

On the Use of ELISA Commercial Test Kits for PCB Screening in Sediments: Practical Implications



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Introduction and Objectives

Polychlorinated biphenyls (PCBs) are a family of persistent organic pollutants (POPs) of anthropogenic origin (209 different congeners) which are widely distributed in the environment. Sediments constitute reservoirs of PCB and are therefore a key compartment in the study of the quality status of water bodies (PCBs have been proposed for the inclusion in the European Water Framework Directive priority list). In addition, PCB levels in dredged sediments need to be monitored within a management context in order to decide on their possible uses (immersion, terrestrial treatment, storage, or valorization). Various screening methods have been employed for PCB determination in environmental matrixes in the last decades, immunoassays (in particular enzyme-linked immunosorbent assay, ELISA) being one of the most employed. The objectives of this work are: (1) to assess the performance of a commercial ELISA test kit for PCB analysis in sediments and to compare with a classical approach (reference method, GC/MS); (2) To assess the suitability of a commercial ELISA test kit within the context of the French legislation on sediments management.

Material and methods

➤ **Reference method for PCB analysis:** Determination of indicator PCB (CB-28, 52, 101, 138, 153 and 180) was based on the US EPA 1668B method. Samples (10g) were Soxhlet extracted with a mixture of n-hexane/acetone 50% for 24h. The analysis was carried out on a Varian 450-GC coupled with a 240-MS ion Trap mass spectrometer. Quantification of individual PCB congeners was achieved working on Selected Ion Storage (SIS) method (i.e. only selected m/z for native and labeled PCB are considered in the detection).

➤ **ELISA method for PCB analysis:** Sediment samples (10g) were extracted with 20ml of methanol by manual shaking for 1 min. For the ELISA detection, both endogenous PCBs and PCB enzyme conjugated (horseradish peroxidase labeled PCB analog) compete for polyclonal antibody binding site. The calibration was performed by using Aroclor 1254 solutions. Thus, the results are expressed as Aroclor 1254 equivalents. Aroclor composition of indicator PCB in Aroclor 1254 is presented in table below. This ELISA test is designed to work in a concentration range of 20 – 25000 ppb.

Aroclor 1254	Composition (%)
MoPCB	0,024
DiPCB	0,3
TriPCB	0,73
TePCB	14
PePCB	55
HxPCB	26
HpPCB	4,1
OcPCB	0,43
NoPCB	0,041
DePCB	0,003
TriCB-28	0,13
TeCB-52	4,8
PeCB-101	9,7
HxCB-138	4,6
HxCB-153	7
HpCB-180	0,95
Σ6PCBs	27,18

- Performances of both methods are assessed with a certified Reference Material (RMs):
 - BCR-536, IRMM-JRC composed of 6 indicators PCB and 7 others PCB on fresh water sediment,
 - CNS339-050 composed of 6 indicators PCB and PBDEs on fresh water sediment,
 - CMR 923-050 composed of Aroclor 1254 on silt loam.
- Repeatability of revelation and detection step (CNS339-050):
 - 1 extraction + 10 independent dilution (1:200) + 2 measurements of each dilution.
- Repeatability of extraction step (CMR 923-050):
 - Procedure: 1 extraction + 1 dilution in triplicate + 2 measurements of each dilution
 - The whole procedure is repeated 3 times for two dilutions (1:200 and 1:800).

Final Remarks

• ELISA could be used as a complementary approach to GC/MS. Indeed ELISA allows determination of PCB total concentration where GC/MS analysis is limited by the matrix effect.

- ✓ ELISA can be used, keeping in mind some issues:
 - non specific reaction (polyclonal antibody).
 - this ELISA test is more adapted when the PCBs proportion is close to Aroclor 1254.
 - If used only for indicator PCB analysis, an antibody against the 6 indicators PCB should be developed in order to provide with a concentration as indicator PCB equivalents.
- ✓ The French legislation for sediment management classify then them in 3 classes : less than 500 ppb (Class 0), between 500-1000 ppb (Class 1), higher than 1000 ppb (Class 2). Available ELISA commercial kits are appropriate to discriminate Class 0 sediments that can be re-submerged. For Class 1 and Class 2 sediments that need treatment a more precise quantification by GC/MS would be required.

Results

➤ Performance of the GC/MS and ELISA methods, concentrations (ng/g, ppb)

PCB in Reference Material	Certified concentration (ppb)	GC/MS (ppb)	ELISA (ppb)
BCR-536 (IRMM-JRC)			
PCB-28	44 5	60	
PCB-52	38 5	38	
PCB-101	44 4	38	
PCB-138	27 5	47	
PCB-153	50 4	18	
PCB-180	22.4 2	17	
Σ6 indicator PCBs	225	218	
Σ13PCBs	345		440
CNS339-050			
Σ 6 indicator PCBs	1330		2500
CMR 923-050 (Aroclor 1254)			
	5470		3400 (1:200) 5070 (1:800)

- For BCR-536, the total concentration of the PCBs indicators obtained by GC/MS is close to the reference value and the total concentration obtained by ELISA is in good agreement with the reference value.
- For CNS339-050, the concentration obtained by ELISA is higher than the reference value, may be due to non-specific reactions with PBDEs also present in the RM (polyclonal antibody).
- For CMR 923-050, the concentration obtained by ELISA is close to the expected value, nevertheless our experience is that for concentrations >5000 ppb, the dilution preconized by the manufacturer (1:200) may not be enough (results using 1:800 were closer to certified values in the RMs).

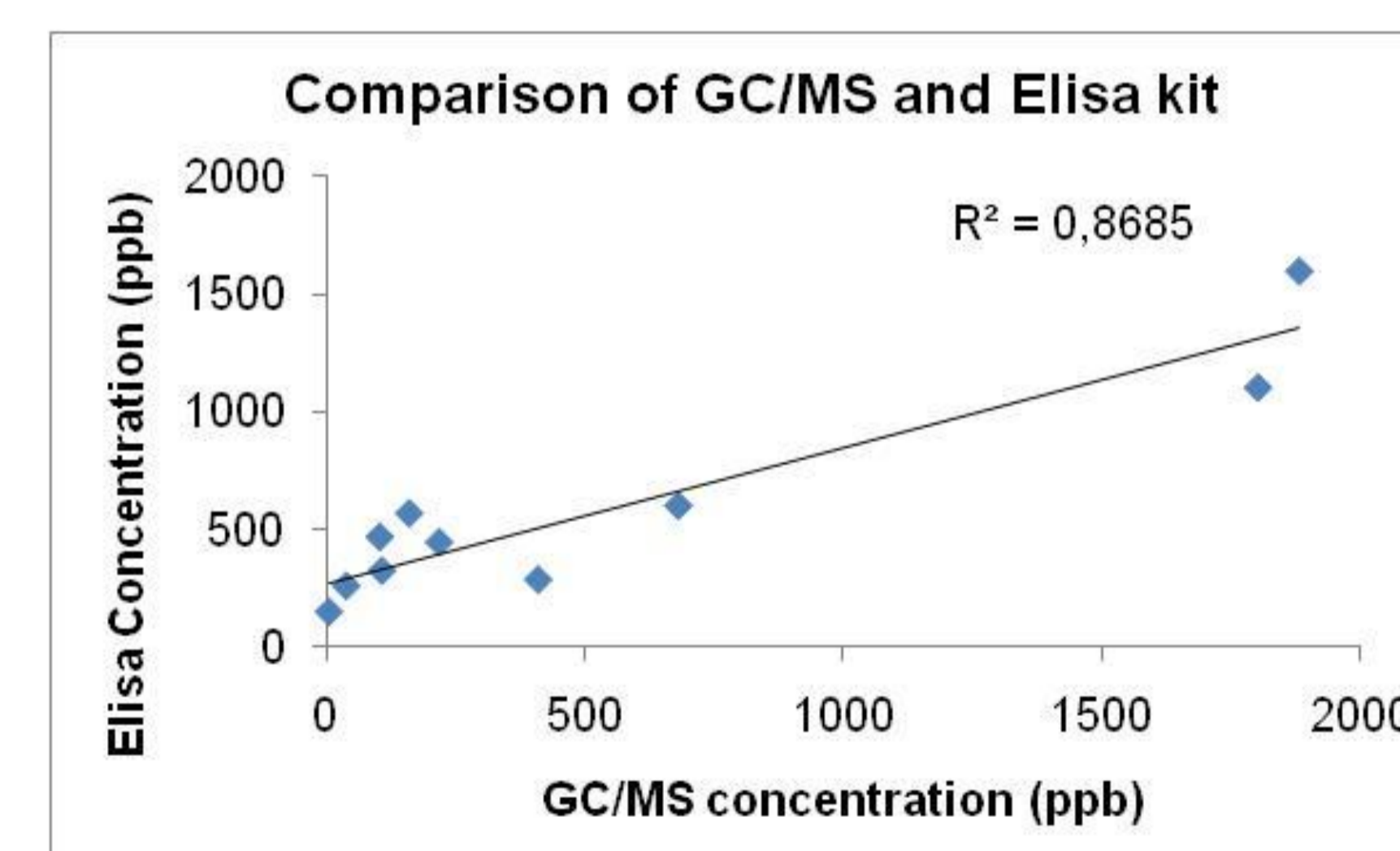
➤ Repeatability of ELISA based on different Reference Materials

- Repeatability of PCB detection (CNS339-050): total variation coefficient obtained is 5,8%. The differences between detection are not statistically significant ($P > 0,05$).
- Repeatability of extraction step (CMR 923-050): total variation coefficient obtained is 8,3% where between extractions variation is 2,4%, between revelation variation is 0,12%. The differences between detection and between extractions are not statistically significant ($P > 0,05$).

➤ Comparison of GC/MS and ELISA results , concentrations (ng/g, ppb)

Sample code	Sediment	GC/MS (ppb)	ELISA kit (ppb)
T1	Marine	107	323
T2	Marine	analysed ^a	140
T3	Marine	analysed ^a	480
T4	Marine	160	568
EU1	Fresh water sediment	680	600
EU2	Fresh water sediment	1880	1600
EU3	Marine	409	285
EU4	Fresh water sediment	1800	1104
C1	Marine	5	148
C2	Marine	analysed ^a	470
C3	Marine	38	258
C4	Marine	103	468
BCR-536 (IRMM-JRC)	Fresh water sediment	218	440

^a Quantification was not possible due to a very important matrix effect



- ELISA provided a total concentration as Aroclor 1254 equivalents whereas GC/MS analysis provided the PCB total concentration as indicator PCB equivalents.
- However, the results obtained by ELISA and GC/MS for studied sediments are in good agreement ($R^2 = 0.8685$).

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