CONTROL ANALYSIS OF EEL USING CALUX AND GC/MS

Ron Hoogenboom[1], Wim Traag[1], Ronald Hoogerbrugge[2], Bert Baumann[2], and Jaap de Vries[3]

[1] RIKILT, Bornsesteeg 45, 6708 PD Wageningen, The Netherlands
[2] RIVM, P.O. Box 1, 3720 BA Bilthoven, The Netherlands
[3] Keuringsdienst van Waren, De Stoven 22, 7200 AE Zutphen, The Netherlands

Introduction

A recent study by the RIVO in fish sampled at the Dutch market, revealed that in particular wild eel may contain high levels of dioxins and dioxin-like PCBs[1]. Average level in five samples of wild fresh water eel was 19.0 TEQ pg/g (range 8.7-36.7), as compared to 7.5 pg TEQ/g (range 3.9-10.7) for 4 samples of farmed eel. A risk evaluation resulted in a consumption advice to eat fresh water eel no more than once a week, followed by a temporary limit in eel of 8 pg TEQ dioxins per gram fish. Dioxin-like PCBs were not included regarding a lack of validated and accreditated methods. However, the consequences of the limit were evaluated based on total TEQ, using a ratio of total TEQ to dioxin TEQ of 5.5, established from the 9 samples measured in the RIVO study. Assuming an average consumption of 50 gram eel per week, it was estimated that the maximum intake of dioxins and PCBs from eel would be 4.8 pg TEQ/kg body weight/day, on top of an average intake of 1.8 pg TEQ/kg body weight per day from other sources. It was considered that the actual intake will be much lower since in general the consumption of eel by the average consumer has been shown to be much lower, and since the average eel (including 90% of farmed eel) will contain much lower levels of dioxins and dioxin-like PCBs. Following the establishment of a residue limit, a control programme for eel was started, based on screening with the CALUX bioassay and confirmation of samples suspected to exceed the limit of 8 pg TEQ/g by GC/MS. In order to evaluate the possible use of the 7 indicator PCBs for screening, samples were analysed for these compounds.

Materials and methods

Sampling and extraction of eel

At least 25 eels were collected at local stores, fish farms and fishermen and mixed into one sample. Part of the homogenized samples were send to the RIKILT, where the oil was extracted using the method developed by Bligh and Dyer.

CALUX-bioassay

A relatively clean sample of fish oil was selected and further cleaned with activated carbon as described previously for butter fat[2]. Part of the cleaned oil was subsequently spiked with a mixture of equal amounts of the 17 dioxin congeners, a mixture of the non-ortho PCBs 77, 126 and 169, and stock solutions of PCB 118 and PCB 156. Final concentrations for dioxins, non-ortho PCBs and PCBs 118 and 156 were respectively 29, 81, 44 and 49 pg TEQ/g oil. This oil was subsequently diluted to samples containing approximately 120, 60 and 30 pg TEQ/g.

Samples of 0.5 gram oil were purified on columns containing 10 gram acid silica (33% H_2SO_4), as described previously[2]. In each series of 26 samples the blanc fish oil and the 4 references were included. Before total evaporation of the hexane/diethylether in a SpeedVac 200 µl of DMSO was added as a keeper. An aliquot of 20 µl was added to 2 ml medium and 250 µl added to 3 wells of a 48 well plate containing p-GudLuc transfected H4IIE cells (Biodetection Systems). After 24 hrs, the medium was aspirated, the cells washed and lysed and an aliquot used for determining the luciferase content in a Luminoskan (Labsystems).

Total TEQ sample in the oil was estimated from a calibration curve of the reference samples whose response was fitted with an exponential curve fit. The level in oil was subsequently translated to the level in eel using the fraction of oil in the fish samples.

GC/MS analysis

Levels of dioxins, non-ortho and mono-ortho PCBs were determined by high resolution GC/MS, basically as described by Tuinstra *et al* [3]. Levels of the 7 indicator PCBs were determined by a newly developed GC/MS method. Fish oil is dissolved in iso-octane, PCB's are isolated from the oil by straight phase HPLC. The PCB fraction transferred on-line to a GC-MS equipped with an Large Volume Injector and an early vapour exit

Results and Discussion

The fact that the limit was set for dioxins only posed some particular problems. Samples were tested and their total TEQ level estimated by comparison with a set of fish oil samples spiked at total TEQ-levels of 30, 60, 120 or 200 pg TEQ/g fat with a mixture of dioxins, non-ortho PCBs and PCBs 118 and 156. Relative contribution of dioxins, non-ortho and mono-ortho PCBs to the total TEQ level was based on the RIVO-study, being respectively 15, 40 and 45%. The latter is important regarding the relatively poor response of the mono-ortho PCBs in the test[2]. For the same reason, a relatively low action limit of 30 pg TEQ/g eel was selected. Testing of the fish oil samples spiked at 30, 60, 120 and 200 pg TEQ/g in six-fold revealed TEQ-levels of respectively 20, 44, 87 and 157 pg TEQ/g with %CV of respectively 17, 14, 17 and 10%, as determined from a TCDD calibration curve. When tested in single in 5 different extraction and test series, %CV was respectively 26, 19, 19 and 14%. The relatively low recoveries are primarily caused by the relatively poor response of compounds with low WHO-TEF values in the CALUX-test. This was

confirmed with fish oil spiked with either dioxins, non-ortho PCBs and mono-ortho PCBs, showing a poor response with the latter sample. For this reason, a calibration curve of the reference samples was used to calculate the levels in eel samples. This implicates that levels in samples with a different pattern of dioxins and PCBs may be under or overestimated. The latter is more likely to be the case, regarding the relatively low contribution of dioxins in the reference samples and the fact that it is unlikely to have samples with mono-ortho but no non-ortho PCBs.

Screening of 153 samples of eel with the CALUX bioassay resulted in the distribution curve of estimated levels shown in Fig. 1, with an average estimated content of 14 pg TEQ/g fish. A total of 21 samples (14%) was estimated to contain total TEQ levels above the action limit of 30 pg TEQ/g fish, with eight samples exceeding the virtual limit of 44 pg TEQ/g, thus indicating dioxin levels above the limit of 8 pg TEQ/g. Analyis by GC/MS of the 21 suspected and 9 samples below the action limit, revealed a very good correlation (r^2=0.93) between CALUX estimated levels and total TEQ-levels of dioxins and dioxin-like PCBs (Fig. 2A). All 9 samples estimated to be lower than the current action limit, were confirmed to be below 30 pg TEQ/g. In the set of 21 samples higher than the action limit, 13 were confirmed to contain levels above 30 pg TEQ/g. Five of the eight results exceeding the virtual limit of 44 were confirmed to do so by GC/MS. GC/MS analysis by RIVM showed no samples exceeding the current limit of 8 pg TEQ/g for dioxins only, analysis by RIKILT one sample just above this limit (Fig. 2B). This observation is in agreement with the known interlaboratory variation of the GC/MS analysis. The low number of

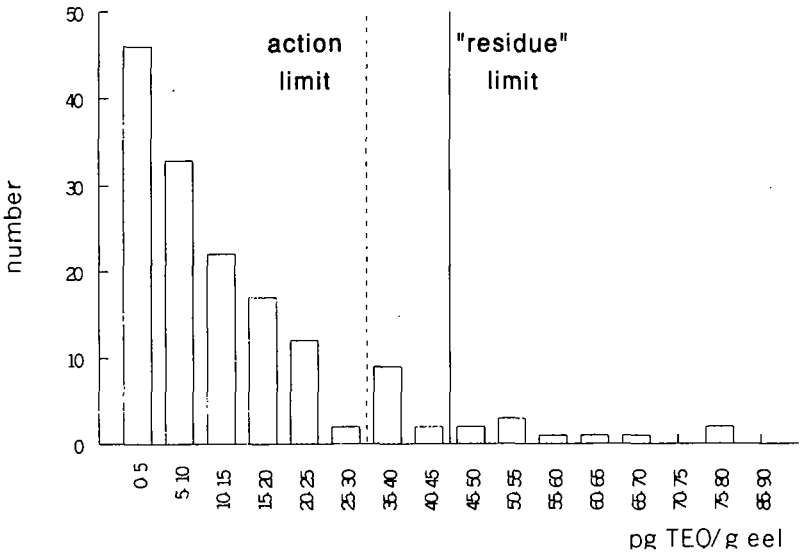

Figure 1. CALUX estimated total TEQ levels in 153 eel samples. The action limit of 30 pg TEQ/g, as well as the virtual residue limit are indicated.

confirmed positives indicates that the current action limit of 30 pg total TEQ/g might be too conservative but more samples above the limit are required to determine a more effective action limit. In general, the correlation between the CALUX response and dioxins appears to be relatively weak (r^2=0.37), which is in accordance with the fact that most of the dioxin-like activity is derived from dioxin-like PCBs. However, only highly contaminated samples were checked by GC/MS and inclusion of data on lower contaminated samples may result in a better correlation, showing that the CALUX-assay is effective enough to select samples with high levels of dioxins. Furthermore, the new set of GC/MS data clearly shows that the ratio of 5.5 between total TEQ and dioxins only, as derived from the RIVO data[1], is too low for highly contaminated samples. In these eels the contribution of dioxins to the total TEQ level tends to decrease to less than 10%, stressing the need to include dioxin-like PCBs in the residue limits.

Current limits for indicator PCBs in eel in the Netherlands are 0.5, 0.2, 0.4, 0.4, 0.5, 0.5 and 0.6 mg/kg for respectively PCBs 28, 53, 101, 118, 138, 153 and 180. Analysis of indicator PCBs in the 153 samples showed 1 sample exceeding the limit for PCB 153. Figures 2C and 2D show a comparison between the level of indicator PCBs and the total TEQ (C) and dioxin (D) levels in part of the eel samples. It is evident that the relation between indicator PCBs and dioxins is even worse (r^2=0.22) than in the case of the CALUX (2B). The relation between indicator PCBs and total TEQ is good (r^2=0.89) and in principle should allow the setting of an action limit for either the 7 congeners or only PCB 153. The relatively good correlation between indicator PCBs and total TEQ is in accordance with the fact that in particular in highly contaminated samples, the non-ortho and mono-ortho PCBs account for respectively 38 and 48% of the total TEQ. PCB 126 is solely (98%) responsible for the non-ortho TEQ, the indicator PCB 118 together with PCB 156 are the two most important mono-ortho PCBs in terms of contribution to the TEQ level, respectively 22 and 16%. Interestingly, the current limit of 0.4 mg/kg for the mono-ortho PCB 118 amounts to a TEQ level of 40 pg TEQ/g product.

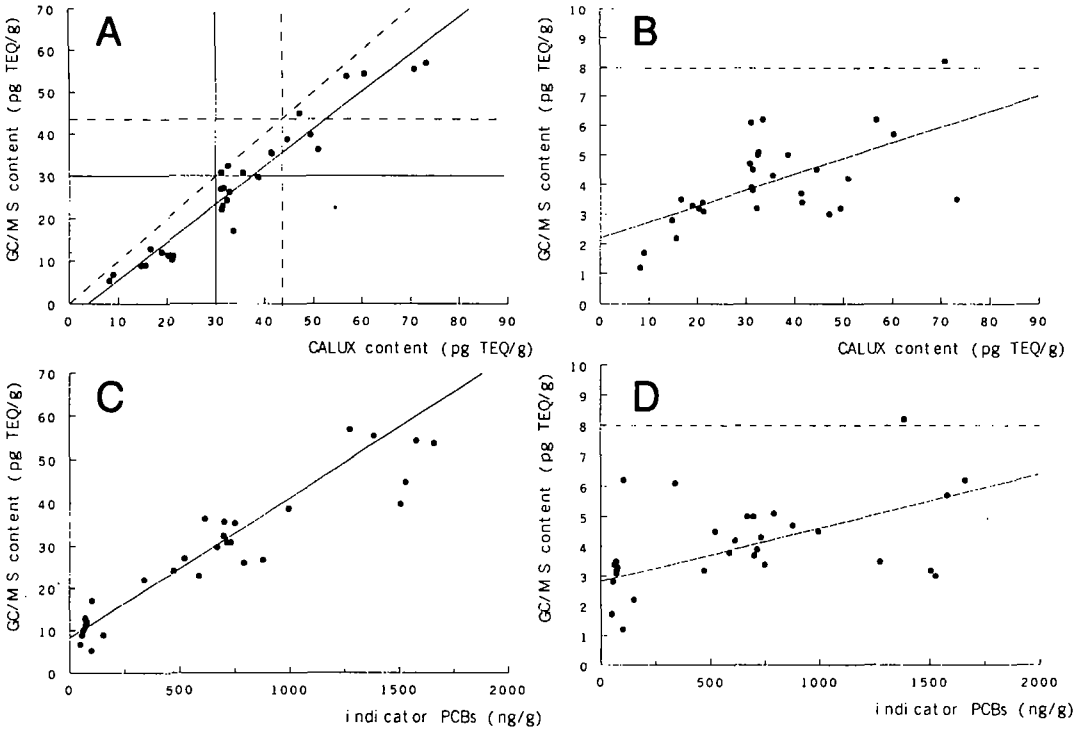

Figure 2. Comparison between total TEQ levels as estimated by CALUX (A,B) or levels of indicator PCBs (C,D) with total TEQ levels (A,C) or dioxin levels (B,D) in 30 samples of eel including 21 samples exceeding the action limit of 30 pg TEQ/g. Solid lines represent linear regression curves, dashed lines the residue and virtual residue limits of 8 and 44 pg TEQ/g.

It is concluded that both the CALUX-assay and the indicator PCBs may be used to select samples that may contain high levels of total dioxins and dioxin-like PCBs. However, the CALUX assay appears to perform slightly better for detection of dioxins only, which is in agreement with the fact that this test is particularly suitable for detection of the most potent Ah-receptor agonists, whereas the indicator PCBs focus more on dioxin-like PCBs and in particular the mono-ortho PCB 118. It should be pointed out that in most other fish products and in food in general, these mono-ortho PCBs are much less important in terms of their contribution to the total TEQ level[1] and that the CALUX-bioassay is likely to perform much better as a screening assay than indicator PCBs.

References

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