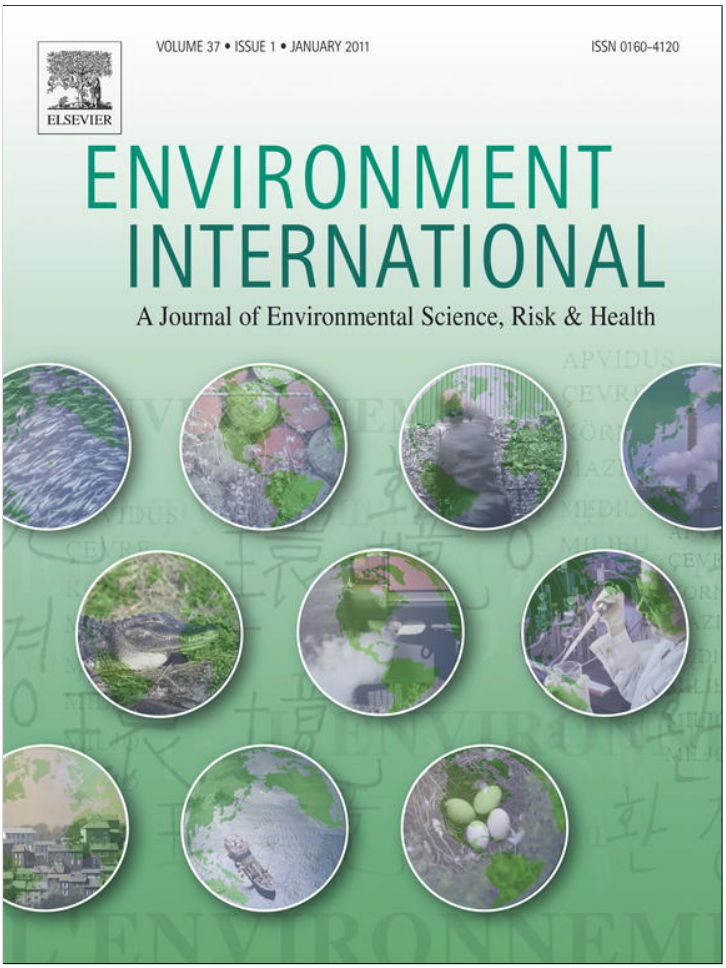

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The predictive power of the elimination of dioxin-like pollutants from pigs: An in vivo study

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ABSTRACT

Pigs accidentally given feed contaminated by dioxin-like pollutants are a serious public health issue. We have examined whether pigs with limited exposure during early periods of fattening would be categorized as non-compliant with the EU limit at slaughtering when growth-dilution, excretion and metabolism effects are considered. Sixteen female and sixteen castrated male weaned pigs were divided into four groups (e.g. DG0, DG1, DG2 and DG3) in week 2 after birth. From weeks 3 to 13, groups DG1, DG2, and DG3 pigs were fed with a polychlorinated dibenzo-*p*-dioxin/dibenzofuran (PCDD/F) and polychlorinated biphenyl (PCB) mixture at dosages of 1, 10 and 100 ng-toxic equivalent (TEQ) per kg dry mass feed in capsules, respectively. From weeks 13 to 23, the animals were nourished with clear feed. Control group DG0 was always fed with clear feed. Subcutaneous fat samples were collected at weeks 13, 18 and 23 by biopsies. The pollutant residues were analyzed by high resolution gas chromatography–high resolution mass spectrometry and quantified by a ^{13}C-isotope dilution method. The results showed the following: (1) when slaughtered at week 23, the TEQ for DG1 pigs (0.66 ± 0.21 pg/g fat) was under the EU limit of 1 pg PCDD/F-TEQ/g fat; (2) PCDD/F congener-specific first-order elimination rates were linearly correlated with their toxicity equivalency factors (TEFs), and the rates were significantly dose-dependent for the more toxic congeners (TEF $\geq$ 0.1). Therefore, the pigs' exposure above the EU limit during the early fattening stage did not necessarily lead to their categorization as non-compliant pork; and the residual TEQ for pork can be predicted from early exposure concentrations based on the models established here.

1. Introduction

Approximately 90% of the dioxin exposure found in humans results from the ingestion of contaminated food (Bocioa et al., 2007). Foods of animal origin are the major source of dioxin exposure, and this arises mainly from the contamination of animal feed (SCAN, 2000). Thus, the ingredients used in animal feed are fundamentally important in terms of both the quality of the resulting food products and the potential human health impacts associated with the animal-based food production chain (Fernandes et al., 2011; Sapkota et al., 2007). In recent years, several cases of contaminated feed were reported, including the following: (a) the use of ball clay from different locations as a flow supporting agent, where the ball clay was believed to be contaminated due to geothermal processes; (b) the drying of grass using wood contaminated with preservatives; (c) the contamination of feed by wood treated with preservatives; and (d) the contamination of feed with wastes originating from industrial sources (e.g., polychlorinated biphenyl (PCB) oils) (Bernard et al., 1999, 2002; Covaci et al., 2002; Hoogenboom et al., 2004b, 2007, 2009; Llerena et al., 2003; Malisch, 2000; Tlustos, 2009a). These accidents have become the potential public health issue.

Although the prediction of the concentrations of lipophilic chemicals in growing pigs was expected (Fries, 1996), for pigs and other animals, there are only a few studies (Hoogenboom et al., 2004a; Marchand et al., 2010; Spitaler et al., 2005; Thorpe et al., 2001; Traag et al., 2006) on the defined exposure to polychlorinated dibenzo-p-dioxin/dibenzofuran (PCDD/F) in relation to their elimination, in the context of food safety. This study was designed to examine the elimination rate of dioxin in adipose tissue of pigs fed with three-level dosages of PCDD/F-PCB

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mixture at 1, 10 and 100 ng-toxic equivalent (TEQ)/kg dry mass food, where TEQs were calculated by multiplying individual congener concentrations by congener-specific toxicity equivalency factors (TEFs) in the feed. The purpose was to determine the change in residual dioxin content based on PCDD/F-PCB toxicant kinetics in pigs due to the early exposure, and to evaluate the safety of pork for consumption after contamination. In addition, due to the very large social and economic losses from dioxin accidents (Tlustos, 2009b), findings from this study may contribute to the knowledge of the categorization of non-compliant pork and the minimization of losses in potential dioxin contamination incidents.

2. Materials and methods

2.1. Chemicals

The PCDD/F-PCB mixture administered consisted of the following: 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (PCDD), 1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin (HxCDD), 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin (HpCDD), 1,2,3,4,6,7,8,9-octachlorodibenzo-*p*-dioxin (OCDD), 2,3,7,8-tetrachlorodibenzofuran (TCDF),2,3,4,7,8-pentachlorodibenzofuran (PCDF), 1,2,3,4,6,7,8,9-octachlorodibenzofuran (OCDF) and 3,3′,4,4′,5-pentachlorobiphenyl (PCB-126). In addition to the administered compounds, the following background PCDD/Fs and PCBs were also measured: 1, 2,3,6,7,8-hexachlorodibenzo-*p*-dioxin, 1,2,3,7,8,9-hexachlorodibenzo-*p*-dioxin, 1,2,3,7,8-pentachlorodibenzofuran, 1,2,3,4,7,8-hexachlorodibenzofuran, 1,2,3,6,7,8-hexachlorodibenzofuran, 1,2,3,7,8,9-hexachlorodibenzofuran, 2,3,4,6,7,8-hexachlorodibenzofuran, 1,2,3,4,6,7,8-heptachlorodibenzofuran, 1,2,3,4,7,8,9-heptachlorodibenzofuran, non-*ortho* PCB-77, -81, -169 and mono-*ortho* PCB-105, -114, -118, -123, -156, -157, -167, -189. All the native and ^{13}C-labeled standards were purchased from Cambridge Isotope Laboratory, MA, USA.

2.2. Dosing capsule

The capsule feeding method was used because it ensured that the entire dose of administered compounds was completely ingested. The mixture was dissolved in soybean oil from the supermarket and was packed in gelatin capsules (Hart-Gelatine-Kapseln, Wepa, Germany). The proportions (% of total TEQ) of the administered PCDD/Fs and PCB-126 in capsule oil were the following (Van den Berg et al., 1998): 25.56% TCDD, 28.40% PCDD, 8.52% HxCDD, 1.14% HpCDD, 0.02% OCDD, 5.40% TCDF, 26.98% PCDF, 0.02% OCDF and 3.98% PCB-126. The capsules were prepared as follows: the chemicals were dissolved in toluene and mixed, and then toluene was evaporated. After the addition of a small amount of ethanol, 40 ml soybean oil dissolved the target compounds for each lot of oil after stirring for 24 h. The oil was stored at room temperature and sealed with Teflon caps in brown glass bottles for light sheltering. The final dioxin content in the oil was measured by high resolution gas chromatography–high resolution mass spectrometry (HRGC–HRMS) to determine if there were possible solubility problems, which could lead to divergences between the real and nominal values of the tested compounds. The capsule was packed using a capsule-filling device (Aponorm, Wepa, Germany), in which up to 60 capsules could be filled simultaneously. Eppendorf pipettes were used to transfer 20 μl of oil into small hard gelatin capsules (white, size 4, 0.21 ml, Wepa, Germany), and then the capsule was filled with soy flour (supermarket quality). The small capsule was then packed into a larger hard gelatin capsule (colorless, size 0, 0.68 ml, Wepa) for safety reasons. The capsules were stored at room temperature in 100 ml Duran® square-lab bottles (Bulkhead, Germany) with Teflon caps and protected from light. Five concentrations were prepared per feeding group to meet the targeted daily dose, maintaining a constant proportion of administered pollutants overall after taking into account the increasing amount of feed given during the growth of the pigs.

2.3. Animals

The local ethics committee approved the experiment. A total of 32 weaned piglets (16 females and 16 castrated males of *Landrasse Pietrain*) were bought from Erzeugergemeinschaft für Ringferkel in Schwaben v.W. Within three days, the piglets were weighted (8.8 ± 1.0 kg), labeled by ear markers (EMs) and divided into four groups. Each group contained eight animals with four males and four females, and the grouped pigs were arranged with four per box (see Table S1 in the Supplementary materials (SM)).

2.4. Treatment

From week 3 to week 13, animals were administered the PCDD/F-PCB mixture in capsules in three dosage groups (DG1, DG2, and DG3) of 1, 10 and 100 ng-TEQ/kg dry mass food, respectively. The amounts of the compounds administered administrated in the capsules were increased with respect to the amount of background contaminated feed consumed so that the intake of compounds was constant in relation to feed consumption. Each pig was given one capsule per day. A total of 616 capsules with 5 different concentrations were given per group. In the 10 weeks following the treatment, all the pigs were nourished with background contaminated feed. The control group (DG0) pigs always received background contaminated feed.

2.5. Sample collection and storage

Subcutaneous fat was sampled at the end of the exposure period (week 13), in the middle (week 18) and at the end of the experiment (week 23) by biopsies while the animal was under anesthesia. The animals labeled EM44 (DG2), EM56 and EM32 (DG3) died after the second surgery, therefore no week 23 samples were available for these animals. All the samples were stored at −18 °C until further analysis.

2.6. Sample preparation and measurement

Sample preparation and measurement procedures have been described elsewhere (Lenk, 2007; Schramm et al., 2009; Simm et al., 2006). Briefly, samples (approximately 2.5 g subcutaneous fat) spiked with a ^{13}C-internal standard were homogenized and extracted by accelerated solvent extraction (ASE 200, Dionex GmbH, Idstein, Germany) at 120 °C and 120 bar with the mixed solvent n-hexane:acetone 75:25. A cleanup procedure (Simm et al., 2006) was applied to the extract before further analysis, where ^{13}C-1,2,3,4-TCDD was used as the recovery standard. The PCDD/F and PCB residuals were analyzed by HRGC (Agilent GC 6890 Agilent Technologies, Palo Alto, CA, USA) in combination with HRMS (Finnigan MAT 95S, Thermo Electron GmbH, Bremen, Germany) separately. For the PCDD/F congener analysis, a 60 m Rtx-Dioxin2 column (0.25 mm ID, 0.25 μm film thickness, Restek) was used, while a 30 m Rtx-CLPesticides2 column (0.25 mm ID, 0.2 μm film, Restek) was used for PCB determination. A ^{13}C-isotope dilution method was used for the quantification, and a three-fold signal/noise ratio was set as the limit of detection.

2.7. Data analysis

All the non-detectable data were set as zero in the data analysis since their limit of detections were <0.005. The statistics and data plotting were achieved with Microsoft Office Excel 2003, S-PLUS 6.2 (Insightful Corp., 2003) and SigmaPlot 9.0 (Systat Software Inc., 2004). In case of statistical method blind spots in the small-size data analysis, both parameter and non-parameter approaches (*t*-test, the Wilcoxon rank-sum test and the Kolmogorov-Smirnov test) were used for comparing the group differences.

3. Results

3.1. Growth rates and lipid contents

The animal body weight was measured at weeks 1, 3, 6, 11, 13, 15, 18 and 23. The overall growth was not significantly different for animals with different sexes, animal pens and treatment dosages. However, during the fattening period, there was a significant increase in lipid content in the subcutaneous fat tissues (Figure S2). More detailed information for the animal growth and lipid changes can be found in the SM.

3.2. Elimination rates of the PCDD/F-PCB mixture

The final pollutant concentrations were expressed as pg/g lipid to adjust the lipid content change. A first-order elimination model, $Con = a_0 e^{-kt}$, was used to assess the pharmacokinetics of the investigated PCDD/F-PCB congeners, where *Con* is the congener concentration or total TEQ in the subcutaneous fat samples, *t* is the age (in days) at the sampling time (weeks 13, 18, and 23), and *k* is the elimination rate of the congener or total TEQ. Here TEQ in total was the summary of the 9 investigated congeners' TEQs (Van den Berg et al., 1998). Considering the concentration change due to growth dilution, the *BW* adjusted elimination rate k_{adj} was calculated by regressing *Con* multiplied by *BW* (i.e., *Con BW*) and *t*. For DG1 pigs, the concentrations of congeners at week 23 were close to the background (i.e., DG0 concentrations) and the trend was not clear during elimination phase (Table 1), therefore DG1 animals were excluded in the further regression analysis. Concentrations of 2,3,7,8-TCDF were below the limit of detection in most of the week 18 and week 23 samples, therefore they were also excluded from the regression analysis. Finally, elimination rates of the other 8 congeners were calculated for DG2 and DG3 animals. Due to the limited sampling points (three data were available for each curve), for all *k* and k_{adj}, only coefficients of determination (R^2) larger than 0.8 were accepted for further analysis. In Fig. 1, *k* and k_{adj} are plotted. The fractions of k_{adj} in *k* (average $k_{adj}/k \pm$ standard deviation) were also calculated; they were 0.751 (±0.033), 0.692 (±0.050), 0.452 (±0.063), 0.530 (±0.093), 0.430 (±0.141), 0.611 (±0.045), 0.489 (±0.131), 0.732 (±0.031) and 0.623 (±0.032), respectively, for 2,3,7,8-TCDD, 1,2,3,7,8-PCDD, 1,2,3,4,7,8-HxCDD, 1,2,3,4,6,7,8-HpCDD, 1,2,3,4,6,7,8,9-OCDD, 2,3,4,7,8-PCDF, 1,2,3,4,6,7,8,9-OCDF, PCB-126 and total TEQ. These data are highly correlated with the number of Cl atoms on the PCDD/F molecules ($R^2 = 0.73$). The difference in the two elimination rates ($k - k_{adj}$) for all the congeners was 0.011 (0.009–0.011).

The possible effect of the pigs' sex on PCDD/F-PCB elimination rates was also analyzed. Both parameter and non-parameter tests were applied due to the relatively small sample set. The statistical results (data not shown) suggested that sex did not affect *k* or k_{adj}. Therefore, the values from both sexes were pooled in the further analysis.

3.3. Dosage, congener toxicity and k or k_{adj}

The congeners of PCDD/F and PCB that were not administered had similar concentrations in all the administered pigs compared to the control pigs (Table S4). The background exposure to the administered congeners (DG0 data in Table 1) were varied at a low level, the biggest variation happened at Week 13 samples (total WHO-TEQ = 1.09 pg/g lipid with standard deviation 2.87 pg/g lipid). For the administered congeners, given the elimination started from week 13 just after the contaminant feeding, these concentrations were set as initial dosage (Con_{w13}). The WHO-TEQs in total (Table 1) at week 13 were 3.30 (1.11), 38.06 (6.46) and 197.86 (45.72) pg/g lipid for DG1, DG2 and DG3 pigs, respectively. The calculated mean *k* values were 0.025, 0.029, and 0.032, respectively, and the related k_{adj} values were 0.013, 0.018 and 0.020. The half-life ($\tau_{0.5}$) and the adjusted half-life ($\tau_{0.5,\ adj}$) can be calculated from *k* and k_{adj} by using formula $\tau_{0.5} = 0.693/k$. The estimated $\tau_{0.5}$ values were 27.8, 23.9 and 21.7 days, respectively, and the corresponding $\tau_{0.5,\ adj}$ were 51.7, 38.1 and 35.0 days. For the congener-specific *k* or k_{adj} in DG2 and DG3 animals, the regressed results of Con_{w13} versus *k* and Con_{w13} versus k_{adj} all suggested that the dosages (Con_{w13}) linearly correlated with the elimination rates for the more toxic congeners 2,3,7,8-TCDD, 1,2,3,7,8-PCDD, 2,3,4,7,8-PCDF and PCB-126 (Fig. 2 and Table S4). The more highly chlorinated congeners (≥6 Cl atoms) were more persistent with smaller *k* and k_{adj} values (Fig. 1) than the less chlorinated congeners. For these congeners, no apparent dosage-dependent elimination rate was observed (Table S4). In addition, by plotting TEFs vs. *k* and TEFs vs. k_{adj}, we observed that *k* and k_{adj} were highly correlated with the TCDD/F congeners' TEFs. However, PCB-126 was an outlier point in the plot (Fig. 3).

3.4. Prediction of residue concentrations at slaughtering

The average TEQ half-life was approximately 23 days for the administered PCDD/F-PCB mixture in this study. Based on the correlations between WHO-TEF and *k* (Fig. 3), we established the models for calculating the elimination half-lives of PCDD/F congeners. We expect that the models will be useful for pork safety assessment in potential accidents involving feed contamination. In the models, Con_{TEQ} is the predicted concentration of residue in fat at time of slaughtering:

$$k_{TEQ} = 0.0202 \sum f_i TEF_i + 0.0216 \quad (1)$$

Table 1
PCDD/F-PCB congeners' TEQs and total TEQ concentrations (pg/g lipid) in the subcutaneous fat at weeks 13, 18 and 23 in the four administered groups (DG0, DG1, DG2, and DG3) with standard deviation (STD).

Congener[a]	DG0 (STD)			DG1 (STD)			DG2 (STD)			DG3 (STD)		
	Week 13 (Con_{w13})	Week 18	Week 23	Week 13 (Con_{w13})	Week 18	Week 23	Week 13 (Con_{w13})	Week 18	Week 23	Week 13 (Con_{w13})	Week 18	Week 23
TCDD	0.00 (0.00)	0.57 (1.47)	0.11 (0.17)	0.56 (0.16)	1.36 (3.57)	0.00 (0.00)	8.01 (1.70)	1.55 (0.42)	0.64 (0.21)	38.04 (9.90)	4.50 (1.28)	1.56 (0.54)
PCDD	0.00 (0.00)	0.33 (0.92)	0.05 (0.13)	0.82 (0.23)	0.46 (0.33)	0.27 (0.17)	9.20 (1.90)	2.65 (0.66)	1.18 (0.35)	40.50 (11.24)	7.79 (2.09)	2.92 (0.85)
HxCDD	0.00 (0.00)	0.02 (0.06)	0.00 (0.00)	0.86 (0.42)	1.14 (1.90)	0.25 (0.13)	8.65 (1.40)	4.25 (1.02)	2.58 (0.55)	57.64 (11.81)	24.01 (3.45)	14.68 (1.17)
HpCDD	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.02 (0.01)	0.01 (0.00)	0.00 (0.00)	0.19 (0.04)	0.08 (0.02)	0.04 (0.02)	0.99 (0.24)	0.36 (0.06)	0.20 (0.04)
OCDD	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.01 (0.00)	0.01 (0.00)	0.00 (0.00)
TCDF	0.00 (0.00)	0.06 (0.18)	0.00 (0.00)	0.00 (0.00)	0.10 (0.29)	0.00 (0.00)	0.08 (0.02)	0.00 (0.00)	0.00 (0.00)	0.54 (0.13)	0.01 (0.01)	0.00 (0.00)
PCDF	0.01 (0.03)	0.10 (0.25)	0.00 (0.00)	0.93 (0.41)	0.34 (0.14)	0.14 (0.05)	10.89 (1.77)	4.07 (0.76)	2.04 (0.34)	55.38 (12.50)	17.51 (4.10)	7.72 (2.36)
OCDF	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.01 (0.00)	0.00 (0.00)	0.00 (0.00)
PCB-126	0.05 (0.02)	0.02 (0.02)	0.02 (0.02)	0.11 (0.06)	0.04 (0.02)	0.01 (0.02)	1.02 (0.21)	0.23 (0.08)	0.09 (0.02)	4.70 (1.52)	0.82 (0.25)	0.28 (0.13)
WHO-TEQ	0.06 (0.04)	1.09 (2.87)	0.17 (0.29)	3.30 (1.11)	3.45 (6.07)	0.67 (0.22)	38.06 (6.46)	12.83 (2.59)	6.57 (1.23)	197.86 (45.72)	55.05 (9.58)	27.39 (4.33)

[a] The investigated congeners were 2,3,7,8-TCDD, 1,2,3,7,8-PCDD, 1,2,3,4,7,8-HxCDD, 1,2,3,4,6,7,8-HpCDD, 1,2,3,4,6,7,8,9-OCDD, 2,3,7,8-TCDF, 2,3,4,7,8-PCDF, 1,2,3,4,6,7,8,9-OCDF, PCB-126.

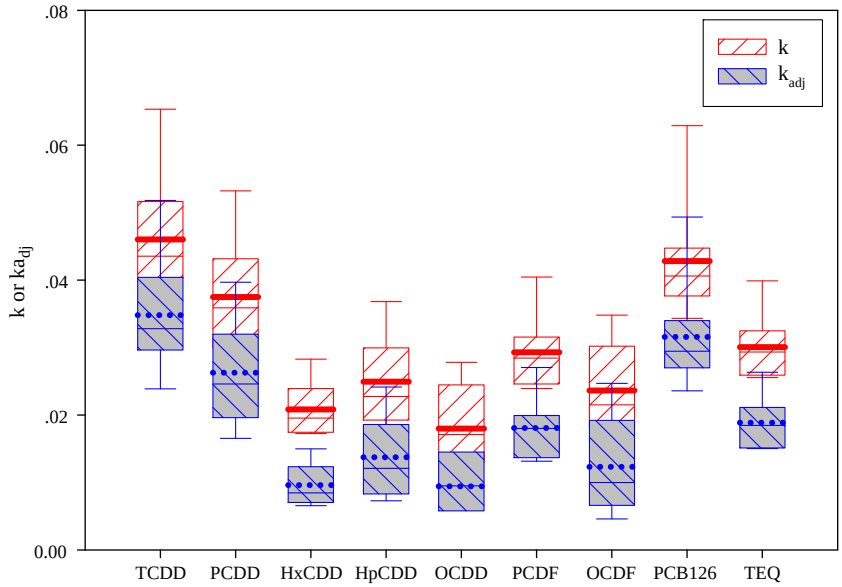

Fig. 1. Vertical boxes plotted the 5th/95th percentile of k or k_{adj} values for 2,3,7,8-TCDD, 1,2,3,7,8-PCDD, 1,2,3,4,7,8-HxCDD, 1,2,3,4,6,7,8-HpCDD, 1,2,3,4,6,7,8,9-OCDD, 2,3,4,7,8-PCDF, 1,2,3,4,6,7,8,9-OCDF, PCB-126 and total TEQ. The red boxes are k and blue ones are k_{adj}, the red solid line is mean value of k and blue dotted line is mean of k_{adj} respectively. 2 of 12 k values and 3 of 12 k_{adj} values for 1,2,3,4,6,7,8,9-OCDF, 5 of 12 k_{adj} values for 1,2,3,4,6,7,8,9-OCDD are not presented due to coefficient of determination (R^2)<0.83.(For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

$$Con_{TEQ} = a_0 e^{-k_{TEQ} t}. \quad (2)$$

Based on Eq. 1, k_{TEQ} can be calculated by using the measured fractions (i.e., f_i is fraction of the congener i) and PCDD/F congeners' WHO-TEFs (TEF_i) in pig fat tissue, and the final total TEQ can be estimated using Eq. 2, where a_0 is the total TEQ of PCDD/Fs when the contaminated feeding stops, and t is the time of the elimination phase.

4. Discussion

4.1. Growth rate and the factors' impact on growth

The growth dilution effect is one of the two important factors we considered in the present study. We first investigated the growth curves. Because the piglet and fattening pig followed different growth curves (Ittner and Hughes, 1938; Schinckel et al., 2003; Taylor and

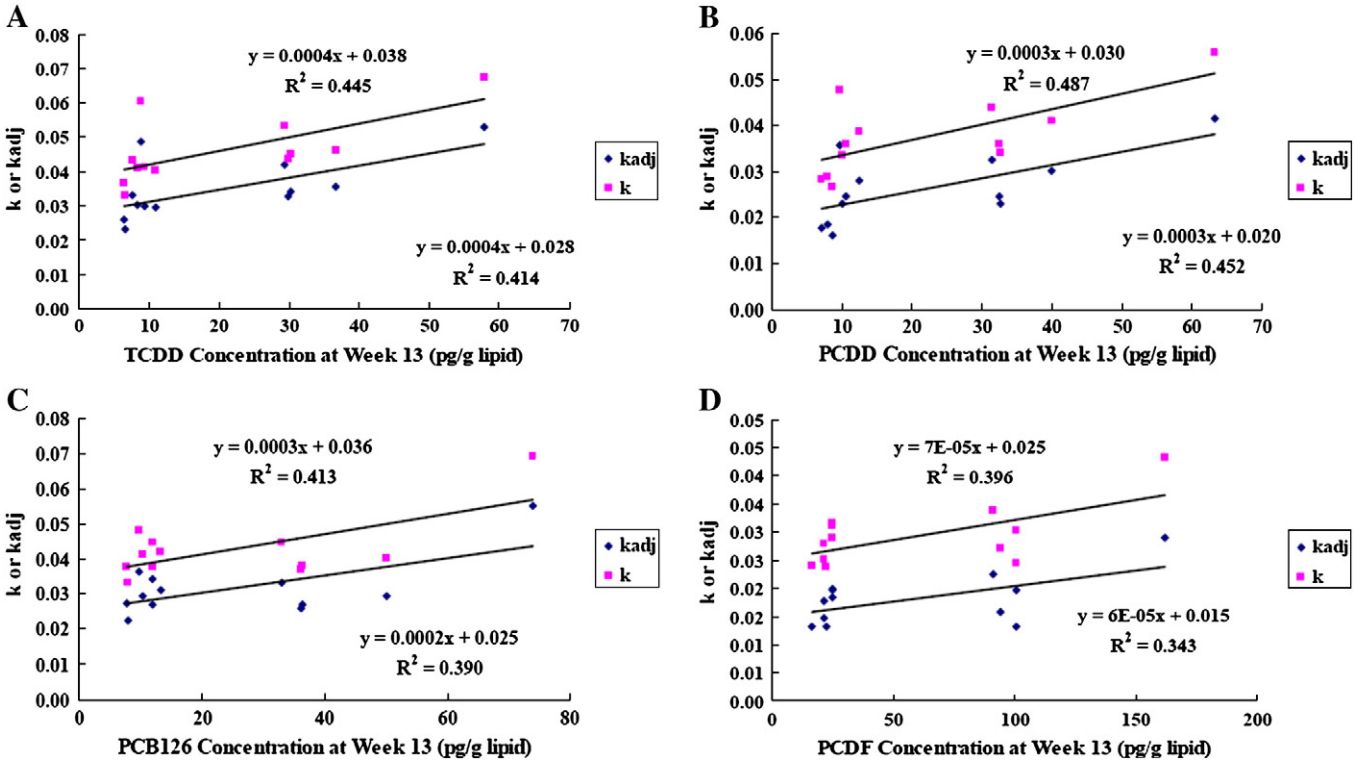

Fig. 2. The trends of dosage (concentrations at week 13 Con_{w13}) versus k and k_{adj} plots for the more toxic congeners 2,3,7,8-TCDD, 1,2,3,7,8-PCDD, PCB-126 and 2,3,4,7,8-PCDF with TEFs≥0.1 (Van den Berg et al., 1998).

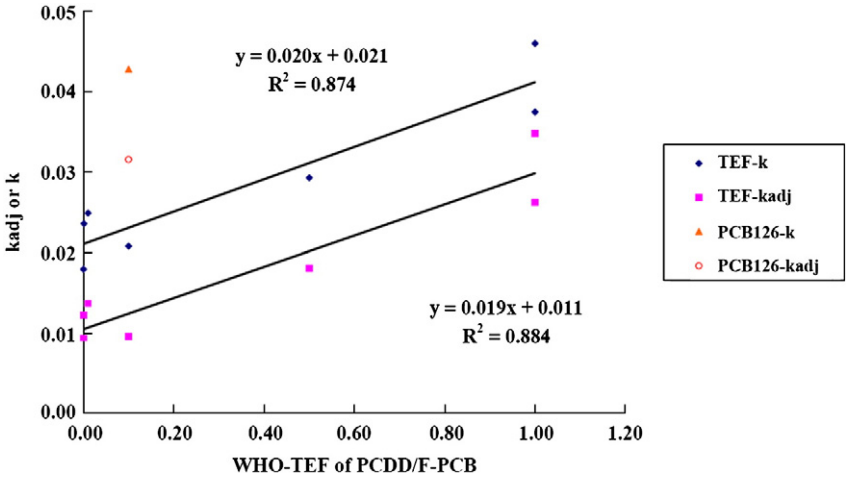

Fig. 3. The regression curves of WHO-TEF (Van den Berg et al., 1998) versus k and k_{adj} for TCDD/F congers 2,3,7,8-TCDD, 1,2,3,7,8-PCDD, 1,2,3,4,7,8-HxCDD, 1,2,3,4,6,7,8-HpCDD, 1,2,3,4,6,7,8,9-OCDD, 2,3,4,7,8-PCDF, and 1,2,3,4,6,7,8,9-OCDF. PCB-126 was excluded from the regression analysis.

Hazel, 1955), life-stage based models were used. The results suggested that the models described the life-stage growing well. The growth rate and the factors (sex, box and dosage) that may impact growth rate were investigated carefully. Generally, sex and box difference did not affect the growth rate, and PCDD/F-PCB exposure did not significantly affect the normal growth of the testing animals. Therefore, the box factor is not included in the further discussion. Due to the increased lipid content in subcutaneous fat tissues, all results were normalized over the lipid content (Kelly et al., 2007).

4.2. Toxicokinetics and growth dilution

A previous study showed that the half-lives of PCDD/Fs and PCBs in the human body were correlated with age, body fat, smoking status, and breast-feeding (Milbrath et al., 2009). For the tested pigs, due to the fact that most conditions were well controlled, we expected that two factors should primarily contribute to the concentration changes: elimination via metabolism and excretion (Thorpe et al., 2001) and growth dilution (Hill et al., 1982). The latter is caused by the increasing body weight and fat pool, which can dilute the pollutants' concentrations (Fries, 1995). The calculated k_{adj} yielded a parameter that is independent of the growth rate (Hill et al., 1982). The ratio of k_{adj}/k may indicate the respective contributions of metabolism and excretion in the total elimination. The results suggested that k_{adj}/k is dependent on the persistent of PCDD/F congeners (the more Cl in PCDD/F congeners, the more persistent they are). On the other hand, the difference between k_{adj} and k may suggest a dilution effect, which was nearly identical for all congeners.

4.3. Dosage-dependent PCDD/F-PCB elimination?

TEQ quantitatively describes the dioxin-like toxicity for PCDD/F-PCB (Van den Berg et al., 1998). The correlations of TEF vs. k and TEF vs. k_{adj} were significant. To our knowledge, this was the first time that the influence of toxicity (i.e., WHO-TEF) on the elimination rates of PCDD/Fs in pigs was elaborated. This may be the result of additional chlorine atoms making the PCDD/Fs more persistent (Fernandes et al., 2011). There is no significant difference when the old or the new TEFs were used (Van den Berg et al., 1998; Van den Berg et al., 2006). For easy comparison with literature data, only 1998 WHO-TEFs were used in the final calculation. The PCB-126 outlier may imply a different toxicological mechanism as compared to the PCDD/Fs. Like the recently developed physiologically based toxicokinetic (PBTK) model using a body-burden-dependent elimination rate (Emond et al., 2005) in human exposure assessment, the observed higher dosage treatment was associated with the higher toxicant elimination rates for the more toxic congeners 2,3,7,8-TCDD, 1,2,3,7,8-PCDD, PCB-126 and 2,3,4,7,8-PCDF (TEFs $\geq$ 0.1), which may support the assumption that dosage determines the PCDD/Fs' elimination.

4.4. Predicting the concentrations of PCDD/F residue at slaughtering

For the assessment of pork safety after feed contamination accidents or general exposure, it is necessary to consider the congener patterns, the interval from exposure to slaughtering and exposure levels. The average TEQ half-life (approximately 23 days) was close to the elimination rate found in rats (Viluksela et al., 1996), but lower than in cattle (Thorpe et al., 2001) and humans (Milbrath et al., 2009; Poiger and Schlatter, 1986). Due to the weak dose-dependent elimination of the more toxic congeners, the assessment should consider the reduced elimination rates for these congeners in the case of low-level contamination. For the most sensitive dose-dependent congener 2,3,7,8-TCDD, the estimated decrease of k is $\delta k = 0.0004$ per pg for the defined exposure range from 60 to 5 pg/g fat (Table S5). By using our prediction models, it may be possible to categorize the pork as safe or not at slaughtering if "clean" feed is offered after the possible accident.

5. Conclusion

Data from the present study suggested that not only the relative content of dioxins in adipose tissues, but also the absolute quantities decreased with time. The contaminated adipose tissues from the feeding of 1.0 ng WHO-TEQ/kg dry food were in compliance with the EU limit for pork after 10 weeks of growth with background contaminated feed. The proposed elimination model based on the first-order elimination rates of PCDD/Fs with reference to their association with congener specific TEF in fat tissues may be further used to predict the TEQ residues at slaughtering. Due to the limited sample sizes used in this study, we suggest that additional practical data should be collected to further refine the use of the current assessment models. Thus, the results may serve as an impetus for further research to develop tools to enable the mitigation of economic and societal losses based on risk assessment and communication.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.envint.2011.08.009.

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