THE GERMAN BAKERY WASTE INCIDENT

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Introduction

In February 2003, a rapid alert was issued by the EU, reporting the presence of elevated dioxin levels in dried German bakery waste used in animal feed. Levels reported varied between 13.3 ng TEQ/kg as measured by the Fresenius Institute in Bayreuth, and 3.3, 5.6 en 5.9 ng WHO TEQ/kg as measured by Food GMBH in Jena. Part of this bakery waste was used by a Dutch company for the production of animal feed. The source of the dioxins was reported to be the use of waste wood for the drying of the bakery waste. Based on the relative amounts of bakery waste used for the feed, it was estimated that only in the case of pigs and calves there was a chance that the EU limits would be exceeded. Intensive tracking and tracing was performed by the Dutch Inspection Services resulting in over 300 samples of bakery waste, feed and calf and pig fat. All samples were screened with CALUX. Part of the samples was investigated by GC/MS. In order to save time, the latter was not always based on the CALUX response, thus resulting in a nice dataset to evaluate the use of the CALUX assay.

Methods and materials

CALUX analysis was performed as described previously. In short, 5 gram of feed or bakery waste was suspended in methanol/water and extracted with hexane/diethyl ether. The extract was subsequently cleaned over a 33% acid silica column. The elute was dried to a small volume, mixed with 40 µl DMSO and further dried to remove the organic solvent. The DMSO was mixed with culture medium, which was then added to the cells. After a 24 h incubation period, cells were lysed and the luciferase measured. The response was compared to that of a set of feed samples containing 0, 0.3, 0.6, 0.8, 1.8 and 3.9 ng TEQ/kg of a mixture of dioxins and dioxin-like PCBs, with a relative contribution of dioxins, no-PCBs and mo-PCBs to the TEQ of 51, 30 and 19%. Samples showing a higher response than the sample of 0.6 ng TEQ/kg were termed suspected and analysed by GC/MS for confirmation.

Fat from calves was mixed with hexane/diethyl ether and directly applied to the acid silica columns. In the case of pigs, 2 gram fat was mixed with 5 ml hydrosulphuric acid, incubated overnight at room temperature and subsequently extracted with hexane/diethyl ether. This extract was applied on the columns. Eventually the extract was evaporated with 20 µl DMSO as keeper. A natural contaminated milk fat sample containing 0.5 ng TEQ/kg and the same fat enriched to levels of 1, 2, 3 and 6 ng TEQ/kg were included in each extraction series, as well as a carbon cleaned fat sample. Samples were declared suspected if the response was higher than the sample containing 0.5 (pig fat) or 2 (calf fat) ng TEQ/kg.

GC/MS analysis was performed as described previously by Tuinstra *et al.*[1], with the exception that 3 gram fat or 50 gram of feed/bakery waste was used.

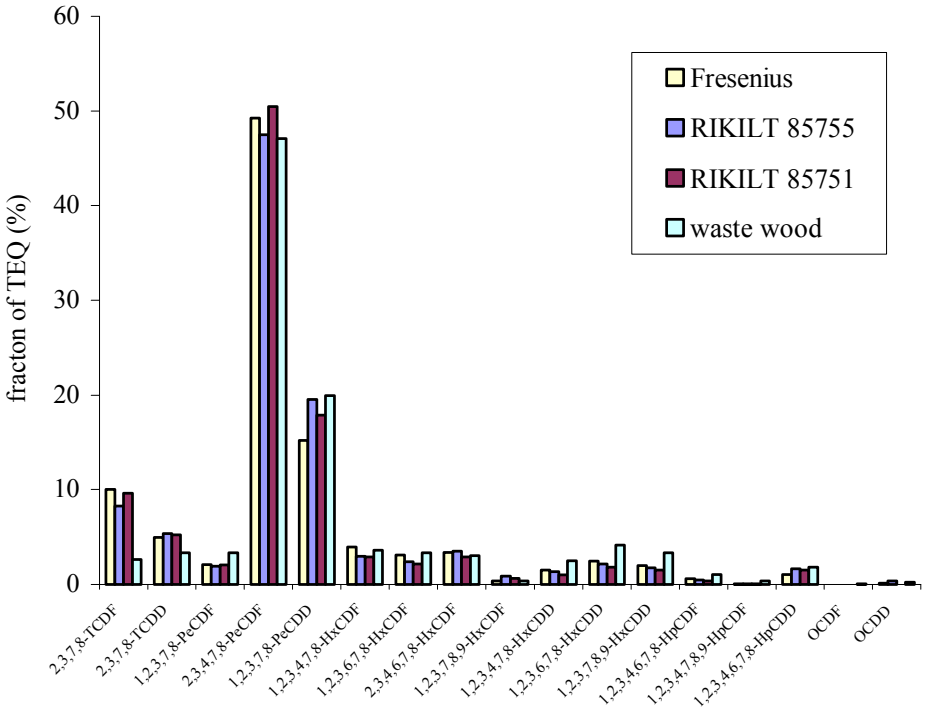

Figure 1. Congener profile of dioxins analysed in bakery waste obtained by the Fresenius institute and RIKILT, as compared to a congener-profile obtained after combustion of waste wood[2].

Results and discussion

Animal feed and bakery waste

A total of 339 samples were screened within a period of 3 weeks, being 168 feed or bakery waste samples and 171 animal fat samples. Bakery waste samples were traced effectively and all samples showed an increased response in the CALUX assay. The highest level measured by GC/MS was 11.5 ng TEQ/kg, the lowest level 1.0 ng TEQ/kg. Figure 1 shows the TEQ based congener profiles of two samples of bakery waste containing 8.1 and 6.4 ng TEQ/kg, as compared to the sample analysed by the Fresenius Institute. It is evident that the profiles are identical with 2,3,4,7,8-PeCDF (TEF 0.5) being the most important congener, followed by 1,2,3,7,8-PeCDD (TEF 1). In terms of absolute amounts, TCDF, HpCDD and OCDD showed similar levels, but don't contribute to the total TEQ level. The profile was similar to data presented by Wunderli *et al.*[2] on congener profiles obtained from the incineration of waste wood, thus confirming the source of the dioxin contamination. A number of feed samples were found suspected in the CALUX-assay, but none of these samples exceeded the current limit of 0.75 ng TEQ/kg as determined by GC/MS.

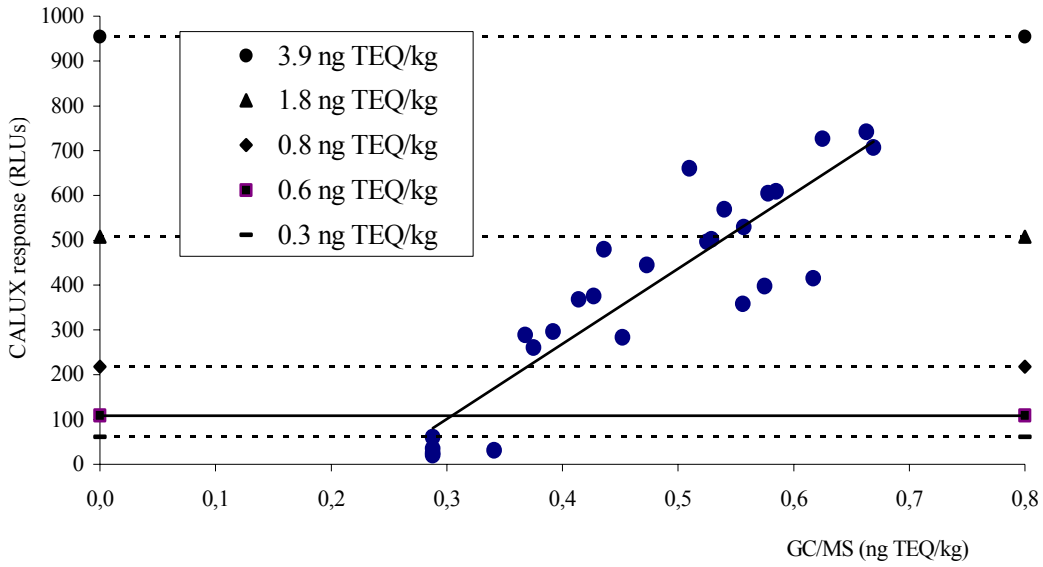

Figure 2. A comparison between the GC/MS determined dioxin level (upper bound levels) and the response in the CALUX assay (expressed in RLUs). The response obtained with the 5 different chicken feed control samples is included as horizontal lines.

However, suspected feed samples always showed levels above background levels in the Netherlands and several exceeded the EU action limit of 0.5 ng TEQ/kg. As shown in Figure 2, results obtained with CALUX tended to overestimate the levels. In this particular case several feed samples showed a higher response than the control feed containing 1.8 ng TEQ/kg, whereas the GC/MS determined level was below the limit of 0.75 ng TEQ/kg. Similar has been observed in other cases of feed ingredients dried on open fires or contaminated by smoke from accidental fires. A possible explanation might be the presence of other Ah-receptor agonists in the feed. Analysis by GC-TOF confirmed the presence of many other compounds in the dried bakery waste, among them polycyclic aromatic hydrocarbons (PAHs) and methylated PAHs.

Table 1 shows the comparison of the results obtained for feed and bakery waste samples with CALUX and GC/MS, based on this action limit. No false-negative results were obtained and 16 false-positives, in all cases animal feed.

Table 1. Comparison of the results obtained with CALUX and GC/MS for bakery waste and feed samples. Suspected/positive indicates a result/level higher than the action limit of 0.5 ng TEQ/kg.

CALUX\GCMS	Negative	Positive
Negative	33	0
Suspected	16	24

Animal fat

A total of 171 animal fat samples were screened with CALUX including 10 calf fat samples. The latter samples, derived from animals fed with suspected feed showed no response above the action limit. GC/MS analysis of these samples showed dioxin levels below 0.5 ng TEQ/kg fat. Based on these results, no further calf samples were examined and all investigations focussed on pig fat. For the use of the CALUX-assay this meant that samples had to be examined for exceeding the very low limit of 0.6 ng TEQ/kg, which is the current action limit in the EU. Using the newly developed clean-up procedure, 14 samples out of 171 were declared suspected and 12 of these samples were confirmed by GC/MS to exceed the action limit (Table 2). Two results were false-positive. Furthermore, one negative sample was shown to contain 0.7 ng TEQ/kg, thus representing a false-negative sample. Most positive samples were just below the limit of 1 ng TEQ/kg, with 3 samples exceeding this limit including one sample with a dioxin level of 2.2 ng TEQ/kg.

Table 2. Comparison of the results obtained with CALUX and GC/MS for animal fat samples.

CALUX\GCMS	Negative	Positive
Negative	60	1
Suspected	2	12

Conclusions

- Based on the results, the use of contaminated bakery waste resulted in elevated levels of dioxins in animal feed, but no levels above the limit of 0.75 ng TEQ/kg
- The use of contaminated feed resulted in slightly elevated levels in pigs but did not endanger human health
- The CALUX bioassay was shown to perform very well, even at these very low limits.
- Results obtained with CALUX indicate the presence of other unknown Ah-receptor agonists in dried bakery waste

Ackowledgements

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References

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