Detection of CB congeners in human umbilical cord.

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Introduction.

The presence of polychlorinated biphenyls (PCBs) in the biosphere has been extensively studied during the last 30 years, both in terms of *total PCBs* (Aroclor® equivalents), and more recently as individual chlorinated biphenyl (CB) congeners and the sum, ΣCB, of these[1]. The load of PCBs and other persistent pollutants in humans have mostly been based on studies using blood and/or human milk as the matrix of interest[2]. The rationale behind this sampling strategy is, that sampling can be done without major inconvenience to the experimental persons. The determination of CB congeners in human umbilical cords has apparently not been reported previously. A sensitive method was needed for the determination of a number of individual CB congeners in human umbilical cord samples from the Faroe Islands where mercury exposures are high[3]. For this type of tissue, a low content of lipids and of CB congeners is expected. The relatively small amount of sample (1-5 gram) that is available for the analyses, required a major revision and refinement of the analytical method. The aim of the present study was to optimize an analytical methodology, hitherto used for high CB congener levels in biological samples, in order to enable identification and quantification of CB congeners present in very low concentrations in the human umbilical cord samples.

Materials and methods.

The method for analysis of marine mammal tissue samples[4] was optimized in order to detect much lower levels of CBs. The method is described in detail[5]. Briefly, the samples were spiked with recovery surrogate standards (5 ng CB/sample), and extracted with 120 ml n-hexane:acetone (1:1 vv) in an Ultra-Turrax blender. The extract was dried and lipid determination was done gravimetrically. Sample clean-up was done on a multi-layer clean-up column containing: 5 gram basic Al_2O_3 (3 % w/w water)/1 gram of silica/5 gram silica containing 40 % H_2SO_4 (w/w)/1 cm of anhydrous Na_2SO_4. The residue was eluted with 100 ml of n-hexane. The internal standards were added, and the volume adjusted to 1 ml (concentration of the internal standards: 5 ng/ml). The samples were analyzed by dual-column capillary GC/ECD, using a revised version of the method published earlier for the seal tissue samples[6]. The two columns were 60 m and 0.25 mm i.d., J&W DB-5 0.11 μm d_f and J&W DB-1701 0.15 μm d_f, respectively, and the injection volume was 2 μl.

Quality assurance. A certified mackerel oil sample BCR-CRM 350[7] was used as control sample. A sample size of 25 mg would yield a similar absolute amount of lipid per sample as expected for the cord samples, and a CB congener level per sample extract about 2-10 times as high as expected in the cord samples (range: 0.6 ng/ml - 8.0 ng/ml). Two control

samples were added in each analytical run, in order to determine the variance components in the method[8,9,10]. A blank sample was included in each analytical run.

The establishment of control charts. Control charts were established based on ANOVA of the results obtained from the repeated analyses of the control sample. In the present method, two types of control charts, namely the X-chart for the average values of the duplicate measurements of the control sample and the Z-chart for the difference between the two measurements, are established. X- and Z-charts are prepared for each variable.

The statistical model for analysis of the control sample data is given as $Y_{ij} = \mu + B_i + E_{ij}$ where μ is the mean, B is the contribution from batches and E is the residual variation of the method. ANOVA of the n set of measurements consisting of r replicates yield the mean square between batches (s_B^2) and the mean square within batches (s_E^2). s_B^2 estimates $r\sigma_B^2 + \sigma_E^2$, and s_E^2 estimates σ_E^2. The estimate of the batch variance, $\hat{\sigma}_B^2$, is then calculated as $\hat{\sigma}_B^2 = \frac{1}{r}(s_B^2 - s_E^2)$ and it has (n-1) degrees of freedom. The estimate of the residual variance, $\hat{\sigma}_E^2$, is $\hat{\sigma}_E^2 = s_E^2$, and this estimate has n(r-1) degrees of freedom.

The standard deviation of the mean of m measurements in a batch is estimated by

$$s_{\bar{Y}}^2 = \hat{\sigma}_B^2 + \frac{1}{m}\hat{\sigma}_E^2 = \frac{1}{r} s_B^2 + \left(\frac{1}{m} - \frac{1}{r}\right) s_E^2 \qquad (1)$$

The variance estimate of Z is $s_z^2 = 2\hat{\sigma}_E^2 = 2s_E^2$. The estimated total variance of *a single measurement* by the method, based on the results of the above ANOVA, is $\hat{\sigma}^2_{Total} = \hat{\sigma}_B^2 + \hat{\sigma}_E^2$.

Results and discussion.

Cord samples A chromatogram of a cord sample is shown in figure 1. Preliminary data (N=60 samples) show that CB-153 has an average value of (0.50±0.28) ng/g wet weight (range 0.15-1.48 ng/g). The lipid% is in average (0.15±0.08)% (range 0.03-0.52 %). The laboratory recovery is in average (83±8)% for CB-40 and (75±10)% for CB-198.

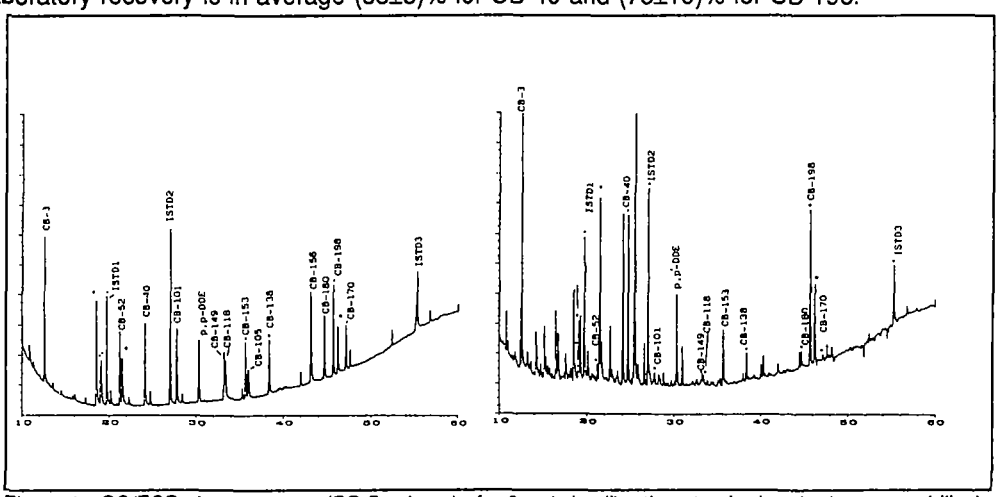

Figure 1. GC/ECD chromatogram (DB-5 column) of a 2 ng/ml calibration standard and a human umbilical cord sample.

Table 1. ANOVA of data from analyses of 4 x 2 replicates. Preliminary limits for the control charts.

Table 1	ANOVA of the control samples (4 batches of 2 samples)			Variance limits for X-, Z-chart		RSD of a single measurement (%)	
Variable	<Y> ng/g	$\hat{\sigma}_B$ (ng/g)	$\hat{\sigma}_E$ (ng/g)	s<Y> ng/g	s_Z ng/g	$\hat{\sigma}_{Total}$ (%)	$\hat{\sigma}_{Repeat}$ (%)
CB-52	59	3.2	3.8	4.2	5.4	8.5	6.5
CB-101	140	7.9	5.2	8.7	7.4	6.7	3.7
CB-149	150	3.7	3.5	4.5	5.0	3.4	2.3
CB-118	112	10	3.3	11	4.6	9.8	2.9
CB-153	270	17	4.4	17	6.2	6.3	1.6
CB-138	210	33	10	34	15	17	4.9
CB-180	58	2.1	1.4	2.4	2.0	4.4	2.4
CB-3 (%)	71	11	15	16	22	27	22
CB-198 (%)	90	5.0	2.3	5.3	3.3	6.2	2.6
CB-40 (%)	96	5.7	2.6	6.0	3.7	6.6	2.7
Lipids (%)	100	6.1	0.8	6.1	1.1	6.1	0.8

Control charts

The results of the ANOVA for the control samples are given in Table 1. An example of a control chart is shown in figure 2. The total variance of a single measurement by the method (given as RSD%) ranged from 3.4% (CB-149) to 9% (CB-118), with exception of CB-138, which had a RSD of 17%. The increased variability for CB-138 may be due to interference from CB-163[11]. The total variance of CB-153 (highest concentration) is comparable to that of CB-52 (lowest concentration), although the concentration of CB-153 exceeds that of CB-52 by about a factor of five. Among the laboratory recovery surrogate standards, the RSD of CB-3 was 27%, while CB-198 had an RSD of 6.2%. The large RSD of CB-3 is believed to be due to partly evaporation of this compound during the gravimetric lipid determination. The RSD of the lipid determination is 6.1%, and this figure is comparable with that of the CB congeners.

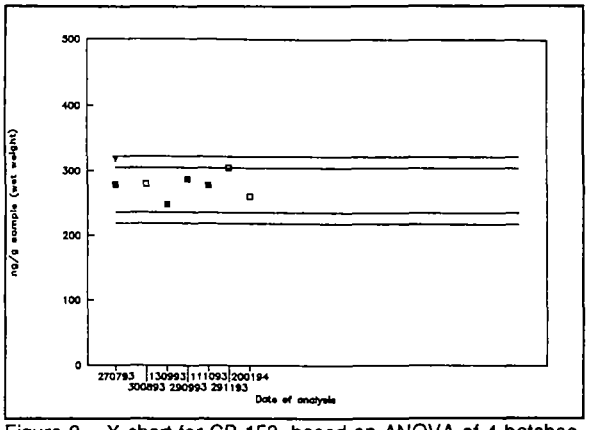

Figure 2. X-chart for CB-153, based on ANOVA of 4 batches of duplicates. ■) ANOVA data; □) data; ▽ certificate value.

Conclusions

A sensitive GC/ECD method has been developed for measurement of CB congeners in human umbilical cord tissues. The use of control samples and control charts is demonstrated. An initial estimate of the method reproducibility was made from four duplicate measurements of a control sample, with a concentration of CBs ranging from 0.5 to 10 ng CB per sample extract. The total RSD was in the range 3 - 10 % with a single exception of 17 % (CB-138). The RSD of the lipid determination step was 6 %. The method was developed from a current method, used for CB congener analyses in marine mammal samples, to enable trace level CB congener analyses in human umbilical cord samples. The major problem of this transition was the small amount of sample available, combined with the low lipid- and CB congener content. CB-153, CB-138, CB-180 and p,p'-DDE are present in detectable amounts, but the levels are close to the detection limit.

Acknowledgments

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