Protein measurement was performed with the BCA Protein Assay Kit (Novagen) according to the manufacturer directly after the EROD assay including a BSA standard protein curve in rising concentrations of 0, 3.91, 7.81, 15.6, 62.5, 125, 250, 500 µg/mL on the same plate. Absorbance was recorded at 540 nm after 90 min incubation.

Sample quantification was interpolated from to the TCDD standard curve by a four-parameter log-logistic curve fit approach by the statistical software "R" and the package "drc" (7). The EROD values were normalized by the protein values and the protein standard curve. Additionally one outlier was removed from the quadruplicate measurement.

Results and discussion

Evaluation of three different cryo media for cells frozen either in 96 well plates or cryo vials revealed varied efficacies in the EROD assay. The EROD assay was performed directly after thawing of cells and compared to the EROD assay performed with cells from permanent culture; results are summarized in fig 1. For cells frozen in 96 well plates maximum relative fluorescence units (RFU) reached only 19.5 % (NFM), 38.4 % (cryoSO) and 36.0 % (standard) compared to permanently cultured cells. For cells frozen in cryo vials maximum RFUs were up to 77.7 % of permanently cultured cells, additionally ED50 values were increased for cryo cells (ED50: 0.11) compared to permanently cultured cells (ED50: 0.21). Therefore, appropriately applied freezing and thawing condition enables the use of cryo preserved cells for the EROD assay without the need of permanently culturing cells.

Quantification of dioxins and dioxin-like compounds by standard addition measurements was based on linear extrapolation of RFU differences between the standard curve and the standard addition curve (fig. 2). In total ten (soil and food) samples were analyzed and the measurements also compared to chemical analysis by HRGC/HRMS (fig. 3). Only for one soil sample the standard addition method compared to external calibration revealed a lower BEQ. In comparison to TEQs, the standard addition method marginally underestimated the BEQs for three samples, whereas the external calibration underestimated the BEQs in eight from ten samples. Therefore using a standard addition method might offer the possibility not to underestimate the amount of dioxins and dioxin-like compounds found in samples which are commonly under food and environmental safety surveillance.

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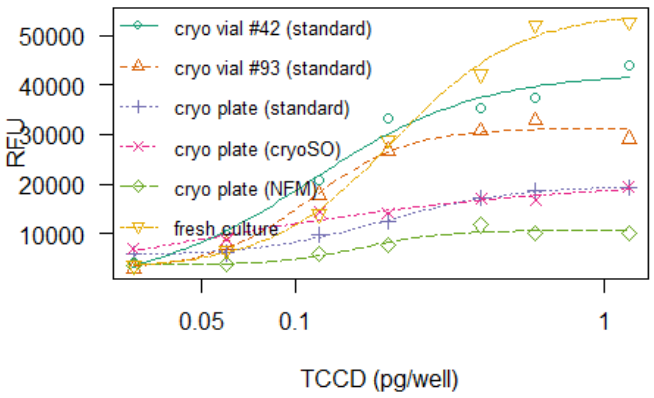
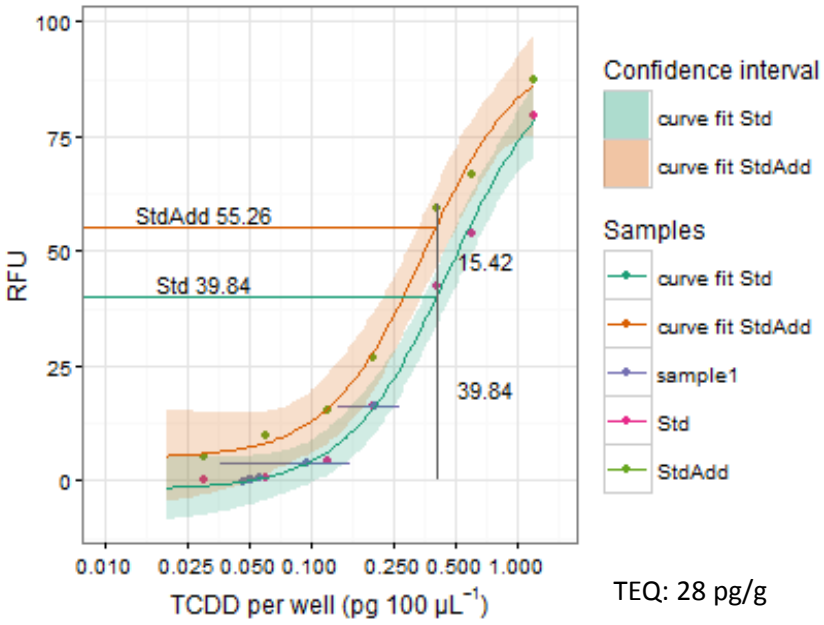


Figure 1: Comparison of cryo preserved cells with permanently cultured cells in EROD assay. Rat hepatoma cells (H4IIE) were cryo preserved with different cryo protectants (standard, cryoSO and NFM). After thawing of cells in 96-well plates and from cryo vials, direct EROD assay using TCDD was performed in comparison to permanently cultured cells.



TEQ: 28 pg/g

Sample concentration by external calibration

dilution	RFU sample	RFU Estimate	BEQ [pg/g]	BEQ z-score	BEQ (z: -1/1)
32	-0.11	0.047	93.9	1.8	22.6
16	0.1	0.051	50.2	0.5	22.6
8	0.06	0.050	24.8	-0.3	22.6
4	0.46	0.056	13.8	-0.6	22.6
2	3.88	0.096	11.9	-0.7	22.6
1	16.2	0.201	12.5	-0.7	22.6

Sample concentration by standard addition

TCDD [pg]	RFU Std	RFU StdAdd	RFU diff	BEQ [pg/g]	BEQ z-score	BEQ (z: -1/1)
0	-1.9	5.0	6.9	0.0	#N/A	39.0
0.03	-1.1	5.9	7.0	48.7	-0.1	39.0
0.06	0.8	8.3	7.5	144.5	2.2	39.0
0.12	6.4	15.8	9.4	43.3	-0.3	39.0
0.2	16.1	28.4	12.3	37.9	-0.4	39.0
0.4	39.8	55.3	15.4	38.5	-0.4	39.0
0.6	56.6	70.5	13.9	36.7	-0.4	39.0
1.2	78.7	86.4	7.7	29.2	-0.6	39.0

Figure 2: Measurement of soil sample by standard addition and external calibration method. For standard addition method, the sample concentration was extrapolated from the difference between standard and standard addition curve at respective TCDD standard points. Sample concertation was calculated within z-score range of -1 to 1. Sample: BFHNR 30504.

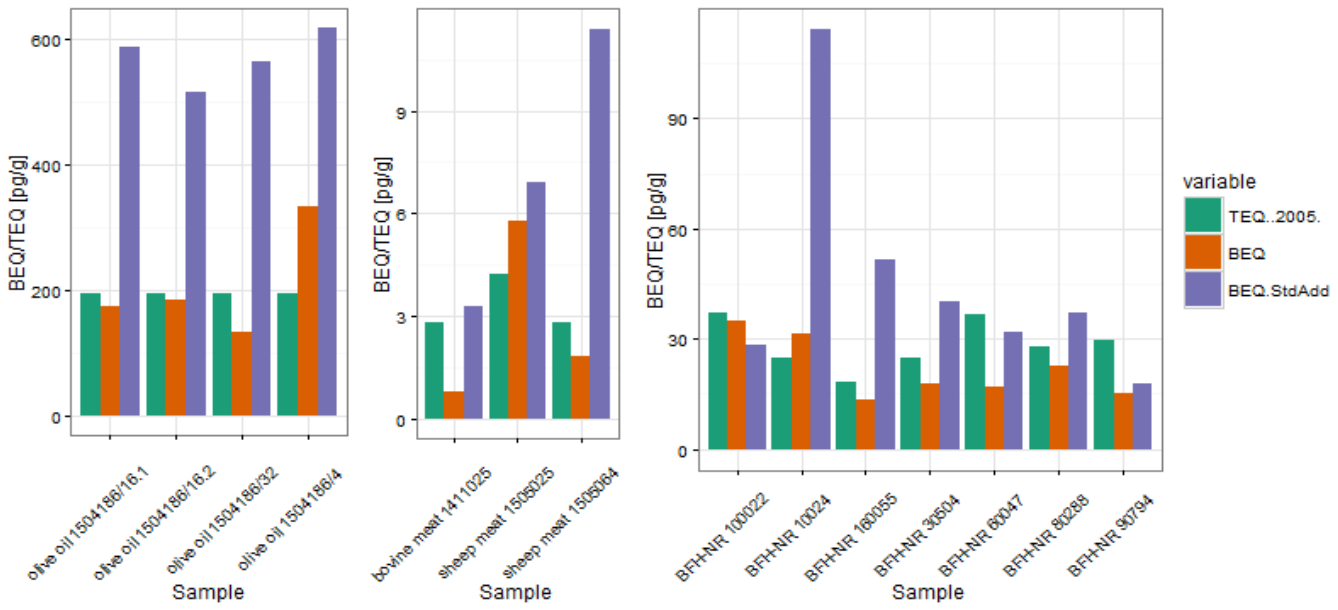


Figure 3: Comparison of measurements by standard addition and external calibration method for food and soil samples.