
IV sandy loam with a low content of organic matter (soil I) spiked with 5% humic acid-Na-salt (Aldrich, Steinheim, FRG).

Soils I–III were obtained as standard soils from LUFA, Speyer, FRG. The characterization of these soils due to the LUFA report is presented in Table 2.

The soils were spiked with different concentrations of the PCB mixture Clophen A40 from a stock solution of 100 µg/mL in cyclohexane (Dr. Ehrenstorfer, Augsburg, FRG). Soils I and IV were spiked with PCB concentrations of 0, 0.3, 3, or 12 mg/kg. Soils II and III were spiked with 0, 3, or 12 mg/kg. The stock solution was added to 60 g of dry soil and mixed for 10 min. The solvent was allowed to evaporate. The soil samples were stored at 4°C for one week and mixed again before analysis. The extracts of the soil samples were analyzed at least in duplicate.

Table 1 Detection limits of cross-reacting PCB mixtures for the test kit Dräger EnviCheck PCB.

PCB mixture Arochlor	*Clophen*	*Fenclor*	*Detection limit [mg/kg soil]*
1248	A40		1.0
1254	A50	54	0.4
1260	A60	64	0.4
1242	A30	42	2.0
1232			4.0
1016			4.0

Table 2 Characteristics of soils I–III according to the report of LUFA, Speyer, FRG.

Soil *Charge no.*	*Soil I* *Sp14693*	*Soil II* *Sp24693*	*Soil III* *Sp34693*
Org. C (%)	0.62 ± 0.11	2.32 ± 0.38	1.22 ± 0.09
N content (%)	0.08 ± 0.02	0.23 ± 0.03	0.15 ± 0.04
pH (0.01 M $CaCl_2$)	5.9 ± 0.2	5.6 ± 0.2	6.4 ± 0.2
Cation exchange capacity (mval/100 g)	5.0 ± 0.0	10.9 ± 1.0	10.2 ± 0.5
Max. water capacity (g/100 g TM)	31± 2.0	48 ± 7	39 ± 4
Volume weight (g/1000 mL)	1410 ± 77	1233 ± 60	1289 ± 7
Particle < 0.02 mm (%)	6.5 ± 1.0	12.1 ± 2.3	22.1 ± 1.5
Grain size analysis			
< 0.002 mm	1.9 ± 1.3	5.5 ± 2.1	9.5 ± 1.7
0.002 – 0.006 mm	1.8 ± 0.9	2.5 ± 1.4	4.3 ± 1.4
0.006 – 0.02 mm	2.8 ± 0.4	4.1 ± 1.4	8.3 ± 0.6
0.02 – 0.063 mm	5.2 ± 1.0	6.8 ± 1.1	17.0 ± 1.1
0.063 – 0.2 mm	24.1 ± 1.8	34.3 ± 2.5	33.0 ± 2.0
0.2 – 0.63 mm	60.7 ± 1.0	45.9 ± 1.6	25.5 ± 0.9
0.63 – 2.0 mm	3.6 ± 0.6	1.0 ± 0.1	2.4 ± 0.6

Sample analysis

The different samples of soil I were analyzed as samples 1–4 and 1a–4a by the single tests *1ABCD, 2ABCD, 3ABCD, 4ABCD, 5ABCD, 6ABCD,* and *7ABCD.* The samples 5–7 of soil II were analyzed within tests *8ABCD,* and *9AB.* The samples 8–10 of soil III were analyzed by the tests *9BC* and *10ABCD.* The samples 11–14 of soil IV were analyzed by the tests *11ABCD* and *12ABCD.* The extraction efficiency was reported by the manufacturer to be 100%. In order to determine the extraction efficiency of this test during the evaluation, the extracts of soil I were measured by GC-ECD and the results were recalculated to the concentration in the soil samples and compared with the nominal value of fortification. Intra-assay and inter-assay reproducibility were determined by measuring the samples of soil I as triplicates within one test kit (intra-assay evaluation, tests *1ABC, 2ABC, 3 ABC, 4ABC*) and as triplicates in different test kits (inter-assay evaluation, tests *1C, 3D, 4D; 2C, 5A, 6A; 3C, 5B, 6B; 1D, 2D, 4C*). The reproducibility of the extraction was tested by extraction of separate parts of the soil samples 1–4 of soil I. The samples of the second extraction were named 1a–4a and were determined in the tests *5CD, 6CD, 7ABCD*). The distribution of the samples within the test kits is presented in Figure 1.

GC-ECD determination

The PCB concentrations of all soil extracts were determined by GC-ECD. The instrument parameters were chosen as presented in Table 3. The PCB congeners #28, #52, #60, and #66 served as indicator PCB because they take the biggest part in Clophen A40. If the concentrations of these indicator PCB are measured, it is possible to recalculate the concentration of the PCB sum because the percental parts of the indicator PCB are known. To preclude false-positive values, not the pure indicator PCB were used

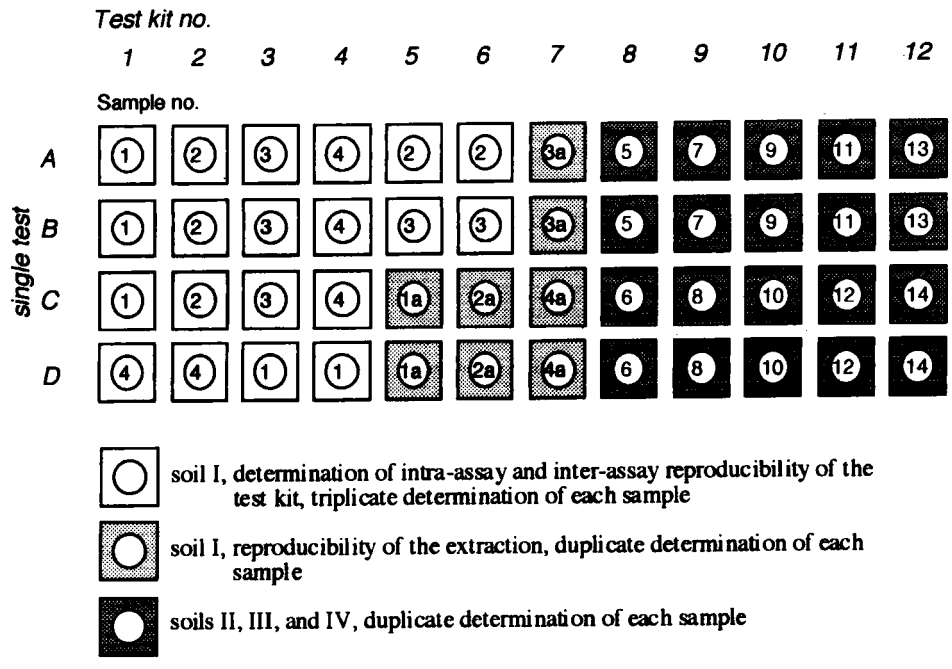

Figure 1 Distribution of soil samples within the different test kits.

Table 3 GC-ECD parameter, Fisons, HRGC 8165.

Carrier gas	Nitrogen	2.3 mL/min	
Injector	SSL-Injector 290°C, splitless injection after 1 min of split opening	Injection volume 0.5 µL	Manual
Retention gap	1 m	0.32 mm ID	Phenyl-Sil deactivated
Column	DB 5	60 m	0.25 ID 0.25 µm df
Detector	ECD	Ni-63	280°C
Make up gas	Argon/methane (95 : 5, v/v)	45 mL/min	
Temperature program	70°C (1 min), with 20°C/min to 170°C, with 14°C/min to 270°C, with 10°C/min to 280°C, 280°C (10 min)		

as external standards but the indicator PCB were determined in a Clophen A40 external standard solution (10 µg/mL). From each indicator PCB results a value for the PCB sum and the final PCB sum concentration is given as average value of the four indicator PCB. Furthermore the percental parts of the indicator PCB in the Clophen A40 standard were

measured and compared with the values in the literature[5] to ensure a good integration. The results are shown in Table 4.

RESULTS

Prior to the determination of PCB concentration in the soil samples by the immunochemical test kit and by GC-ECD the percentage of the different indicator PCB determined by GC measurement were compared to data from literature. Table 4 shows that the results of our GC measurements are comparable to literature data except for the sample of PCB #28 because of coeluating PCB #31, so that reproducibility and correct integration can be assumed.

The four different soils were spiked with varying concentrations of the PCB mixture Clophen A40 and analyzed by the immunochemical test kit Dräger EnviCheck PCB. Intra- and inter-assay reproducibility and the reproducibility of the extraction were examined. GC-ECD measurement of the extracts determined the efficiency of the extraction. The results of the immunochemical test kit were evaluated by comparing the data to the results of GC-ECD measurements.

Soil I

Soil I (sandy loam with a low content of organic matter) served for the determination of intra-assay reproducibility and inter-assay reproducibility (samples 1–4). The samples were spiked with PCB concentrations of 0, 0.3, 3, or 12 mg/kg. The extracts were measured as triplicates. Furthermore the reproducibility of the extraction was evaluated (samples 1a–4a). 28 single test kit analyses were carried out. 27 of the results corresponded well with the spiked concentrations. The analysis *4D*, a replicate of sample 1, gave a false-positive result. It was determined to contain 'more than 10 mg/kg' instead of 'less than 1 mg/kg'. The concentrations determined by GC-ECD corresponded mainly with the spiking data. Samples 4 and 4a, which had a fortified concentration of 12 mg/kg, showed an underestimation by GC-ECD. The GC-ECD data were 4.95 mg/kg (sample 4) and 5.40 mg/kg (sample 4a) instead of 12 mg/kg. The results are presented in Figure 2 and in Table 5.

Reproducibility of the extraction

The different extractions of the same sample showed reproducible results. Samples

Table 4 Detection limits and percentage of the indicator PCB in the Clophen A40 mixture.

PCB congener	*Detection limit [mg/kg]*	*Percentage of indicator PCB in Clophen A40 [%]; literature [5]*	*GC measurement [%]*
PCB #28	0.08	4.0	6.5
PCB #52	0.14	7.3	7.6
PCB #60	0.08	3.1	3.2
PCB #66	0.05	5.7	7.3

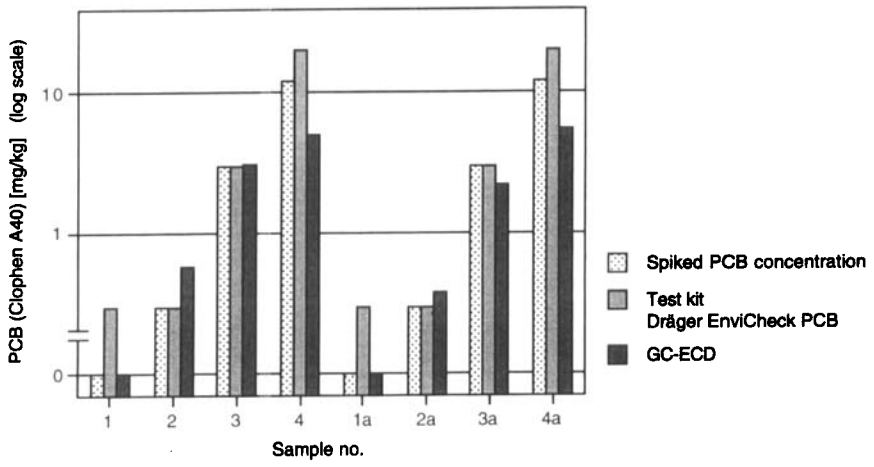

Figure 2 Comparison of PCB concentrations in spiked soil samples (soil I) determined by the test kit Dräger EnviCheck PCB and by GC-ECD. The test kit classifies the samples into the categories 'less than 1 mg/kg soil', 'between 1 and 10 mg/kg soil', or 'at least 10 mg/kg soil'. These cateories are represented by the fictitious values 0.3 ('less than 1 mg/kg soil'), 3 ('between 1 and 10 mg/kg soil'), and 20 ('at least 10 mg/kg soil') in the logarithmic scale.

Table 5 PCB concentrations of different spiked soil samples determined by the test kit Dräger EnviCheck PCB and by GC-ECD. The test kit classifies the samples into categories 'less than 1 mg/kg', 'between 1 and 10 mg/kg', or 'at least 10 mg/kg'.

Soil type	*Sample no.*	*Spiked PCB-concentration Clophen A40 [mg/kg soil]*	*Test kit Dräger EnviCheck PCB [mg/kg soil]*	*GC-ECD [mg/kg soil]*
soil I	1	0	< 1	–
sandy loam with a	2	0.3	< 1	0.58
low content of	3	3	1 – 10	3.05
organic matter	4	12	> 10	4.95
soil I	1a	0	< 1	–
sandy loam with a	2a	0.3	< 1	0.38
low content of	3a	3	1 – 10	2.21
organic matter	4a	12	> 10	5.40
soil II				
sand with a low	5	0	< 1	–
content of organic	6	3	1 – 10	2.30
matter	7	12	> 10	14.40
soil III				
loamy sand with a	8	0	< 1	–
high content of	9	3	> 10	3.50
organic matter	10	12	> 10	3.90
soil IV	11	0	< 1	–
soil I spiked with	12	0.3	1 – 10	0.30
5% humic acid-Na-	13	3	1 – 10	1.60
salt	14	12	>10	9.90

1a–4a (test kits *5CD, 6CD, 7ABCD*) yielded the same results as samples 1–4 (test kits *1ABC, 2ABC, 3ABC*). They corresponded well with the spiking data.

Efficiency of the extraction

A high efficiency of the extraction was found with yields about 100% or more (193%) in case of concentrations up to 3 mg/kg. However, except for a few samples the extraction yield decreased to 30% (sample 10; soil III) with higher PCB concentrations as determined by GC-ECD. The efficiency did not depend on the soil type.

Intra-assay reproducibility

The intra-assay reproducibility (Test kits *1ABC, 2ABC, 3ABC*) was very exact. The measurements of samples 1–4 (soil I) gave the same result for each of the replicates and corresponded well with the values of fortification. There were no false-positive or false-negative detections. The results are presented in Table 6.

Inter-assay reproducibility

The inter-assay tests showed reproducible results (tests *1C, 3D, 4D; 2C, 5A, 6A; 3C, 5B, 6B; 1D, 2D, 4C*). All values except one replicate of sample 1 (test *4D*) were conform with the values of fortification. This replicate showed a false-positive result with 'at least 10 mg/kg' instead of 'less than 1 mg/kg'. The results are shown in Table 6.

Table 6 Intra-assay and inter-assay reproducibility of the immunochemical test kit Dräger EnviCheck PCB.

Sample no.	*Spiked PCB-concentration Clophen A40 [mg/kg soil]*	*GC-ECD [mg/kg soil]*	*Replicates of test kit intra-assay evaluation [mg/kg soil]*	*Replicates of test kit inter-assay evaluation [mg/kg soil]*
1	0	–	< 1	< 1
			< 1	< 1
			< 1	> 10
2	0.3	0.58	< 1	< 1
			< 1	< 1
			< 1	< 1
3	3	3.05	1 – 10	1 – 10
			1 – 10	1 – 10
			1 – 10	1 – 10
4	12	4.95	> 10	> 10
			> 10	> 10
			> 10	> 10

Soil II

Soil II (sand with a low content of organic matter) was spiked with three different PCB concentrations (0, 3, or 12 mg/kg) for analysis in 6 single tests (samples 5–7, test kits *8ABCD, 9AB*). The results corresponded well with the values of fortification. Determination of PCB concentrations by GC-ECD showed good correspondence. The results are presented in Figure 3 and Table 5.

Soil III

The analyses of three samples of soil III (loamy sand with a high content of organic matter) were carried out in duplicate (samples 8–10, test kits *9CD, 10 ABCD*). Out of these 6 single test the results of samples 8 and 10 corresponded well with the values of fortification. The two replicates of sample 9 (test kit *10AB*) were overestimated as 'at least 10 mg/kg' instead of 'between 1 and 10 mg/kg'. GC-ECD analysis corresponded well but resulted in an underestimation of sample 10 (3.9 mg/kg instead of 12 mg/kg). The results are shown in Figure 3 and Table 5.

Soil IV

Soil IV (soil I spiked with 5% humic acid-Na-salt) was fortified with PCB concentrations

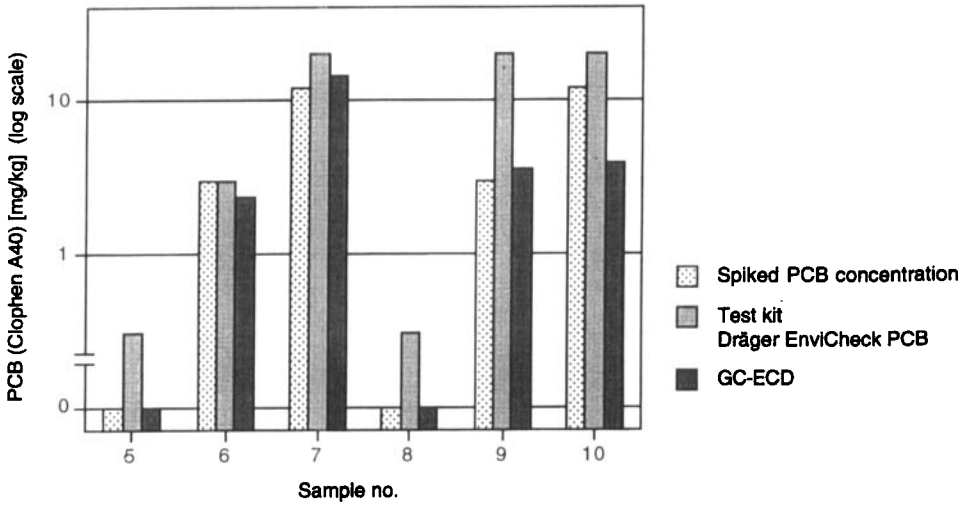

Figure 3 Comparison of PCB concentrations in spiked soil samples of soils II and III as determined by the test kit Dräger EnviCheck PCB and GC-ECD. The test kit classifies the samples into the categories 'less than 1 mg/kg soil', 'between 1 and 10 mg/kg soil,' or 'at least 10 mg/kg soil'. These categories are represented by the fictitious values 0.3 ('less than 1 mg/kg soil'), 3 ('between 1 and 10 mg/kg soil'), and 20 ('at least 10 mg/kg soil') in the logarithmic scale.

of 0, 0.3, 3, or 12 mg/kg (samples 11–14). They were analyzed in 8 single tests (tests *11ABCD, 12ABCD*). Six out of 8 analyses corresponded with the values of fortification, the two replicates of sample 12 (tests *11AB*) were overestimated as 'between 1 and 10 mg/kg' instead of 'less than 1 mg/kg'. GC-ECD analysis corresponded with all samples except for sample 13 which was underestimated. The results are presented in Figure 4 and Table 5.

DISCUSSION

Immunoassays have gained growing importance in environmental analytics in the last years[8,10–12]. They offer advantages when many samples need to be screened for pollutants in a short time. Samples can be measured after simple extraction steps. Clean-up steps and sample concentration are not necessary. For conventional analytical methods well equipped laboratories and instruments as gas chromatographs or mass spectrometers are necessary. When immunoassays are introduced to the market for commercial use careful evaluation of the method and validation of the results by a different analytical method is necessary. The immunochemical test kit Dräger EnviCheck PCB for the determination of PCB concentrations in soil was evaluated. Four different types of soil were spiked with varying concentrations of PCB Clophen A40 and analyzed by the test kit and by GC-ECD. The different soil types contained varying amounts of organic matter. In the case of the enzyme immunoassay the results corresponded mainly with the nominal values after spiking. False-negative results did not occur. Sample 9 (soil III) and sample 12 (soil IV), which had a high content of organic matter, showed reproducible overestimations by the enzyme immunoassay. In this case the enzyme tracer or the antibody binding site was probably inhibited by organic matter, which resulted in a lower absorption and therefore

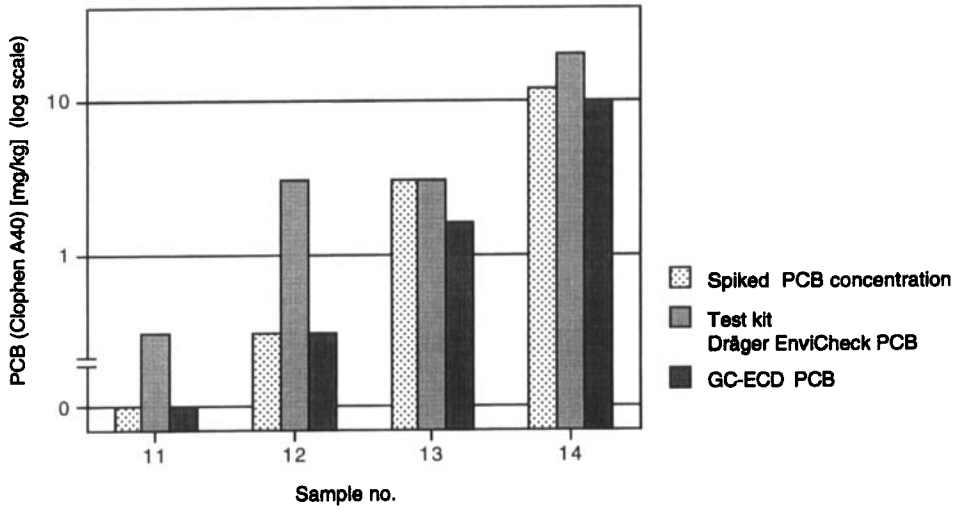

Figure 4 Determination of PCB concentrations in spiked samples of soil IV by the test kit Dräger EnviCheck PCB and by GC-ECD. The test kit classifies the samples into the categories 'less than 1 mg/kg soil', 'between 1 and 10 mg/kg soil', or 'at least 10 mg/kg soil'. These categories are represented by the fictitious values 0.3 ('less than 1 mg/kg soil'), 3 ('between 1 and 10 mg/kg soil'), and 20 ('at least 10 mg/kg soil') in the logarithmic scale.

in a false-positive detection. Johnson and Van Emon[15] reported on this effect. They estimated that cross reacting compounds were extracted by methanol and reacted in the ELISA. To remove this effect a further clean-up step might lead to a higher accuracy.The extraction efficiency for the 1-minute extraction was found to be 100% or more with concentrations up to 3 mg/kg soil. They decreased with higher PCB concentrations. Lawruck *et al.*,[16] tested the extraction efficiency as a function of time (1 minute, 30 minutes, 18 hours). They showed that the 1-minute extraction was sufficient and that longer extractions did not improve the extraction efficiency. The reproducibility of the extraction was tested by separate extraction of two parts of the same soil sample. The results corresponded well. The intra-assay and inter-assay reproducibility showed good results. The results corresponded well with the spiked PCB concentrations.

In the case of GC determination the recovery varied in dependence of the spiked concentrations. In the case of low spiking concentrations the recovery is quantitative. However, recoveries were lower when the soil was spiked with PCB concentrations > 10 mg/kg (sample 4, 4a, and 10). Baek[17] reported on extraction yields around 20% using methanol as extracting solvent. He spiked his soils with a non-aqueous phase liquid containing many chlorinated aliphatics and aromatics in addition to PCBs. As possible explanations for the insufficient extraction yield he states saturation of the extractant as well as blocking contact with an extractant caused by other soil components. The saturation of the extractant might apply to the case of higher PCB concentrations. Chiou *et al.*,[18] reported enhanced solubility of unpolar compounds such as DDT and PCB in water in case of high concentrations of dissolved organic matter (DOM). Then PCB are adsorbed and/or absorbed by DOM and might be retained while filtering the soil/solvent mixture. The measured values are affected by the following errors:

- dilution of the standard solution
- incomplete extraction of the soil sample with methanol
- losses during the evaporation of the sample
- incomplete phase separation (soil/methanol)

The immunochemical test kits are easy to handle and cheaper than gas chromatographic analysis. Problems arise when complex matrices exist, e.g. soils with high biological activity, or when sewage sludges need to be analyzed. Then false-positive results may occur by molecules blocking the antibodies or the enzyme tracer. For validation more test kits should be studied considering different PCB compositions. Moreover, tests should be carried out with real soil samples, especially in the range near 1 mg/kg because this is the detection limit given by the manufacturer. The results reveal that the immunoassay yields reliable results within the given limits. This technique is suitable when fast decisions are required, for example in the case of field analysis at contaminated sites. On-site analysis can be carried out with simple laboratory equipment such as balance, pipettes, and photometer. For this purpose immunoassays are superior to conventional analytical methods.

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