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COMPARISON OF DR-CALUX® AND HRGCMS- DERIVED TEQs: INTRODUCTION OF CONVERSION FACTORS

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Introduction

Food safety is a high priority issue for the feed and food industry, retailers and governmental regulators nowadays. At the present time stringent EU limit values are enforced for dioxins and furans in feed and food. The use of bioassays, like the DR-CALUX® system for monitoring dioxins in food and feed, allows the (pre)-selection of samples suspected of being contaminated above limit values with dioxins and/or furans.
BDS' DR-CALUX® bioassay system, in its present configuration, measures the contribution of both dioxins/furans and dioxin-like PCBs. For some matrices such as fishoil, the expected contribution of dioxin-like PCBs may be considerable. By applying the C-SPLIT® [1] method in combination with the DR-CALUX® bioassay, it is possible to discriminate between TEQ contributions by dioxins/furans on one hand and dioxin-like PCBs on the other hand.

Although the overall comparison of DR-CALUX®-derived TEQs with HRGCMS-derived TEQs is good, some consistent differences in results have been observed for some matrices as well. Here we report on the introduction of conversion factors to improve the comparison of HRGCMS and bioassay results for fish based products.

HRGCMS-derived TEQ vs DR-CALUX®-derived TEQ

Overall good correlations between total HRGCMS TEQs (TCDDs, TCDFs and PCBs) and DR-CALUX® TEQs in fishoil are observed. However, when the combined DR-CALUX®/C-SPLIT® technology is applied, the correlation between HRGCMS derived TEQs and DR-CALUX®/C-SPLIT® derived TEQs for TCDD/Fs and PCBs separately decreases. The observed discrepancy between HRGCMS and DR-CALUX®/C-SPLIT® derived TEQs can at least partly be explained by the fact that for the calculation of HRGCMS TEQs, WHO-TEFs for the individual analysed dioxin, furan and PCB congeners are used whereas the relative potencies (REP) of individual dioxin, furan, or PCB congeners as measured in the DR-CALUX® bioassay, may deviate from the WHO-TEF values.

WHO-TEF values are toxic equivalent factors for dioxin, furan and dioxin-like PCB congeners, derived from both *in vivo* and *in vitro* studies. The relative potencies of congeners determined by the DR-CALUX® bioassay are expressed as CALUX®-REPs (CALUX® Relative Potencies). The CALUX® REP values are actual "TEF" values for the congeners in the CALUX® bioassay and represent the actual potency of the specific congener to activate the Ah-receptor pathway. A number of authors have compared WHO-TEFs and DR-CALUX®-REPs [2,3,4]. Some

differences between WHO-TEFs and DR-CALUX®-REPs are apparent (Table 1). As a consequence, DR-CALUX®-TEQs may differ from HRGCMS-TEQs for a given sample.

Determination of conversion factors

Since the EU limit values for dioxins/furans in feed and food are based on HRGCMS-TEQs (WHO-TEQs), DR-CALUX®-TEQs should be converted to WHO-TEQs for comparison. The factor to convert DR-CALUX®-TEQs to WHO-TEQs can be determined using actual HRGCMS mass data, WHO-TEFs and DR-CALUX®-REPs. As an example, a TCDD/F conversion factor is calculated using actual fishoil HRGCMS analysis results (Table 2). In table 2 it can be seen that the calculated TCDD/F TEQ depends on whether the WHO-TEFs or DR-CALUX®-REPs are used for calculation. Using WHO-TEFs, the calculated WHO-TEQ for TCDD/Fs in the analysed sample is 6.4 pg 2,3,7,8-TCDD TEQ/g oil as compared to 9.1 pg 2,3,7,8-TCDD TEQ/g oil in case DR-CALUX®-REPs are used.

From the example given, it is clear that DR-CALUX® analysis results will overestimate the TEQ value for dioxins and furans in case the same sample is analysed using HRGCMS. Therefore, both results can not be compared directly to each other. DR-CALUX® TEQ results can be compared more properly after conversion of the DR-CALUX® analysis results by applying the conversion factors with HRGCMS analysis results (WHO-TEQ). The conversion factor can be calculated using the example given. In case of the present fishoil sample, DR-CALUX® TCDD/F

Table 1. WHO Toxic Equivalency Factors (TEFs) and DR-CALUX® relative potencies (REPs) used to express the toxic potency of mixtures of PCDDs, PCDFs and PCBs

PCDDs and PCDFs			**PCBs**			
Structure	WHO-TEF	CALUX®-REP	IUPAC No.	Structure	WHO-TEF	CALUX®-REP
Furans			*Non-ortho PCBs*			
2,3,7,8-TCDF	0.1	0.32	81	3,4,5,3'-TCB	0.0001	0.0001
1,2,3,7,8-PeCDF	0.05	0.21	77	3,4,3',4'-TCB	0.0005	0.0013
2,3,4,7,8-PeCDF	0.5	0.5	126	3,4,5,3',4'-PeCB	0.1	0.067
1,2,3,4,7,8-HxCDF	0.1	0.13	169	3,4,5,3',4',5'-HxCB	0.01	0.0034
1,2,3,6,7,8-HxCDF	0.1	0.039				
2,3,4,6,7,8-HxCDF	0.1	0.18	*Mono-ortho PCBs*			
1,2,3,7,8,9-HxCDF	0.1	0.11	118	2,4,5,3',4'-PeCB	0.0001	0.000000001
1,2,3,4,6,7,8-HpCDF	0.01	0.032	114	2,3,4,5,4'-PeCB	0.0005	0.000048
1,2,3,4,7,8,9-HpCDF	0.01	0.041	105	2,3,4,3',4'-PeCB	0.0001	0.000012
OCDF	0.0001	0.0001	167	2,4,5,3',4',5'-HxCB	0.00001	0.00001
			156	2,3,4,5,3',4'-HxCB	0.0005	0.00021
Dioxins			157	2,3,4,3',4',5'-HxCB	0.0005	0.00008
2,3,7,8-TCDD	1	1	189	2,3,4,5,3',4',5'-HpCB	0.0001	0.0001
1,2,3,7,8-PeCDD	1	0.54				
1,2,3,4,7,8-HxCDD	0.1	0.3				
1,2,3,6,7,8-HxCDD	0.1	0.14				
1,2,3,7,8,9-HxCDD	0.1	0.066				
1,2,3,4,6,7,8-HpCDD	0.01	0.05				
OCDD	0.0001	0.0001				

Ahlborg et al., 1994; Hosoe et al., 2002

Table 2. Determination of the TCDD/F conversion factor (fishoil). The conversion factor was calculated using HRGCMS mass data, WHO-TEFs and DR-CALUX®-REPs.

TCDD/TCDF					
Congeneer	Mass (HRGCMS analysis)	WHO-TEF	CALUX®-REP	WHO-TEQ	CALUX®-TEQ
(-)	(pg/g)	(-)	(-)	(pg 2,3,7,8-TCDD/g)	
2378-f	15	0.1	0.32	1.5	4.8
12378-f	1.3	0.05	0.21	0.065	0.273
23478-f	4.1	0.5	0.5	2.05	2.05
123478-f	0.25	0.1	0.13	0.025	0.0325
123678-f	0.25	0.1	0.039	0.025	0.0098
234678-f	0.33	0.1	0.18	0.033	0.0594
123789-f	0.25	0.1	0.11	0.025	0.0275
1234678-f	0.25	0.01	0.032	0.0025	0.008
1234789-f	0.25	0.01	0.041	0.0025	0.0103
ocdf	0.43	0.0001	0.0001	0.000043	0.00004
2378-d	0.63	1	1	0.63	0.63
12378-d	1.9	1	0.54	1.9	1.026
123478-d	0.25	0.1	0.3	0.025	0.075
123678-d	0.5	0.1	0.14	0.05	0.07
123789-d	0.25	0.1	0.066	0.025	0.0165
1234678-d		0.01	0.05		
ocdd	3.4	0.0001	0.0001	0.00034	0.0003
				6.358	9.088

TCDD/F conversion factor HRGCMS to DR-CALUX® = sum DR-CALUX® -TEQ/su, WHO-TEQ = **1.429**
TCDD/F conversion factor DR-CALUX® to HRGCMS = sum WHO-TEQ/sumDR-CALUX®-TEQ **= 0.700**

analysis results have to be multiplied by a factor of 6.4/9.1 = 0.7. A similar calculation can be performed for the mono- and non-ortho PCBs (data not shown). The conversion factor for conversion of DR-CALUX®-derived PCB TEQs into HRGCMS-derived PCB TEQs is 1.7.

The method described above to calculate conversion factors for both TCDD/Fs and PCBs was based on 33 fish oil samples analysed by HRGCMS. In table 3 the calculated conversion factors are given. The average DR-CALUX® to HRGCMS conversion factor for TCDD/Fs in fishoil was calculated to be 0.7 ± 0.1. The average DR-CALUX® to HRGCMS

Table 3. Calculated DR-CALUX® to HRGCMS conversion factors for TCDD/F in a large number of fishoil, fish, fishfeed and fishmeal samples.

TCDD/TCDF							
Matrix	n	min	max	median	average	SD	RSD%
Fishoil	33	0.3	0.8	0.7	0.7	0.11	17
Fish	24	0.3	1.1	0.7	0.7	0.18	27
Fishfeed	23	0.3	0.8	0.6	0.6	0.11	18
Fishmeak	20	0.5	0.8	0.7	0.7	0.10	15
Average					0.7		
SD					0.02		
RSD%					3.0		

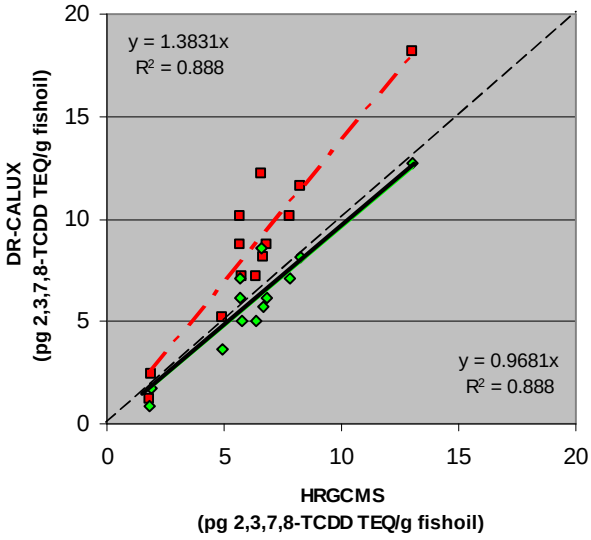

Figure 1 Comparison of DR-CALUX® determined TCDD/F specific TEQs and HRGCMS determined TCDD/F specific TEQs in fishoil samples before (dashed line) and after (solid line) conversion using the calculated average conversion factor (0.7)

conversion factor for PCBs in fishoil was calculated to be 1.6 ± 0.2 (data not shown). In addition to fish oil samples, conversion factors were also determined for fish feed, fish meal ad fish filet. HRGCMS data of 23, 20, and 24 samples respectively were used for the calculations. The resulting conversion factors are presented in table 3. The calculated conversion factor for fishoil was used on a number DR-CALUX® and HRGCMS TCDD/F specific analyses of fishoil samples. The results are presented in figure 1. As can be observed, the slope of the converted datapoints (solid line) is close to 1 indicating that converted DR-CALUX® analysis results can directly be compared to HRGCMS analysis results.

Conclusion

1. Differences between WHO-TEF and DR-CALUX®-REP values create some discrepancy in results when comparing bioassay (DR-CALUX®) and chemical analytical HRGCMS results.
2. Conversion factors were determined that allow a proper comparison of DR-CALUX® and HRGCMS results.
3. These factors, e.g. 0.7 for dioxins/furans and 1.6 for PCBs, were determined based on a large number of HRGCMS-analysed samples and appeared to be highly consistent.
4. However, it is advised to use matrix specific conversion factors until the data-set from which conversion factors are derived, has been extended.

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