DR-CALUX® INTERLABORATORY VALIDATION STUDY FOR SEDIMENTS.

Harrie Besselink[1], Cor Schipper[2], Hans Klamer[2], Pim Leonards[3] Henk Verhaar[4], Emiel Felzel[1] and Bram Brouwer[5]

[1]BioDetection Systems bv (BDS), Badhuisweg 3, 1031 CM Amsterdam, The Netherlands
[2]National Institute for Coastal and Marine Management/RIKZ, P.O.Box 8039, 4330 EA Middelburg, The Netherlands
[3]Netherlands Institute for Fisheries Research (RIVO), P.O. Box 68, 1970 AB IJmuiden, The Netherlands
[4]OpdenKamp•Registration & Notification (OR&N), Koninginnengracht 23, 2514 AB The Hague, The Netherlands
[5]Vrije Universiteit, Institute for Environmental Studies, de Boelelaan 1115, 1081 HV Amsterdam, The Netherlands

Introduction

In the Fourth National Policy Document on Water Management in the Netherlands [Ministry of Transport, Public Works and Water Management, 1997], it is defined that in 2002, in addition to the assessment of chemical substances, special guidelines for the assessment of dredged material should be recorded. The assessment of dredged material is based on integrated chemical and biological effect measurements. Among others, the DR-CALUX® bioassay has tentatively been recommended for inclusion in the dredged material assessment. To ensure the reliability of this bioassay, a Quality Control Management system is being developed. One of the issues to be investigated in relation to the development of this Quality Control Management system is the accuracy and repeatability of the DR-CALUX® bioassay. To this end, an inter-laboratory validation study, or ring test, was performed, organized by the Dutch National Institute for Coastal and Marine Management/RIKZ in cooperation with BioDetection Systems B.V. (BDS). The results of this study are intended as a starting point for implementing the integrated chemical-biological assessment strategy, and for systematic monitoring in the coming years.

Methods and materials

Six laboratories, located in the Netherlands (BioDetection Systems bv (BDS); Institute for Environmental Studies (IVM), Wageningen University and Research Centre, Toxicology Group), the UK (CEFAS), Japan (KANEKA Corporation), and Belgium (VITO) were invited to participate. The DR-CALUXâ inter-laboratory study consisted of 3 phases: phase 1: DR-CALUXâ analysis of 2 defined standard solutions (2,3,7,8-TCDD in DMSO; TCDD/ PCB mix in DMSO); phase 2: DR-CALUXâ analysis of a sediment extract, prepared by the RIVO; phase 3: DR-CALUXâ analysis of a sediment extracts prepared by the participants. Sediment samples were extracted and cleaned prior to analysis of dioxin and/or dioxin-like compounds using the DR-CALUX® bioassay, using the protocol supplied by RIVO, version 1. Total 2,3,7,8-TCDD TEQ content was determined using the DR-CALUX® bioassay according to the guidelines from BioDetection Systems (BDS-version 9a) or RIKZ (RIKZ/Specie-o7-version 2). Each of these laboratories analysed blind samples in each of three phases of the study. Statistical analysis to interpret the results of the inter-laboratory validation study, was performed by OpdenKamp•Registration & Notification (OR&N).

Results and Discussion

For all of the participants, Shewart control charts were constructed using the 3 pM 2,3,7,8-TCDD concentration of the 2,3,7,8-TCDD calibration curve. Over the analysis period, none of the participants exceeded the action levels (data not shown).

Phase 1: In table 1, the averaged DR-CALUX® results for the concentration of 2,3,7,8-TCDD equivalents per participant and per sample are given. The results for the dioxin sample are slightly higher then the expected 2,3,7,8-TCDD concentration. The percentage standard deviation for the dioxin sample between participants was calculated to be 7.0% (avg. = 4.62; SD. = 0.32). The percentage standard deviation for the mixed sample between participants was calculated to be 11.4% (avg. = 4.00; SD. = 0.45).

Phase 2: Three sediment extracts, obtained from the same extract, were provided to the participants. Samples were analysed in triplicate on three separate 96-microtiterplates. In Fig. 1, the DR-CALUX® results for 30-times diluted extracts, are presented. The percentage standard deviation for the sediment extract sample between participants was calculated to be 15.2% (avg. = 35.68; SD. = 5.42). All participants also analysed a 10-times dilution of the sediment extract samples. DR-CALUX® analyses for this dilution showed slightly lower dioxin-like TEQ contents (data not shown). In addition to the sediment extract, all participants also received a procedure blank and were kindly asked to analyse this sample as well. DR-CALUX® analysis results of this procedure blank for all participants were lower then 1 pM 2,3,7,8-TCDD TEQ/well. This is according to the standards set for the DR-CALUX® bioassay.

Table 1. Result of the DR-CALUX® analyses of phase 1 (dioxin and mixed sample) of the interlaboratory validation study.

Participant	Dioxin sample			Mixed sample			
	Avg.conc (pM)	SD.	% SD.	Avg. Conc (pM)	SD.	%	SD.
Lab A	4.49	0.42	9.4	3.72	0.45	12.1	
Lab B	4.52	0.95	21.0	3.24	0.45	13.9	
Lan C	5.14	0.43	8.4	4.52	0.51	11.3	
Lab D	4.59	0.52	1.0	4.28	0.45	10.5	
Lab E	4.81	0.55	11.4	4.17	0.54	12.9	
Lab F	4.18	1.43	34.2	3.96	1.42	35.9	

The actual 2,3,7,8-TCDD concentration of the sample analysed is 4.29 pM. The mixed samples consisted of 2,3,7,8-TCDD, PCB-126 and PCB-169. The expected DR-CALUX® 2,3,7,8-TCDD TEQ concentration was 4.06 pM.

Phase 3: In phase 3, the participants were asked to prepare extracts themselves, using a soxhlet extraction procedure provided by the coordinator. It should be noted that some of the participants did not have prior experience using the supplied extraction protocol. Furthermore, the participants were free to use the dilution factor of their choice. In Fig.2, the averaged DR-CALUX® results are presented graphically. The data shown are results from 0-200x diluted extract. The average concentration 2,3,7,8-TCDD equivalents in the analysed sediment was 4.13 ± 1.14 pg 2,3,7,8-TCDD TEQ/g sediment (%SD = 27.6).

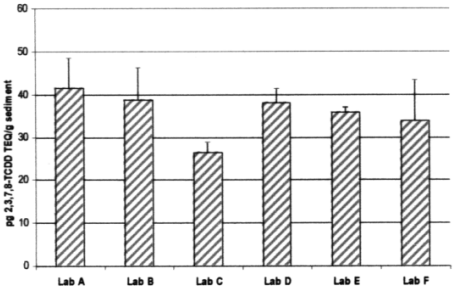

Figure 1. Averaged DR-CALUX® results by participant for the analysed sediment extracts (phase 2). The data presented are the 30X diluted samples.

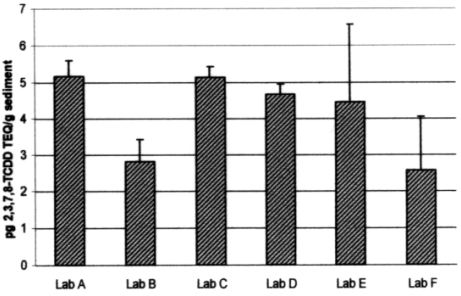

Figure 2. Averaged DR-CALUX® results by participant for the sediment extracted by the participants (phase 3). Participants were free to choose their own dilution factor for analysis. Data presented are 0-200X diluted samples.

The repeatability of the DR-CALUX® bioassay was calculated for the four samples (2,3,7,8-TCDD sample, mixed sample, sediment extract and sediment sample) analysed by the participants over the 3 phases of the interlaboratory validation study (table 2).

Table 2. Repeatability and reproducibility for the DR-CALUX© bioanalysis of the various phases of the present interlaboratory validation study.

		Dioxin sample	Mixed sample	Sediment extract	Sediment (0-200x diluted)
Repeatability (%)	Lab A	9.4	12.1	17.1	8.3
	Lab B	21.0	13.9	19.4	20.6
	Lab C	8.4	11.3	8.9	5.8
	Lab D	1.0	10.5	8.6	5.8
	Lab E	17.0	15.5	19.5	37.8
	Lab F	34.2	35.9	28.4	56.8
Average repeatablitiy (%)		14.6	16.0	15.0	26.1
Reproducibiltiy (%)		6.5	10.5	18.0	27.9

Note: Repeatability was calculated as % standard deviation of 9 individual analysis results for the indicated analysed samples. Reproducibility was calculated as % standard deviation of the averaged analysis results of the participating laboratories per analysed sample.

The average repeatability for the participating laboratories ranged from 14.6% for the dioxin sample analysis to 26.1% for the sediment samples that had to be extracted by the participants. Repeatability was lowest for phase 3 during which the participants were asked to extract, clean-up and perform a DR-CALUX® bioassay on a supplied sediment sample. Since none of the participants had

prior experience using the supplied extraction procedure, it can be anticipated that with increasing experience using the supplied extraction protocol, the repeatability will also increase. Table 2 also shows the reproducibility for the various analysed samples. The percentage standard deviations over the DR-CALUX® bioanalysis results for the analysed samples ranged from 6.5% for the dioxin sample to 27.9% for the supplied sediment sample. Again, the biggest differences in analysis results were observed in the sediment sample that had to be extracted and cleaned-up by the participants themselves. Increasing experience using the supplied extraction protocol should improve the extraction performance and hence the repeatability and reproducibility.

Limit of detections ('LOD'), defined as the average control response per well plus three times the standard deviation, were recorded for all experiments. LOD varies between 0.51 pM and 1.17 pM , with an average of 0.34 ± 0.3 pM. Analysis of variance indicated no significant differences in limit of detection between laboratories could be identified.

Overall conclusions

Several overall conclusions can be drawn, based on the statistical evaluation of the data submitted by the participants of the DR-CALUX® inter-laboratory validation study:

- The average results, over all participants, are very close to the expected concentration, expressed in DR-CALUX® TEQs 2,3,7,8-TCDD for the analytical samples.
- There are significant differences in the results obtained by the individual participants, such that the inter-laboratory variability exceeds the intra-laboratory variability. This is true for analytical sample measurements, for sediment extracts as provided by the coordinator, as well as for sediments extracted by the participants themselves.
- The inter-laboratory variation for the different sample types can be regarded as estimates for the method variability.
- The analytical method variability is estimated to be ±10.5 % for analytical samples and ± 22.0 % for sediment extracts.
- Responses appear dependent on the dilution of the final solution to be measured. This is hypothesized to be due to differences in dose-effect curves for different dioxin-responsive-element active substances. For 2,3,7,8-TCDD,this effect is not observed. It is suggested that this effect may, in principle, be exploited to derive additional information on the major dioxin-responsive-element active substances in an unknown sample.

Acknowledgements

The authors would like to thank the following persons and organizations for participating in the present DR-CALUX® interlaboratory validation study: Dr. Ir. Juliette Legler, Institute for Environmental Studies (IVM), Amsterdam, the Netherlands; Dr. Albertinka Murk, Wageningen University and Research Centre, Toxicology Group, Wageningen, the Netherlands, Dr. John Thain, CEFAS, Burnham-on-Crouch, UK; Dr. Peter Behnisch, KANEKA Corporation, Takasago-shi, Japan and Dr. Greet Schoeters, VITO, Mol, Belgium.