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Effect-based and chemical analysis of polycyclic aromatic hydrocarbons in smoked meat – a practical food monitoring approach

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Abstract

Polycyclic aromatic hydrocarbons (PAHs), which are generated by heat treatment and smoke curing of meat, pose a risk to human health. At present, the determination of these unwanted contaminants requires costly, time-consuming chemical analysis of smoked meat. An alternative is effect-directed high throughput bioassays, which could also be used as a pre-screening method. We recently adapted the *in vitro* chemical-activated luciferase expression (CALUX) assay as a rapid, sensitive and inexpensive screening technique for compounds such as dioxins, polychlorinated biphenyls and PAHs. The aim of the present study was to apply a practical approach under realistic conditions. Custom-made meat samples produced under defined conditions with different PAH levels were analysed using this bioassay and GC/MS to determine the influence of different smoking conditions (temperature and duration) on PAH levels. We found that cold smoking for up to six hours did not result in strong PAH contamination, whereas hot (65 °C) and longer smoking times caused a considerable increase in both the bioassay response and the levels of 31 individually determined PAHs. The response in the effect-based bioassay was in good agreement with the values of chemical analysis. The bioassay made it possible to determine accurately the degree of contamination. Our results show that this assay is suitable for high throughput screening for unknown levels of toxicologically relevant PAHs in meat samples and is sensitive enough to differentiate between different PAH levels generated under various smoking conditions. Effect-based screening techniques therefore provide a new instrument for official food monitoring.

Keywords:- Smoked meat, polycyclic aromatic hydrocarbons (PAHs), CALUX, bioassay, effect-directed analysis

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a widespread group of environmental contaminants, many of which, including the representative compound benzo[*a*]pyrene (B[*a*]P), are known to exert teratogenic, mutagenic and carcinogenic effects (Guillen and Sopelana 2003; Shaw 1994). PAHs in food may result from their uptake from a contaminated environment or from food processing (Gomaa 1993). In general, PAHs are formed as complex mixtures during heat treatment processes such as smoke curing, barbecuing, deep frying, or drying and they contaminate food by direct contact with these combustion products. Contamination of food during the smoking process is highly variable and depends on the composition of the wood, the type of generator (internal or external), oxygen accessibility, the temperature of smoke generation and smoking time (European Union 2002; Simko 2005).

At the moment, PAHs in smoked meat samples are determined by costly, time-consuming chemical analysis. Toxic equivalence factors (Nisbet and LaGoy 1992) or potency equivalency factors (Collins 1998) for individual PAHs as to their carcinogenic potency relative to B[*a*]P are used for risk assessment of PAH mixtures in smoked meat and other heat-treated food items. We adapted an effect-based screening assay using the *in vitro* chemical-activated luciferase expression (CALUX) assay (Kuhn et al. 2008). This bioassay can evaluate the total activity of the sample compounds that interact with the aryl hydrocarbon receptor (AhR). The AhR mediates most of the toxic effects of dioxins, polychlorinated biphenyls and PAHs (Guillen and Sopelana 2003). The total carcinogenic potential is in good accordance with AhR-mediated activities of individual PAHs both *in vitro* (Machala et al. 2001) and *in vivo* (Shimada et al. 2002). Following the binding of the ligand to the receptor, the resulting ligand-receptor complex is activated and translocated to the nucleus, where it binds to a dioxin-responsive element (DRE) and stimulates the transcription of target genes including those of PAH-metabolising enzymes such as cytochrome P450 1A1 (Denison and Heath-Pagliuso 1998). The CALUX assay is based on a genetically engineered

cell line (rat hepatoma H4IIE cells) which contains a stable transfected vector including the firefly luciferase gene under the transcriptional control of several DREs. This transgenic cell line responds to ligand exposure with the induction of the luciferase, which in the presence of its substrate produces a luminescent signal proportional to the cell's response. Because of its specificity to AhR, this bioassay does not allow identification of the chemicals that are responsible for the enhanced signal but it does provide information about the effect of the specific compounds in a meat sample on the receptor.

Effect-directed analysis (EDA) is routinely used for screening of environmental contaminants occurring in soil, water and air (Giesy et al. 2002, Vondracek et al. 2001) and it should also be possible to use it as a pre-screening method to test smoked meat samples, contaminants and the potency of both known and unknown ingredients. Such a pre-screening method can handle a high sample throughput and analyse large numbers of samples for the presence of potentially toxic compounds, thus improving food monitoring and consumer safety.

In a previous study we showed that higher molecular weight PAHs, particularly B[a]P and dibenzo[a,h]anthracene (DBA), could be detected by the CALUX assay (Kuhn et al. 2008). The potency of the meat samples to cause a defined biological response mediated by the AhR activity of the PAHs present in the sample was expressed relative to the response of the two standards, benzo[k]fluoranthene or 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (Kuhn et al. 2008). The biological potency of the contaminated meat samples was expressed as induction equivalents (IEQs).

The aim of our present study was to apply a practical approach using an effect-based screening assay under real-life conditions with custom-made meat samples with different degrees of PAH accumulation. The robustness of this method was investigated both in terms of the influence of smoking conditions (temperature and duration) on PAH levels and in regard to their distribution in the smoked meat sample. The robustness of the bioassay was

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75 confirmed by the analysis of 31 individual PAHs which were determined in every meat
76 sample by gas chromatography-mass spectrometry (GC-MS).

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Material and methods

Samples

Grade E (premium) raw pork bellies were purchased from a local slaughter house and smoked in an FPC100 smoking chamber (Fessmann, Winnenden, Germany) using beech wood shavings. Five types of samples were produced: unsmoked products, products smoked cold (for 3 or 6 h) and products smoked hot (for 1 or 3 h). The raw pork bellies were deboned and the meat was rubbed with a mixture of curing salt and white pepper to yield final concentrations in the meat of 2% and 0.2%, respectively. The bellies were packed in polyamide-polyethylene foil under vacuum and stored for two weeks at 7 °C. The salt was removed by washing and the bellies were dried for two days at 7 °C. Two types of indirect smoking were applied: cold (18 °C) and hot (65 °C). Smoke was generated in a separate chamber from which the smoke was passed onto the meat. The pork bellies were then stored at 7 °C for four days before sampling. The unsmoked pork bellies were prepared (deboned, rubbed, packed, washed) and stored in the same way and served as negative controls.

According to Toth et al., (1971) PAH accumulation takes place in the outer part of a meat sample. We therefore used two sampling regions, the "middle" and "edge". The middle samples were taken more than 1 cm from the edge of the pork belly. The smoke thus reached the pork belly only from the top and the bottom, but not from the sides. The edge samples were taken from the outer 1 cm of the pork belly.

All 30 samples of pork belly (100 g each) were minced and blended in a chopper (Rondo 1000, Tefal, Offenbach, Germany), packed in aluminium boxes, frozen and stored at -18 °C until the extractions were performed.

Five 10-g aliquots (four for the bioassay and one for the GC-MS) were collected from each of the 30 pork belly samples and prepared for further analysis. Although a recently published study (Kuhn et al. 2008) showed that one aliquot would be sufficient for meat monitoring, we prepared more to ensure reproducibility of the bioassay. All aliquots were

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3 103 prepared in the same manner, with particular care taken to assure equal reaction and storage
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5 104 times.
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8 105 **Chemicals**
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10 106 The solvents employed were cyclohexane (CAS No. 110-82-7),
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12 107 *N,N*-dimethylformamide (CAS No.68-122), spectroscopic grade acetone (CAS No. 67-64-1;
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14 108 Merck, Darmstadt, Germany) and Pestilyse® grade methanol (CAS No. 67-56-1; Roth,
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16 109 Karlsruhe, Germany). TCDD (CAS No. 1746-01-6; LGC Promochem Wesel, Germany) was
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18 110 dissolved in spectrophotometric grade dimethylsulfoxide (DMSO, CAS No. 67-68-5; Acros
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20 111 Organics, Geel, Belgium). The final concentration of TCDD was 300 nM. Silica gel 60 (0.06
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22 112 – 2 mm) was purchased from Roth (Karlsruhe, Germany). All other chemicals used were of
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24 113 high purity grade.
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29 114 For the identification and quantification of individual PAHs, an internal standard
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31 115 mixture was added at the beginning of the extraction to the samples intended for GC-MS
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33 116 analysis. This mixture included the deuterated or fluorinated PAHs *d*₈-naphthalene, *d*₈-
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35 117 acenaphthylene, *d*₁₀-acenaphthene, 2-fluorofluorene, *d*₁₀-phenanthrene, *d*₁₀-fluoranthene, *d*₁₀-
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37 118 pyrene, *d*₁₂-benzo[*a*]anthracene, *d*₁₂-benzo[*b*]fluoranthene, *d*₁₂-benzo[*a*]pyrene, indeno[1,2,3-
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39 119 *cd*]pyrene and *d*₁₂-benzo[*ghi*]perylene. The internal standard mixture was provided by the
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41 120 Biochemical Institute for Environmental Carcinogens, Grosshansdorf, Germany, which also
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43 121 performed the GC-MS analyses on an Agilent 6890N GC with an Agilent 5973N MSD
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45 122 (Agilent, Santa Clara, CA, USA). The analyses were made with slight modifications
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47 123 according to a previously published procedure implementing an isotope dilution technique
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49 124 (Grimmer and Bohnke 1975; Grimmer et al. 1997) and carried out in the single-ion
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51 125 monitoring (SIM) modus; quantification was obtained by comparing the area responses of the
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53 126 target ions in relation to the area responses of the internal standards after a three-point
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55 127 calibration.
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128 *Sample extraction and cleanup procedure*

129 The methodology used for the extraction of the PAH of the pork belly samples and for the
130 cleanup procedure has been described previously (Kuhn et al. 2008). Briefly, this includes an
131 alkaline hydrolysis followed by a two-step liquid-liquid partition with cyclohexane and *N,N*-
132 dimethylformamide and a final cleanup through a silica gel column. Prior to use, all glassware
133 and equipment were rinsed with acetone and cyclohexane.

134 The purified extract was further concentrated under a gentle stream of N₂, and 100 µl of
135 dimethylsulfoxide were added just prior to the complete evaporation of the solvent. Samples
136 intended for analysis by GC-MS were dissolved instead in 1 ml of a mixture of cyclohexane
137 and toluene (9:1, vol/vol). The samples were concentrated and an aliquot was used for GC-
138 MS analysis.

139 *The CALUX bioassay*

140 A rat hepatoma cell line (H4IIE), stably transfected with the luciferase reporter gene plasmid
141 pGudLuc1.1 (Garrison et al. 1996), was provided by Prof. Dr. A. Brouwer (BDS, Amsterdam,
142 The Netherlands). All media and supplements for cell culture were purchased from PAA
143 (Cölbe, Germany). As described previously (Kuhn et al. 2008), the cells were grown at a
144 density of 30,000 per well in 96-well culture plates (Nunc, Wiesbaden, Germany) in 200 µl
145 DMEM/F-12 with 10% FBS (Mycoplex®, PAA) and 1% glutamine. The cell layer reached
146 90% confluence 24 h after seeding and 50 µl of culture medium containing the test extracts or
147 the standard dilutions were added to the medium in the wells. Exposures were performed in
148 quadruplicate. Solvent control, TCDD calibration standards (between 0.3 pM and 300 pM)
149 and one pork belly sample with a known grade of PAH contamination were included on each
150 well plate. The final concentration of DMSO in each well was 0.1% (vol/vol).

151 The measurement of the IEQ relative to the TCDD standard was carried out as
152 previously described (Kuhn et al. 2008). In brief, the cells were harvested after 6 hours of
153 incubation in cell lysis reagent and transferred to a white 96-well plate (Luminunc, Nunc,

Wiesbaden, Germany). Luminescence was measured using a multi-label Mithras LB 940 reader (Berthold, Bad Wildbad, Germany) immediately after automatic injection of assay buffer and luciferin solution. Results were converted into x-fold induction of the mean of the negative samples (the control). The sigmoid dose-response standard curve was fitted using this ligand binding function of SigmaPlot 9.01 (Systat, San Jose, CA, USA):

$$f(x) = \min + \frac{\max - \min}{1 + 10^{(\log EC_{50} - x) \text{Hillslope}}}$$

The extracts were interpolated on this curve. Results were expressed as TCDD-IEQ and converted into nanograms per kilogram of meat. The limit of detection was set to the DMSO response plus three times the standard deviation (SD); the limit of quantification (LOQ) was set to the DMSO response plus ten times SD.

Statistical evaluation

As described recently (Kuhn et al. 2008), certain parameters were calculated and evaluated in every assay for quality assurance: curve fitting was carried out with R² greater than 0.985. The results of a culture plate were excluded if the results for the 3- and 10-pM dilutions deviated from the expected values by more than three times SD or if those of one known meat extraction sample deviated by more than two times SD.

All calculations were made using Microsoft Excel 11.0 (Microsoft, Redmond, WA, USA) and SAS 8.02 (SAS Institute Inc., Cary, NC, USA) software.

For PAH levels determined by GC-MS to be less than LOQ, we used half of the LOQ value for statistical calculations as recommended by the task group for scientific cooperation SCOOP (European Union 2004).

Results and Discussion

The repeatability of the CALUX bioassay (variability of extraction samples analysed in the same run) performed in quadruplicate was between 0% and 15%. The mean of the extraction samples was calculated from at least four assays in independent runs (different days, different plates); the average variation coefficient between the results of these plates was 16% (ranging from 3% to 37%). The pork belly samples were extracted four times, and the reproducibility (mean coefficient of variation of the IEQ) was 24% (3% to 55%).

We manufactured a total of 30 pork belly samples: 5 unsmoked, 13 cold smoked (for 3 to 6 h) and 12 hot smoked (for 1 to 3 h). The total number of samples includes four from the preliminary trial (two cold smoked for 1 or 3 h and stored not in aluminium boxes but in plastic boxes and two hot smoked for 1 h and additionally cold smoked for 1 h). Those four samples are included in the overview presented in table 1, but not in the more detailed presentation in the figures.

The effect-based bioassay evaluates the total activity of the specific compounds in the meat samples interacting with the AhR. The induction equivalents measured by effect-based analysis correspond to the degree of smoking. There were significant differences in the averaged bioassay results ($p < 0.01$) between the three smoking procedures: unsmoked (28 ng TCDD-IEQ/kg), cold smoked (248 ng TCDD-IEQ/kg) and hot smoked (661 ng TCDD-IEQ/kg). This bioassay thus basically categorises the pork belly samples according to smoking technique. The maximum bioassay value was 52 ng TCDD-IEQ/kg for the unsmoked meat samples; 429 for the cold smoked samples and 1,346 for the hot smoked samples. Figure 1 shows the mean IEQ and the median of the pork belly samples including the distribution according to smoking time. The average TCDD-IEQ for samples cold smoked 3 h was 221; for samples cold smoked 6 h, 282; for samples hot smoked for 1 h, 476; and for those hot smoked for 3 h, 1,093. Significant differences ($p < 0.05$) were found between unsmoked samples and all smoked samples and between those hot smoked for 1 h and those

smoked cold. Additionally, the median is marked in Figure 1. It is notable that the duration of cold smoking seemed to have little effect on the results of the bioassay, whereas one hour of hot smoking led to a significant increase in IEQ levels ($p < 0.05$).

PAHs are generated as complex mixtures and their profiles in food are variable. Table 1 shows the levels of 31 individual PAHs in these pork belly samples, including the 16 “EFSA-priority PAHs” (marked with an asterisk) designated by the European Food Safety authority (EFSA). These 16 PAHs are confirmed as toxicologically important by the International Agency for Research on Cancer (Straif et al. 2005). They include the list of 15 priority PAHs for which the former Scientific Committee on Food (SCF) of the European Commission has determined there is evidence of carcinogenic activity, as well as those PAHs designated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) as important for food safety monitoring (European Union 2002; Simon et al. 2008). There were significant differences ($p < 0.05$) in the mean levels of EFSA-priority PAHs in the pork belly samples depending on the type of smoking for all 16 priority PAHs. However, for two, chrysene and 5-methylchrysene, there were significant differences ($p < 0.05$) only between the unsmoked and hot smoked samples. The question therefore arises as to the meaningfulness of a determination of chrysene and 5-methylchrysene in a risk analysis of smoked meat. The average B[a]P level of $0.43 \mu\text{g kg}^{-1}$ found in the hot smoked samples agrees with the results of the EFSA data collection of 2007, which found an average of $0.386 \mu\text{g kg}^{-1}$ in heat-treated meat (European Union 2007). Figure 2 presents the total amounts of the 16 EFSA-priority PAHs in the pork belly samples (mean, SD and median) as determined by GC-MS according to the different smoking times. As shown by the screening test, there was no significant difference in PAH levels between 3 and 6 h of cold smoking.

In 2002, the SCF determined that the lower molecular weight PAHs such as fluorene, anthracene, phenanthrene, fluoranthene and pyrene showed markedly higher variability than those of higher molecular weight (European Union 2002). We investigated whether there

were significant differences in the levels of the lower molecular weight PAHs depending on different smoking treatments and found significant differences ($p < 0.01$) for fluorene and anthracene, but this was not the case for acenaphthene, phenanthrene, fluoranthene and pyrene. This supports the hypothesis that determination of the lower molecular weight PAHs in smoked meat products may not be useful for risk analysis.

The SCF has classified B[a]P as a marker compound for the presence and carcinogenicity of PAHs in food (European Union 2002) based on the fact that B[a]P levels remain fairly constant in relation to those of B[a]A, benzo[*b+j+k*]fluoranthene (B[*b+j+k*]F), indeno[1,2,3-*cd*]pyrene (IP) and DBA. With the exception of B[a]A, these PAHs contribute substantially to the overall PAH-induced AhR-mediated activity (Machala et al. 2001). For this reason, those PAHs were the focus of this study. Figure 3 shows the amounts of these compounds in the meat samples. The increases in the individual compounds depending on smoking technique are obvious, while the relative proportions of the individual PAHs remained relatively constant at every treatment level. However, it is questionable to apply conclusions drawn for one PAH to others. A better tool for food risk monitoring and analysis is the estimation of the total carcinogenic potential of a smoked meat sample.

We determined the distribution of PAHs in the meat samples more precisely by subdividing the pork belly samples according to sampling location. Figure 4 shows the induction equivalents of the two types of cut meat samples: those taken from the middle and from the edges of the pork belly. There were no significant differences ($p = 0.6$) in the unsmoked samples depending on the sampling zone. The differences between the two sampling zones became more pronounced with increasing smoking temperature and duration. The induction equivalents detected for the edge zone samples differed significantly ($p < 0.01$) from those of the middle zone when the samples were subjected to hot smoking for one hour. There appeared to be an even more pronounced difference between sampling zones between the meat samples subjected to hot smoking for three hours, but no statistical significance

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could be demonstrated here due to the small number of samples. This unusual technique was used only once in order to induce a high PAH contamination.

In summary, it appears that cold smoking of up to six hours did not cause strong contamination of the pork belly. In fact, there was no indication of any influence of cold smoking for up to six hours, but the results for hot smoking at 65 °C were very different. In particular, the duration of hot smoking seems to have substantially influenced the level of PAH contamination, which was clearly detected by the screening test. These findings are supported by those of Lijinsky (Lijinsky and Ross 1967) and by the EFSA statistical report of 2007, which showed increased concentrations of B[a]P with increasing temperatures (European Union 2007). The EFSA also reported low B[a]P levels resulting from the use of liquid smoke or from filtering the smoke before application (European Union 2007). The EFSA found no correlation between smoking time and B[a]P levels (European Union 2007). This was most likely due to the combined effects of smoking time and smoking temperature, as cold smoking is normally carried out for a much longer time than hot smoking.

Machala et al. (2001) cited two types of induction equivalent factors (IEFs) of PAHs relative to TCDD as standard substance in the CALUX assay. Those investigators derived IEFs from EC₅₀ or EC₂₅ values in 6-h exposure assays. Figure 5 shows the IEQs we determined in relation to the theoretical IEQs of the smoked meat samples, which are calculated using the GC-MS values of the samples and the IEFs of 17 PAHs cited by Machala (fluorene, anthracene, fluoranthene, pyrene, B[a]A, B[b]F, B[k]F, B[j]F, B[a]P, DBA, IP, benzo[e]pyrene, chrysene, benzo[ghi]perylene, cyclopenta[c,d]pyrene, 5-methylchrysene, benzo[c]phenanthrene). It is striking that the IEQs determined from the bioassay of the unsmoked samples were lower than the calculated IEQs; these products can thus easily be considered safe and the likelihood of false positive results is minimal. On the other hand, our results for the cold smoked and the samples smoked hot for 1 hour are in good agreement with the IEQs calculated using Machala's induction equivalent factors. However, the most

striking results were found for the samples smoked hot for three hours: here, the IEQs determined by bioassay were noticeably higher than the calculated theoretical values so that true positive results are easily detectable.

In conclusion, the results of our study provide further confirmation that the CALUX bioassay is a practicable pre-screening method for detecting AhR-active compounds such as PAHs in smoked meat. The toxic potential of the total meat sample can thus be determined using this method, although the specific agents still would have to be confirmed by chemical analysis. Large numbers of samples can thus be pre-screened and analysed for potential non-compliant samples. Further chemical analyses could then be limited to those samples. Effect-based screening tests like this bioassay have been proven to be sensitive in a range much lower than the European threshold value for B[a]P of 5 $\mu\text{g kg}^{-1}$ smoked meat and also lower than the former German threshold value of 1 $\mu\text{g kg}^{-1}$. This study lends further support to the conclusion that effect-based analysis applied as pre-screening method is suitable for food monitoring and risk control.

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Legends to figures

Figure 1: Mean (column), SD and median (cross) of TCDD - induction equivalents (TCDD-IEQ) of 26 samples of pork belly, determined with the CALUX bioassay as described in “Material and Methods”. All samples were prepared in the same manner; then some were cold smoked at 18 °C (for 3 or 6 hours), hot smoked at 65 °C (for 1 or 3 hours), or remained unsmoked (LOQ = 6 ngkg⁻¹ TCDD-IEQ)

Figure 2: Mean (column), SD and median (cross) of total 16 EFSA-priority PAHs in 26 samples of pork belly, determined with GC-MS as described in “Material and Methods”. All samples were prepared in the same manner; then some were cold smoked at 18 °C (for 3 or 6 hours), hot smoked at 65 °C (for 1 or 3 hours), or remained unsmoked.

Figure 3: Content and SD of 7 PAHs in 26 samples of pork belly, determined with GC-MS: benzo[a]anthracene (B[a]A), total benzo[b+j+k]fluoranthene (B[b+j+k]F), benzo[a]pyrene (B[a]P), indeno[1,2,3-cd]pyrene (IP) and dibenzo[a,h]anthracene (DBA).

Figure 4: TCDD induction equivalents (TCDD-IEQ) and SD of 26 samples of pork belly, determined with the CALUX bioassay. Samples were taken from both the middle (left column) and the edges (right column) of the pork belly after the smoking process. There were significant ($p < 0.01$) differences between sampling locations for the samples hot smoked for 1 h (LOQ = 6 ngkg⁻¹ TCDD-IEQ)

Figure 5: Mean and SD of calculated IEQs, using the contents measured with GC-MS and the induction equivalent factors (IEFs) of 17 PAHs published by Machala et al. 2001, who derived IEFs from EC₅₀ or EC₂₅ values in 6 h exposure assays with CALUX. Comparable to them, the means and SD of measured IEQs are shown.

Table 1. Mean, median and maximum levels ($\mu\text{g kg}^{-1}$) of 31 PAHs, measured with GC-MS as described in “Material and Methods” in 30 pork belly samples: 5 unsmoked, 13 cold smoked (for 3 or 6 h) and 12 hot smoked (for 1 or 3 h). EFSA-priority list PAHs are indicated with an asterisk.

PAH	Unsmoked			Cold smoked (3 or 6h)			Hot smoked (1or 3h)		
mean, median, maximum:	(n = 5)			(n = 13)			(n = 12)		
$\mu\text{g kg}^{-1}$	mean	median	max	mean	median	max	mean	median	max
Naphthalene	9.2	9.7	10.7	227.6	186.4	425.4	125.4	94.8	317.3
Acenaphthylene	1.1	1.0	1.7	28.7	27.5	66.0	92.9	64.0	267.1
Acenaphthene	32.3	25.7	61.8	45.6	47.6	100.2	65.8	80.6	118.7
Fluorene	15.8	13.7	25.2	53.5	49.0	103.2	149.0	115.0	333.0
Phenanthrene	85.6	58.7	165.0	86.6	54.1	205.4	227.1	196.6	393.5
Anthracene	2.6	2.7	4.6	7.3	7.0	12.3	41.3	30.9	119.6
Fluoranthene	10.1	11.4	16.5	13.8	17.3	27.1	31.2	26.4	55.3
Pyrene	4.3	3.9	7.9	6.4	8.4	11.0	17.9	14.6	37.7
*Benzo[c]fluorene	0.04	0.02	0.1	0.2	0.2	0.3	0.8	0.5	2.1
Benzo[ghi]fluoranthene	0.1	0.1	0.2	0.2	0.17	0.3	0.6	0.5	1.4
Benzo[c]phenanthrene	0.07	0.04	0.1	0.1	0.2	0.2	0.4	0.3	0.9
*Benz[a]anthracene	0.2	0.04	0.5	0.5	0.5	1.0	1.3	1.1	3.1
*Cyclopenta[cd]pyrene	<LOQ	<LOQ	<LOQ	0.1	0.2	0.2	1.0	0.8	2.7
*Chrysene	0.5	0.5	0.5	0.5	0.6	1.3	0.9	0.9	2.5
Triphenylene	0.1	0.1	0.2	0.3	0.3	0.5	0.3	0.3	0.6
*5-Methylchrysene	<LOQ	<LOQ	<LOQ	0.02	0.02	0.05	0.07	0.06	0.2
*Benzo[b]fluoranthene	0.07	0.06	0.1	0.2	0.2	0.3	0.4	0.4	0.6
*Benzo[k]fluoranthene	0.03	0.02	0.06	0.1	0.1	0.4	0.2	0.2	0.3
*Benzo[j]fluoranthene	0.06	0.07	0.1	0.1	0.1	0.2	0.3	0.2	0.4
Benzo[e]pyrene	0.04	0.02	0.09	0.2	0.2	0.2	0.3	0.3	0.5
*Benzo[a]pyrene	0.03	0.02	0.05	0.2	0.2	0.3	0.4	0.4	0.7
Perylene	<LOQ	<LOQ	<LOQ	0.03	0.03	0.05	0.07	0.07	0.1
*Indeno[1,2,3-cd]pyrene	0.02	0.01	0.04	0.1	0.1	0.2	0.3	0.3	0.5
*Dibenz[a,h]anthracene	<LOQ	<LOQ	<LOQ	0.02	0.02	0.03	0.05	0.05	0.08

*Benzo[ghi]perylene	0.02	0.02	0.04	0.08	0.07	0.1	0.2	0.2	0.4
Anthanthrene	<LOQ	<LOQ	<LOQ	0.03	0.04	0.07	0.1	0.1	0.2
Coronene	<LOQ	<LOQ	<LOQ	0.01	0.01	0.03	0.04	0.04	0.09
*Dibenzo[a,e]pyrene	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.01	0.01	0.04
*Dibenzo[a,h]pyrene	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.01	0.01	0.01
*Dibenzo[a,i]pyrene	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.01	0.01	0.02
*Dibenzo[a,l]pyrene	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.02	0.02	0.01	0.08
Total 16 EFSA-PAHs	0.8	0.3	1.6	2.2	2.0	3.7	5.8	4.8	13.1
Total 31 PAHs	162	189	228	473	450	821	760	649	1657

LOQ: 0.02 μgkg^{-1}

Mean (column), SD and median (cross) of TCDD - induction equivalents (TCDD-IEQ) of 26 samples of pork belly, determined with the CALUX bioassay as described in "Material and Methods". All samples were prepared in the same manner; then some were cold smoked at 18 °C (for 3 or 6 hours), hot smoked at 65 °C (for 1 or 3 hours), or remained unsmoked (LOQ = 6 ngkg⁻¹ TCDD-IEQ)

203x123mm (72 x 72 DPI)

Mean (column), SD and median (cross) of total 16 EFSA-priority PAHs in 26 samples of pork belly, determined with GC-MS as described in "Material and Methods". All samples were prepared in the same manner; then some were cold smoked at 18 °C (for 3 or 6 hours), hot smoked at 65 °C (for 1 or 3 hours), or remained unsmoked.

194x143mm (72 x 72 DPI)

Content and SD of 7 PAHs in 26 samples of pork belly, determined with GC-MS: benzo[a]anthracene (B[a]A), total benzo[b+j+k]fluoranthene (B[b+j+k]F), benzo[a]pyrene (B[a]P), indeno[1,2,3-cd]pyrene (IP) and dibenzo[a,h]anthracene (DBA).
213x133mm (72 x 72 DPI)

TCDD induction equivalents (TCDD-IEQ) and SD of 26 samples of pork belly, determined with the CALUX bioassay. Samples were taken from both the middle (left column) and the edges (right column) of the pork belly after the smoking process. There were significant ($p < 0.01$) differences between sampling locations for the samples hot smoked for 1 h (LOQ = 6 ng kg^{-1} TCDD-IEQ)

213x137mm (72 x 72 DPI)

Mean and SD of calculated IEQs, using the contents measured with GC-MS and the induction equivalent factors (IEFs) of 17 PAHs published by Machala et al. 2001, who derived IEFs from EC50 or EC25 values in 6 h exposure assays with CALUX. Comparable to them, the means and SD of measured IEQs are shown.

213x106mm (72 x 72 DPI)