



# High levels of dioxins and PCBs in meat, fat and livers of free ranging pigs, goats, sheep and cows from the island of Curaçao

Ron L.A.P. Hoogenboom<sup>a,\*</sup>, Guillaume ten Dam<sup>a</sup>, Stefan P.J. van Leeuwen<sup>a</sup>, Harry van Egmond<sup>a</sup>, Jennyfer Nicolina<sup>b</sup>, Arnold J.S. Dwarkasing<sup>b</sup>

<sup>a</sup> Wageningen Food Safety Research (WFSR), Wageningen University and Research, Akkermaalsbos 2, Wageningen, the Netherlands

<sup>b</sup> Department of Veterinary Affairs of the Ministry of Health, Environment and Nature, Abattoirweg 15, Willemstad, Curaçao

## HIGHLIGHTS

- High levels of PCDD/Fs and PCBs detected in fat and meat of animals.
- Lipid based levels in fat and meat were in general comparable.
- Lipid based liver levels were much higher, pigs being most sensitive.
- Patterns point to burning as the major source, but also PCBs contribute.

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## ABSTRACT

Samples of adipose tissue, meat and livers from pigs, cows, sheep and goats from Curaçao were analysed for polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs), and dioxin-like (dl-) and non-dioxin-like (ndl-) PCBs (polychlorinated biphenyls). Levels in many samples of adipose tissue were higher than the EU maximum levels (MLs) for PCDD/Fs and the sum of PCDD/Fs and dl-PCBs (sum-TEQ), indicating unusually high levels. Median sum-TEQ (Toxic Equivalents) levels for pigs, cows, sheep and goats were 0.9 (range 0.3–35), 3.0 (0.5–14), 5.7 (0.3–28) and 6.5 (0.5–134) pg TEQ g<sup>-1</sup> fat. For most samples, the congener pattern pointed to the burning of waste as the major source, in line with the fact that most animals forage outside. MLs for ndl-PCBs were also exceeded in some of the samples, indicating that some areas are additionally contaminated with PCBs. Meat levels showed similar lipid based levels as adipose tissue, contrary to liver levels, which were much higher in most animals. Pigs showed liver sequestration at lower levels in adipose tissue than the ruminants. The relatively high levels observed in this study are likely to result in high exposure of consumers and measures were taken to reduce the contamination of areas where animals forage.

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## 1. Introduction

Polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) and dioxin-like polychlorinated biphenyls (dl-PCBs) in food remain a concern. A new risk assessment by the European Food Safety Authority (EFSA) resulted in a lowering of the Tolerable Weekly Intake (TWI) from 14 to 2 pg TEQ/kg body weight (bw) per week, based on effects on sperm quality observed in humans (EFSA, 2018). It was also shown that most of the European population exceeded this new TWI. This may also apply to many countries

outside Europe. It is therefore important to identify remaining sources and lower the exposure of consumers.

Animal derived food, including fish, contributes most to human exposure, due to the accumulation of these contaminants in fatty tissues and liver (EFSA, 2018). Although much effort has been placed in monitoring and reducing the levels in these products, it remains an important question to which extent this is really feasible for the general background level. However, there are also food products with relatively high levels resulting in an elevated exposure of certain consumers. This was e.g. shown for consumers of eel from contaminated rivers (Van den Dungen et al., 2016), or eggs from hens foraging on contaminated soil (Hoogenboom et al., 2014). In the latter case, the source for these contaminants was soil

\* Corresponding author.

E-mail address: [ron.hoogenboom@wur.nl](mailto:ron.hoogenboom@wur.nl) (R.L.A.P. Hoogenboom).

rather than feed. It is less clear to what extent this also applies to other animal species, although this has been shown for fat and livers of sheep which normally forage outside (Bruns-Weller et al., 2010; Rose et al., 2010), and milk of cows and buffaloes raised on soil with elevated levels of PCDD/Fs and dl-PCBs (Liem et al., 1991; Diletti et al., 2008, 2009; Esposito et al., 2010).

The island of Curaçao has 160,000 inhabitants and a relatively small animal husbandry sector with an average self-supply percentage of 3% for meat and meat products. As part of a project regarding the assessment of the food safety of locally produced meat and meat products, the Department of Veterinary Affairs requested RIKILT (now WFSR) in 2014 to analyse a set of fat, meat and liver samples from pigs, goats, sheep and cows raised on the island. With the experience of rather low background levels in pigs in the Netherlands (Adamse et al., 2017), samples of fat tissue were screened with the Dioxin Responsive Chemical Activated Luciferase gene eXpression (DR CALUX)-bioassay resulting in almost exclusively suspected test results. This was confirmed by GC-HRMS and resulted in follow-up studies on pigs but also other animal species like goats, sheep and cows, free-ranging on the island. Also the levels in livers were examined, regarding the specific accumulation of certain PCDD/Fs and dl-PCBs in liver. This has been shown in controlled studies with rodents (Abraham et al., 1988; Diliberto et al., 1995, 1999; Hurst et al., 2000), but also sheep (Hoogenboom et al., 2015a), cows (Feil et al., 2000; Thorpe et al., 2001), goats (Ounnas et al., 2010) and pigs (Spitaler et al., 2005). At least in mice and rats, this so-called sequestration was shown to be due to the binding to cytochrome P450 1A2, an enzyme that is induced at high exposure levels (Diliberto et al., 1995, 1999; Harril et al., 2016). The relatively high levels in livers of sheep raised under field conditions, as reported by Bruns-Weller et al. (2010) and Rose et al. (2010), was the reason for a specific risk assessment by EFSA on the consumption of such livers (EFSA, 2011). Field studies on pigs also showed this sequestration (Watanabe et al., 2010; Brambilla et al., 2011). The current study allowed to further contribute to this issue for foraging farm animals raised under field conditions and at relatively low exposure levels. In addition, both adipose tissue and meat were analysed, since this provides insight in whether adipose tissue is a suitable target tissue for monitoring meat levels, as generally assumed. This was confirmed in controlled studies with beef cows (Feil et al., 2000; Huwe et al., 2001), and sheep (Hoogenboom et al., 2015a). However, Thorpe et al. (2001) observed 5- to 7-fold higher levels in meat than in body fat. The latter implies that focusing on adipose tissue for routine control might lead to an underestimation of the exposure of consumers.

## 2. Materials and methods

### 2.1. Samples

Samples of animal fat, meat and livers (up to a hundred gram), were provided by Curaçao authorities. The samples were collected in 2014 at two local slaughterhouses from animals offered by farmers from different parts of the island, but in general these represented single animals rather than a herd of animals. Samples were frozen and shipped as such to the RIKILT (now WFSR).

### 2.2. Analysis

The first samples of adipose tissue were screened with the DR CALUX (Dioxin-Responsive Chemical Activated Luciferase gene Expression) bioassay, as described previously (Hoogenboom et al., 2007), but since most samples showed an elevated result, these and subsequent samples were also analysed with GC/HRMS.

Analysis by GC/HRMS for PCDD/Fs and PCBs was performed by

routine methods applied at WFSR, as described previously (Van Dam et al., 2016). Fat from adipose tissue was extracted using microwave heating, and either sent to DR CALUX analysis or spiked with the  $^{13}\text{C}$  labelled standards (CIL) for GC-HRMS analysis. Extraction of fat from liver and muscle tissue was performed according to Smedes (1999) after addition of the  $^{13}\text{C}$  labelled standards (CIL). To 45 g of whole liver or 65 g of muscle tissue, 15 ml water, saturated with sodium chloride, was added, as well as 80 ml propan-2-ol and 120 ml cyclohexane. This mixture was blended using an ultraturrax for 2 min. Afterwards, 120 ml demineralized water was added and the mixture was blended for another 2 min and then centrifuged for 10 min at 3000 r.p.m. at 10 °C. The upper layer of cyclohexane, containing the fat, was transferred over a funnel with sodium sulphate into a flask. The extraction was repeated twice by adding 120 ml of cyclohexane. The combined solvents were evaporated using a rotary evaporator from Buchi.

After drying and determining the fat content, the fat was purified using a PowerPrep™ system (FMS). The clean-up resulted in the collection of two purified fractions, one with the mono-ortho dl-PCBs and ndl-PCBs, the other one with the PCDD/Fs and non-ortho dl-PCBs. Both fractions were concentrated in a Power-Vap (FMS) and analysed by GC-HRMS using an Agilent (Wilmington, USA) GC 6890 N (GC column DB5 MS 60 m, 0.25 mm i.d., 0.25 µm; J&W, Folson, USA) and an AutoSpec Ultima HRMS (Waters, Milford, USA), operated in electron impact ionization mode using selected-ion monitoring and controlled by Masslynx data system. GC-HRMS data were processed using Masslynx Targetlynx software to determine the concentrations and subsequently the TEQ levels, based on WHO-TEFs (Toxic Equivalency Factors) 2005 (Van den Berg et al., 2006). Internal reference samples as well as blank controls were included in each series of samples. WFSR is the Dutch National Reference Laboratory for these substances and participates annually in proficiency tests of the European Reference Laboratory, Folkehelse and FAPAS. The method meets the performance criteria as set in Commission Regulation (EU) 2017/644.

The limit of quantification (LOQs) varied depending on the sample amount and lipid content of the sample. In general LOQs were around 0.01–0.1 pg g<sup>-1</sup> lipid/product for PCDD/Fs and non-ortho PCBs, and 10 and 100 pg g<sup>-1</sup> lipid/product for mono-ortho and ndl-PCBs, respectively. Both lower- and upperbound levels were determined but the latter were used for comparison with the MLs and as such presented below. For lowerbound levels, non-detected levels are assumed to be zero, for upperbound levels these are assumed to be equal to the LOQ. At higher levels and certainly those above the MLs, the lower- and upperbound levels are similar. The measurement uncertainty was 10% for PCDD/Fs and dl-PCBs, and 15% for ndl-PCBs (expanded measurement uncertainties based on in-house validation studies).

## 3. Results and discussion

### 3.1. Levels in adipose tissue

#### 3.1.1. Levels in pig fat

In a first batch, 17 samples of pig fat were analysed with DR CALUX, the routine strategy in the Netherlands (Adamse et al., 2017). This also applies to the choice to test samples of adipose tissue rather than meat, assuming that persistent lipophilic compounds like PCDD/Fs and PCBs distribute evenly throughout the fat compartment, meaning that lipid based levels in body fat and muscle are assumed to be similar. Sixteen of these 17 samples tested as suspected in the bioassay, with estimated levels of 1.5–8.1 pg BEQ (bioequivalents) g<sup>-1</sup> fat (for pig fat a cut-off of 0.7 pg BEQ g<sup>-1</sup> fat is applied). This was unexpected based on monitoring results from pigs raised in the Netherlands, rarely showing

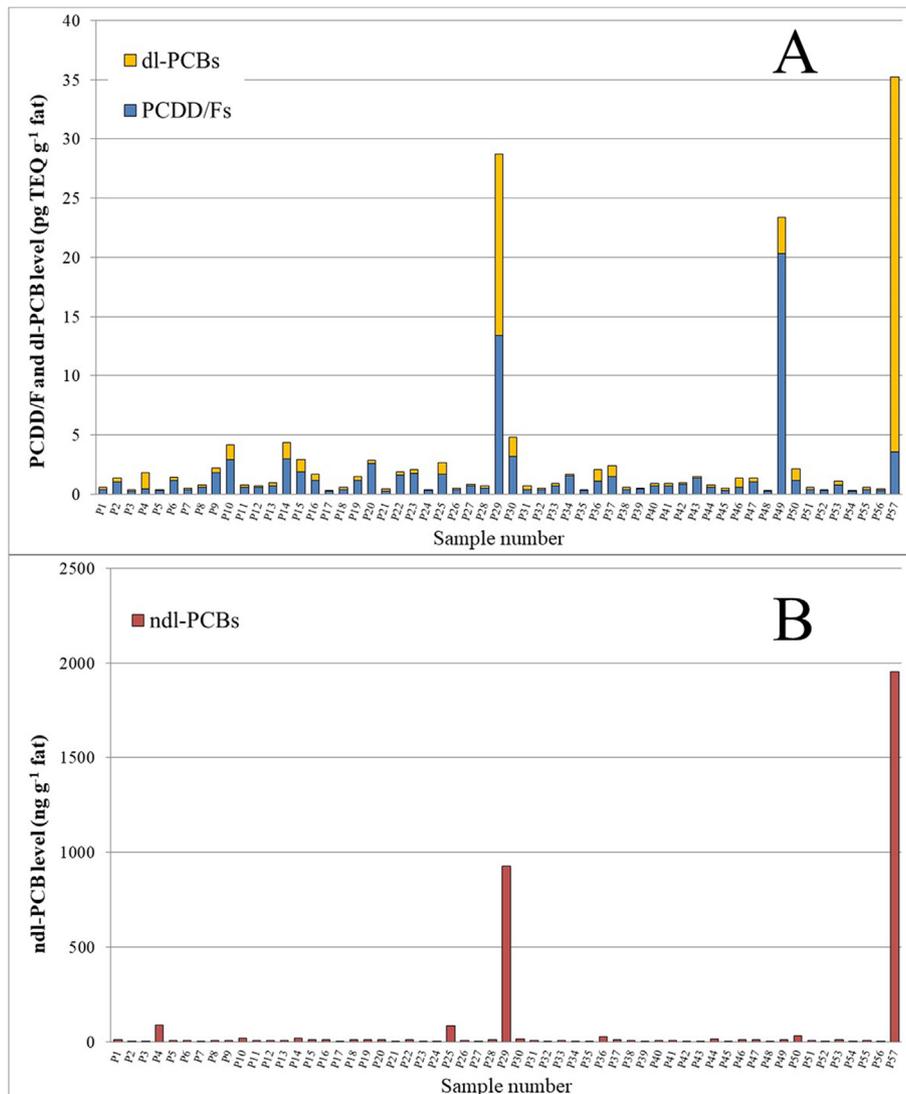
suspected test results (Adamse et al., 2017). Therefore, all 17 samples as well as 4 additional samples were analysed by GC/HRMS and levels were compared with the MLs applied in the EU, which for pigs are 1 and 1.25 pg TEQ g<sup>-1</sup> fat for respectively PCDD/Fs, and the sum of PCDD/Fs and dl-PCBs (from now on termed sum-TEQ). These MLs were set according to the principle “strict but feasible” and reflect the relatively low background levels for pigs reported by EU member states, as compared to other animal species. Since 2012, there is also an ML for the sum of the 6 non-dioxin-like (ndl-) PCBs of 40 ng g<sup>-1</sup> fat, based on levels reported for animal fat in general.

As shown in Fig. 1A (samples P1 to P21), of the first set of 21 samples, 8 exceeded the ML for PCDD/Fs, 8 the ML for sum-TEQ and 9 either one of these MLs (taking into account 10% measurement uncertainty). Levels of ndl-PCBs were also relatively high for pig fat, but only one sample (P4) exceeded the ML with a level of 88 ng g<sup>-1</sup> fat. Based on the levels in this first set of 21 samples it was decided to start a follow-up study, including other animal species foraging outside.

The new set of samples included 36 additional samples of pig fat, of which respectively 10, and 11 exceeded the MLs for PCDD/F-TEQ and/or the sum-TEQ, and 12 either one or both of these MLs.

Highest levels exceeded those in the first set (Fig. 1A, samples P22–P57), with three samples showing rather high sum-TEQ levels of 28.7 (P29), 23.4 (P49), and 35.2 (P57) pg TEQ g<sup>-1</sup> fat. In these three samples, there was a clear variation in the contribution of the dl-PCBs to the sum-TEQ, being respectively 53, 13 and 90%. Two of these samples were also among the three samples exceeding the ML for ndl-PCBs, with levels of 929 (P29) and 1953 (P57) ng g<sup>-1</sup> fat (Fig. 1B). The third sample (P25) showed a level of 84 ng g<sup>-1</sup> fat.

When combining all 57 samples, 21 (37%) of the samples would not comply with MLs in the EU. Median upperbound levels of PCDD/Fs and sum-TEQ were, respectively, 0.67 (range 0.21–20.3) and 0.90 (range 0.26–35.2) pg TEQ g<sup>-1</sup> fat. Adamse et al. (2017) reported much lower levels for pig fat from the Netherlands sampled in the period 2001–2011, with median and P95 upperbound levels for the sum-TEQ of respectively 0.24 and 0.35 pg TEQ g<sup>-1</sup> fat. Similar levels were reported by EFSA (2018), based on data on pig fat (lard) reported by the member states, the mean being respectively 0.20 and 0.44 pg TEQ g<sup>-1</sup> fat. The median level for ndl-PCBs for the 57 samples was 6 ng g<sup>-1</sup> (range 1.2–1953). These levels are also much higher than reported by EFSA (2012) with median and P95 levels of respectively 0.50 and 2.45 ng g<sup>-1</sup>. It should be



**Fig. 1.** Levels of PCDD/Fs and dl-PCBs (A) and ndl-PCBs (B) in 57 samples of pig fat from Curaçao. Current EU MLs for pig meat and fat are 1.0 and 1.25 pg TEQ g<sup>-1</sup> fat for PCDD/Fs and sum-TEQ, respectively, and 40 ng g<sup>-1</sup> fat for ndl-PCBs.

mentioned that in many countries in the EU, almost all pigs are raised indoors and fed compound feed, meaning that exposure levels are rather low. However, for laying hens, sheep and cows foraging outside, it has been shown that contaminated soil and grass can be an important source for these contaminants (Bruns-Weller et al., 2010; Rose et al., 2010; Fernandes et al., 2011; Hoogenboom et al., 2014). In addition, Watanabe et al. (2010) observed elevated levels in pigs living on a dumpsite.

### 3.1.2. Levels in bovine fat

Samples of adipose tissue of six cows were analysed and compared with the current EU MLs for PCDD/Fs and sum-TEQ of 2.5 and 4.0 pg TEQ g<sup>-1</sup> fat and for ndl-PCBs of 40 ng g<sup>-1</sup> fat. As shown in Fig. 2A (C1 to C6), two samples exceeded the ML for PCDD/Fs, one the ML for sum-TEQ and that same sample also exceeded the ML for ndl-PCBs, showing a level of 198 ng g<sup>-1</sup> fat (Fig. 2B). Median levels and range were 1.8 (0.33–4.0) and 3.0 (0.48–14.4) pg TEQ g<sup>-1</sup> fat for PCDD/Fs and sum-TEQ, and 5 (1–198) ng g<sup>-1</sup> fat for ndl-PCBs.

### 3.1.3. Levels in ovine fat

Adipose tissue samples of twenty sheep (Figure 2, S1 to S20)

were analysed and compared with the current EU MLs for PCDD/Fs and sum-TEQ of 2.5 and 4.0 pg TEQ g<sup>-1</sup> fat and for ndl-PCBs of 40 ng g<sup>-1</sup> fat. Of these samples respectively 11, 12 and 3 samples exceeded these MLs. Median levels were 3.3 (range 0.24–27) and 5.7 (range 0.31–28) pg TEQ g<sup>-1</sup> fat for PCDD/Fs and sum-TEQ, and 14 (range 1–281) ng g<sup>-1</sup> fat for ndl-PCBs.

### 3.1.4. Levels in caprine fat

Adipose tissue samples of thirteen goats (Fig. 2, G1 to G13) were analysed. In the absence of EU MLs for products from goats, the MLs for other ruminants were used for comparison. Of these samples, 7 showed a PCDD/F level higher than 2.5 pg TEQ g<sup>-1</sup> fat and 8 a sum-TEQ level higher than 4 pg TEQ g<sup>-1</sup> fat. One sample showed a sum-TEQ level of 135 pg TEQ g<sup>-1</sup> fat, primarily due to PCDD/Fs. None of the samples showed an ndl-PCB level higher than 40 ng g<sup>-1</sup> fat. Median levels were 3.9 (range 0.23–133) and 6.5 (range 0.46–135) pg TEQ g<sup>-1</sup> fat for PCDD/Fs and sum-TEQ, and 9 (range 1–30) ng g<sup>-1</sup> fat for ndl-PCBs.

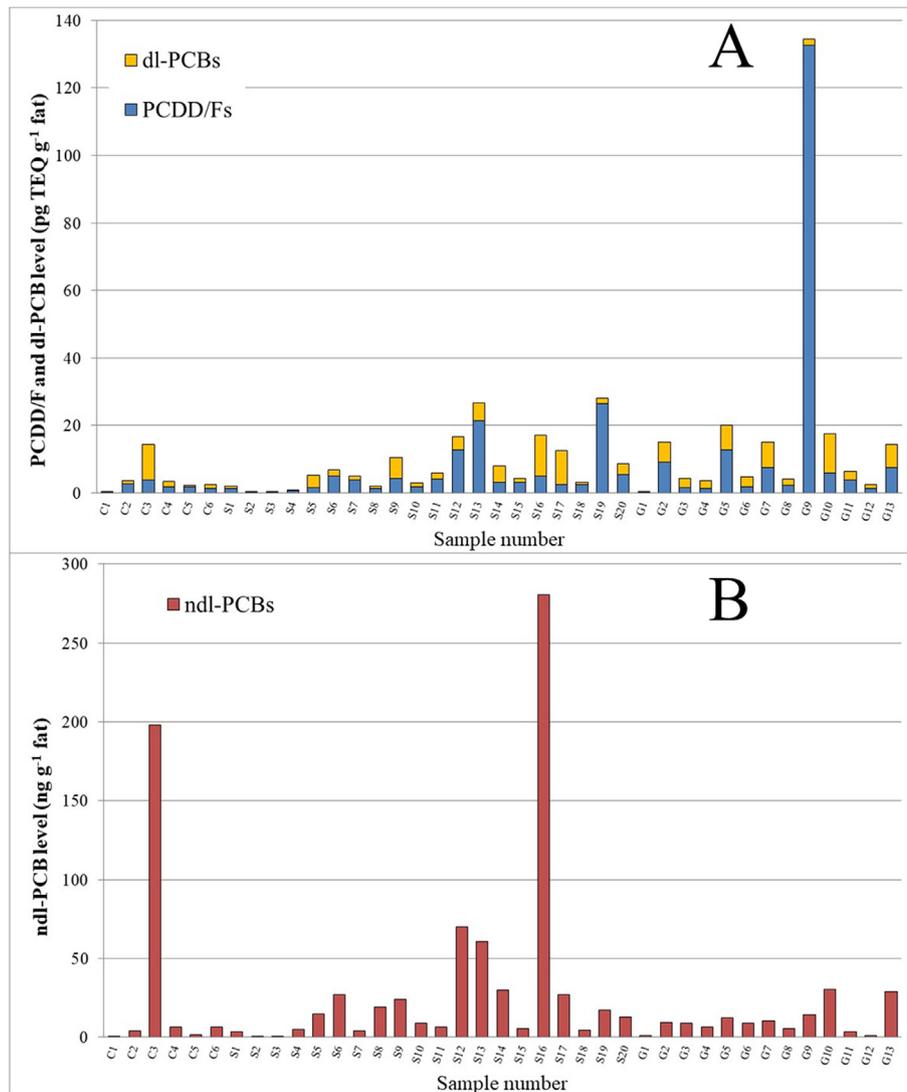
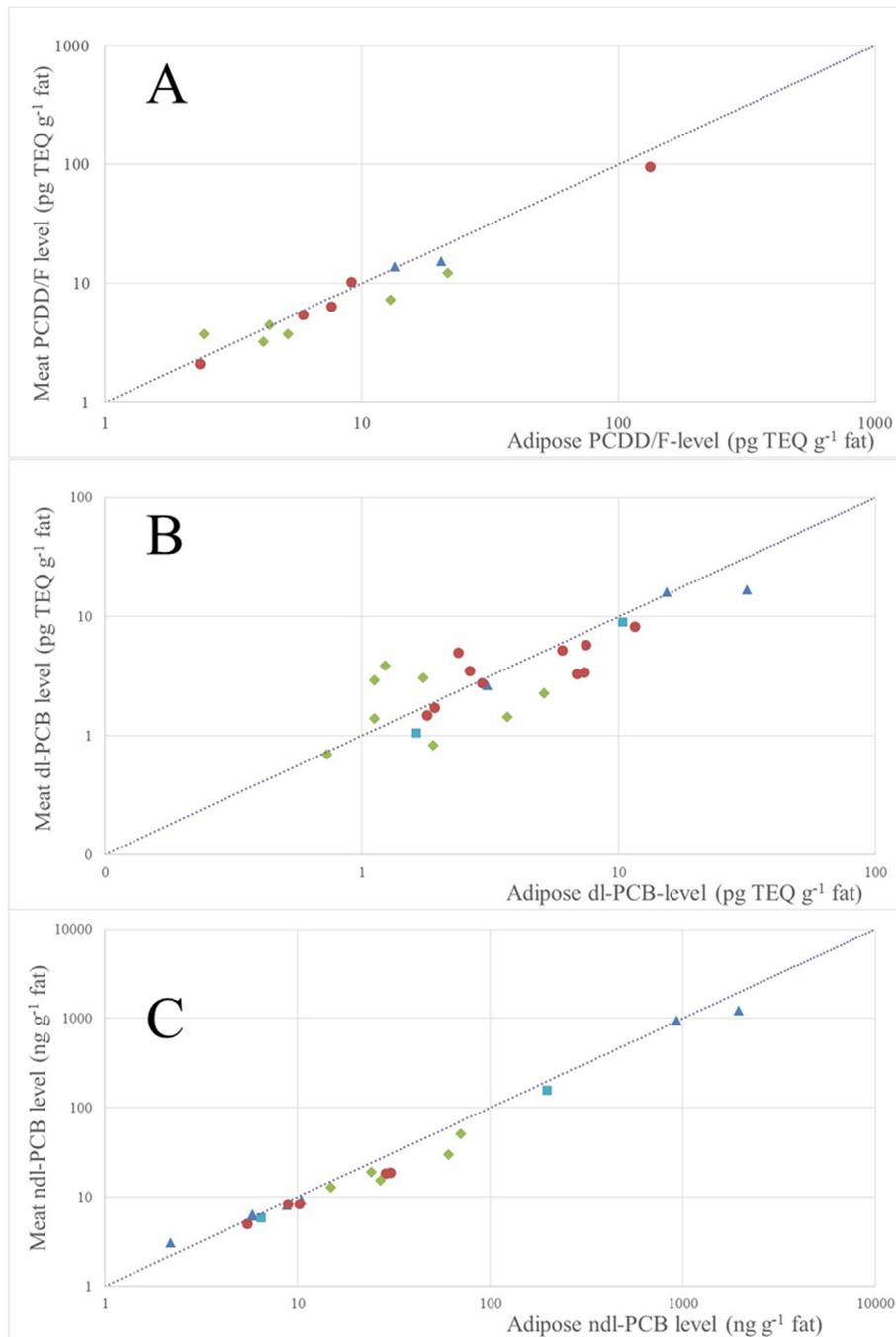


Fig. 2. Levels of PCDD/Fs and dl-PCBs (A) and ndl-PCBs (B) in 6 samples of bovine (C), 20 samples of ovine (S) and 13 samples of caprine fat (G). Current EU MLs for bovine and ovine fat are 2.5 and 4.0 pg TEQ g<sup>-1</sup> fat for PCDD/Fs and sum-TEQ, respectively and 40 ng g<sup>-1</sup> fat for ndl-PCBs (no MLs for caprine fat).

### 3.2. Levels in meat

Meat samples from 10 pigs, 10 goats, 11 sheep and 4 cows were analysed, based on their levels in adipose tissue, i.e. the higher levels were preferred. Sample volumes were quite small and lipid contents low, with average (range) levels of 4.7% (1.1–13.7), 1.9% (0.6–4.6), 1.8 (0.5–3.5) and 1.2% (0.5–1.6) for pigs, goats, sheep and cows, respectively. As a result, the LOQs were rather high, in particular for PCDD/Fs and only few data could be used for comparison of levels in meat and adipose tissue. Therefore, the numbers differed for PCDD/Fs, dl-PCBs and ndl-PCBs. Fig. 3A

compares the PCDD/F-TEQ levels in meat and fat for those samples with a ratio between upper- and lowerbound smaller than 1.5 (non-detects assumed to be either zero (LB) or equal to LOQ (UB)). A log scale is used regarding the wide variation in the levels. The data show that in general the lipid based levels in meat are similar to those in adipose tissue, with a median ratio of adipose to meat levels of 1.2. In 3 samples the levels in meat were slightly higher than those in adipose tissue. In the case of dl-PCBs, absolute levels in both fat and meat were in general higher and differences between upper- and lowerbound levels much smaller (in most cases non-existent). Fig. 3B shows the dl-PCB-TEQ levels in the 31



**Fig. 3.** Comparison of lipid based upperbound levels of PCDD/Fs (A), dl-PCBs (B) and ndl-PCBs (C) in meat versus adipose tissue. Only samples with an upper-to lowerbound ratio smaller than 1.5 were taken into account. The line represents the situation where lipid based levels in meat and adipose tissue are equal. Blue triangles: pigs, red dots: goats, green diamonds: sheep, light blue squares: cows. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

samples of meat as compared to those in adipose tissue. The median ratio of levels in adipose tissue to meat was 1.2. Only 3 samples showed a meat level (slightly) higher than that in adipose tissue.

Fig. 3C shows the ndl-PCB levels in 24 samples of meat as compared to adipose tissue, with a median ratio of 1.1 between adipose tissue and meat. Only a few samples showed slightly higher levels in meat than in adipose tissue.

Therefore these data are in line with those reported for cows (Feil et al., 2000), pigs (Spitaler et al., 2005) and sheep (Hoogenboom et al., 2015a), and could not confirm those reported by Thorpe et al. (2001), showing 5- to 7-fold higher levels in meat than in adipose tissue. In that study, animals received capsules daily for 4 weeks and animals were slaughtered 1, 14 and 27 weeks later. It is unclear whether the discrepancy could be caused by differences in the dosing, the application route or the time between dosing and slaughtering of the animals. However, the current study and also the other studies mentioned reflect normal practice with exposure via contaminated soil or feed. Therefore, the strategy to analyse adipose tissue rather than meat appears not to lead to a substantial over- or underestimation of levels in meat and thus human exposure. The present study also demonstrates that the analysis of adipose tissue allows an easier and more sensitive detection of these contaminants.

### 3.3. Levels in livers

Liver samples of 35 of the animals were analysed, collected from 10 pigs, 4 cows, 11 sheep and 10 goats. Fig. 4 shows the levels of PCDD/Fs and dl-PCBs, expressed on fresh weight base, Fig. S1 (Supplementary files) the levels based on lipid weight. The first allows a comparison with the EU limits which are based on fresh weight, the latter to compare with the lipid based levels in adipose tissue. The median fat content (and range) of the livers was 4.1% for pigs (0.7–5.4), 4.1% (3.8–7.0) for cows, 4.4% (3.5–7.6) for sheep and 4.8% (3.7–7.1) for goats.

For pigs, lipid based sum-TEQ levels showed a median of 77 pg TEQ g<sup>-1</sup> fat (range 8.0–2126). As shown in Fig. S1, several pigs showed high levels, clearly exceeding those in the adipose tissue. This confirms the strong sequestration of these compounds, reported for pigs by Spitaler et al. (2005), Watanabe et al. (2010) and Fernandes et al. (2011). With the exception of P57, PCDD/Fs always contributed much higher to the sum-TEQ level than dl-PCBs. When comparing the levels with the EU MLs, taking into account measurement uncertainty, 70% of the samples exceeded the ML for PCDD/Fs of 0.3 pg TEQ g<sup>-1</sup> liver and 60% the ML for sum-TEQ of 0.5 pg TEQ g<sup>-1</sup> liver. Samples P29 and P57 showed rather high levels of ndl-PCBs, being respectively 1178 and 2215 ng g<sup>-1</sup> fat, whereas in all other livers the levels were below 100 ng g<sup>-1</sup> fat (Fig. S2A).

The 4 samples of cow's liver showed a median level of 8.9 pg TEQ g<sup>-1</sup> fat (range 4.3–57). Of these, 50 and 25% exceeded the aforementioned MLs for PCDD/Fs and sum-TEQ respectively. Levels of ndl-PCBs were rather low with one exception (C3), showing a level of 975 ng g<sup>-1</sup> fat (Supplementary files, Fig. S2A).

In the EU, sheep showed on average the highest levels in livers, causing some concern for frequent consumers (EFSA, 2011). The 11 samples from Curaçao showed a median sum-TEQ level of 80 pg TEQ g<sup>-1</sup> fat, being comparable to that in pigs. When expressed on fresh weight the average was 3.3 pg TEQ g<sup>-1</sup> liver, with a range of 0.7–32 pg TEQ g<sup>-1</sup> liver (Fig. 4). Seven (64%) of the samples exceeded the MLs for both PCDD/Fs and sum-TEQ, being respectively 1.25 and 2 pg TEQ g<sup>-1</sup> liver. As in pigs, in most samples the PCDD/Fs contributed most to the sum-TEQ level. Levels of ndl-PCBs showed a median level of 36 ng g<sup>-1</sup> fat or 1.8 ng g<sup>-1</sup> liver (Fig. S2B). Compared to pigs and cows, the lipid based levels varied much less

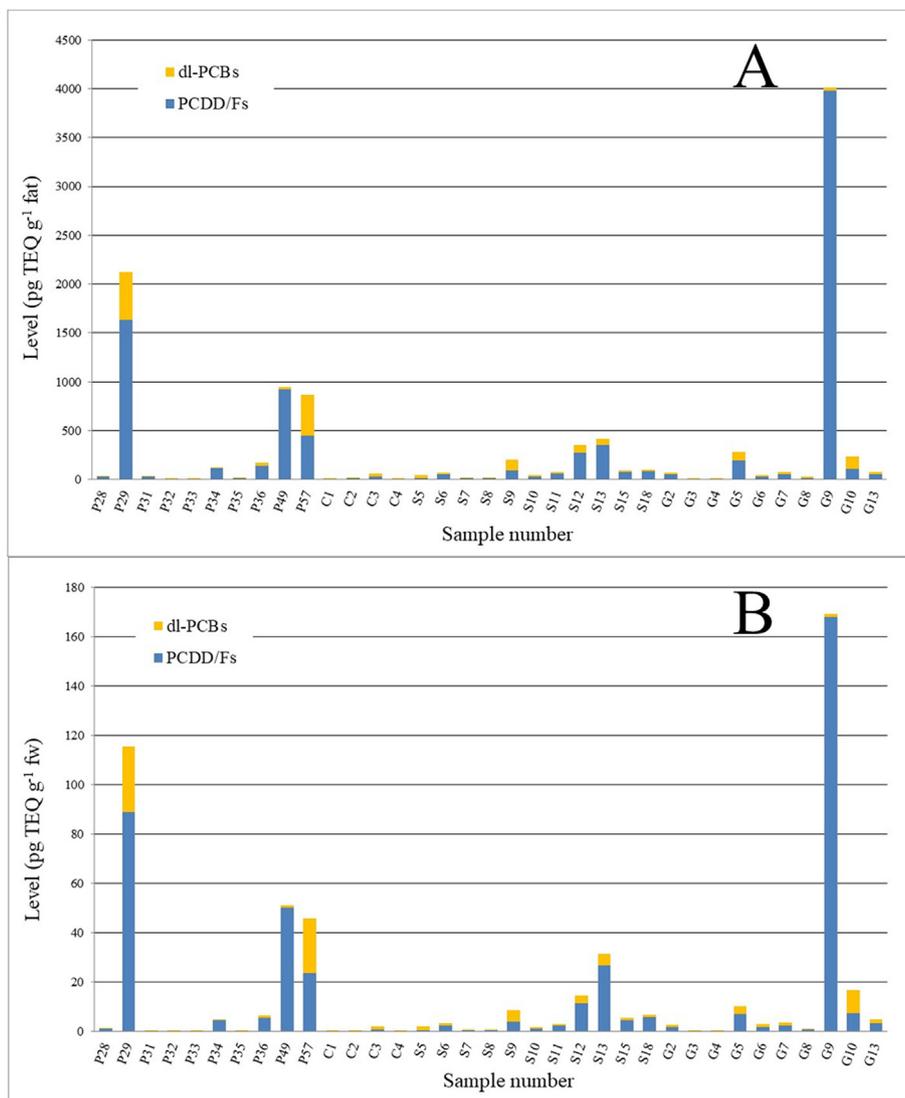
and the highest level was much lower, with a range of 12–129 ng g<sup>-1</sup> fat (Fig. S2A).

The sum-TEQ levels in goat livers showed a median of 73 pg TEQ g<sup>-1</sup> fat, comparable to pigs and sheep (when expressed on fresh weight being 3.3 pg TEQ g<sup>-1</sup> liver). One goat, G9, showed by far the highest level observed, being 4012 pg TEQ g<sup>-1</sup> fat, primarily due to PCDD/Fs. There are no EU-MLs for goat liver, but when applying those for sheep, 70% would exceed both the ML for PCDD/Fs and that for the sum-TEQ. Levels of ndl-PCBs showed a median level of 64 ng g<sup>-1</sup> fat or 3.8 ng g<sup>-1</sup> liver, i.e. at least 2-fold higher than those in the other species (Fig. S2). However, the highest level was 180 ng g<sup>-1</sup> fat, being 12- and 5-fold lower than those observed in cows and pigs.

Fig. 5A shows a comparison between the lipid based levels in adipose tissue and livers, Fig. 5B the ratio of sum-TEQ levels in liver and adipose tissue (both on a log scale). Based on this ratio, it is clear that all 4 species show some degree of sequestration, i.e. ratio higher than 1. The data also suggest that pigs show the highest sequestration, and that this is observed already at relatively low levels in adipose tissue, i.e. levels below the ML for pig meat and fat. For pigs, the median ratio between sum-TEQ levels in liver and adipose tissue was 44 (range 9–84). Sheep and goats seem comparable with median ratios of 13 (range 4–31) and 6 (range 3–30). Cows seem the least sensitive, showing a median of 4 (range 1–9), although the number of data for cows was rather limited.

Regarding the lack of a clear dose-related increase in the ratios (Fig. 5B), it seems unlikely that the exposure levels are high enough to cause an induction in the levels of cytochrome P450 1A2, which is thought to be responsible for the sequestration. It has been shown in rodents but also humans that the induction of this enzyme results in a dose-related increase in the ratio of the levels in liver and adipose tissue (Carrier et al., 1995), with a clear increase at body burdens higher than 10 ng TEQ kg<sup>-1</sup>. (corresponding to 40–100 pg TEQ g<sup>-1</sup> fat at body fat contents of 10 and 25%, respectively). It seems more likely that the normal background expression of this enzyme or the binding of certain PCDD/Fs and dl-PCBs to this enzyme is higher in pigs than in the other species. However, also other contaminants might contribute to the increased expression of CYP 1A2.

To examine potential congener related differences in the sequestration, ratios of the levels in liver and adipose tissue were determined for each congener and then averaged for each species. Average values are shown in Fig. 6A for PCDD/Fs and Fig. 6B for the various non-ortho and mono-ortho dl- and the ndl-PCBs. Data on individual animals are shown in Figs. S3 and S4 (supplementary files). A number of congeners could not be detected in either fat or liver and as such the ratio could not be determined. This applied in particular to the lower chlorinated PCDD/Fs, i.e. TCDF and 1,2,3,7,8-PeCDF, which are known to disappear rapidly in cows and pigs, probably due to metabolism (Schuler et al., 1997; Malisch, 2000; Huwe and Smith, 2005; Hoogenboom et al., 2004, 2015b; Spitaler et al., 2005). Based on the current data this also seems to apply for sheep but not for goats. Also the lower chlorinated ndl-PCBs 52 and 101 were rarely detected, and to a lesser extent this also applied to PCB 28. The ratios presented in Fig. 6A indicate that in pigs, sheep and goats the PCDFs show a higher sequestration than the PCDDs. This has been reported before for sheep (Hoogenboom et al., 2015a). However, for cows, this seems to be the opposite and the lower sequestration of the PCDFs may underlay the lower liver to fat ratios observed for cows in this study. The average ( $\pm$ SD) ratios for the upperbound PCDD/F-TEQ levels was 71  $\pm$  42 for pigs (range 10–135, n = 10), 17  $\pm$  8 for sheep (range 4–35, n = 10), 11  $\pm$  8 for goats (range 4–30, n = 10) and 6  $\pm$  5 for cows (range 2–12, n = 4), also showing a higher sequestration of PCDD/Fs in pig livers. In the case of the dl-PCBs, pigs and sheep showed higher ratios for



**Fig. 4.** PCDD/F and dl-PCB levels in 35 samples of liver from pig (P), cow (C), sheep (S) or goat (G) from Curaçao, expressed on fresh weight base. Current EU MLs for livers from pigs and cows are 0.3 and 0.5 pg TEQ g<sup>-1</sup> liver for PCDD/Fs and dl-PCBs respectively, and for sheep livers 1.25 and 2.0 pg TEQ g<sup>-1</sup> liver (no MLs for goat livers).

the more potent PCBs 126 and 77 (Fig. 6B). This was also reflected in the liver to adipose tissue ratios for PCB-TEQ, being on average  $16 \pm 9$  for pigs (range 3–32),  $11 \pm 6$  for sheep (range 3–21),  $7 \pm 5$  for goats (range 2–16) and only  $2 \pm 1$  for cows (range 1–3). In the case of the PCBs, there seems to be some sequestration of the ndl-PCBs 138 and 153 in livers of ruminants and in particular goats, but much less for pigs and sheep.

Overall, these data clearly show that even at relatively low levels in fat and meat, the levels in liver can be quite high and can result in a much higher exposure of consumers than via meat or fat. On the island of Curaçao, livers are regularly consumed.

#### 3.4. Patterns and potential sources

No investigations were performed to investigate the source of the contaminations. The investigations of the locations of the animals from which the samples were collected, generally revealed the presence of activities or findings related to the incineration of organic materials, like garden waste or wood in most of the cases. The overall housing and hygienic conditions were considered as being poor. Feeding on the ground was also noticed as a regular

activity which may suggest the ingestion of contaminated soil to be an important source of contamination. Congener patterns may help to confirm the potential sources of the PCDD/Fs (Hoogenboom et al., 2020). Fig. S5 (supplementary files) shows the patterns for the most contaminated samples of adipose tissue for each species. Fig. 7 shows some examples for the more contaminated samples.

In pigs, samples P57, P29 and P49 showed the highest sum-TEQ levels and the first two showed high contributions of the dl-PCBs, as well as high levels of ndl-PCBs. These samples also showed a relatively high contribution of PCDFs to the TEQ, being respectively 84 and 71%, as compared to the median of 44% for all pig fats. Considering that TCDF and 1,2,3,7,8-PeCDF are metabolized in pigs, the original contribution of PCDFs to the exposure might have been even higher. More general, the relation between a high contribution of PCBs and PCDFs was not so clear, since a number of the more contaminated samples showed a high contribution of PCDFs, but low contribution of PCBs, like samples P14 and P20, with sum-TEQ levels 4.4 and 2.9 pg TEQ g<sup>-1</sup> fat, PCDF contributions for both samples of 74% and dl-PCB contributions of 32 and 11%. Sample P14 showed a relatively high contribution of 1,2,3,4,6,7,8-HpCDF of 40%, suggesting another source (Fig. 7B). When focusing on median TEQ

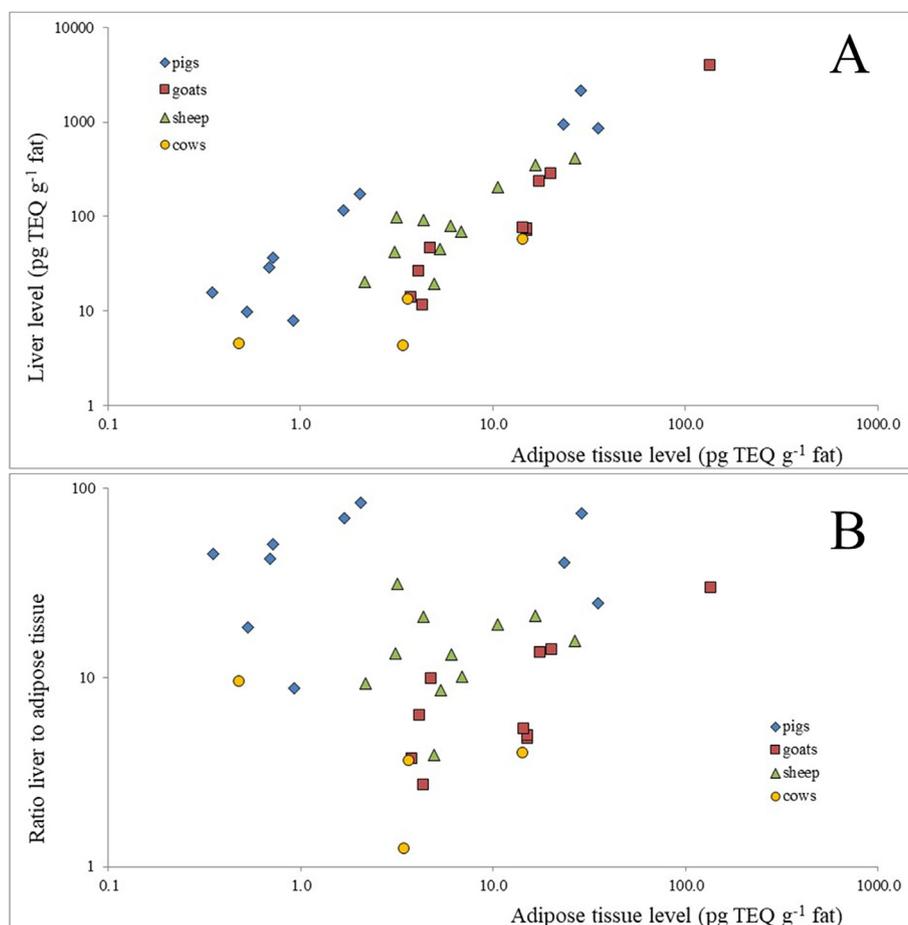


Fig. 5. Comparison of lipid based sum-TEQ levels in adipose tissue and livers (A) and the ratio of levels in liver and adipose tissue (B).

contributions for all samples, PeCDD showed the highest value (20%), followed by 2,3,4,7,8-PeCDF (17%), 1,2,3,6,7,8-HxCDD (11%) and 1,2,3,4,7,8-HxCDF (9.6%). This suggests that the burning of waste may be the most important source for the lower contaminated samples. Sample P30 is a typical example of this pattern, also showing a high contribution of TCDD, keeping in mind that the other typical congener for burning patterns (Hoogenboom et al., 2020), TCDF, cannot be observed in pigs.

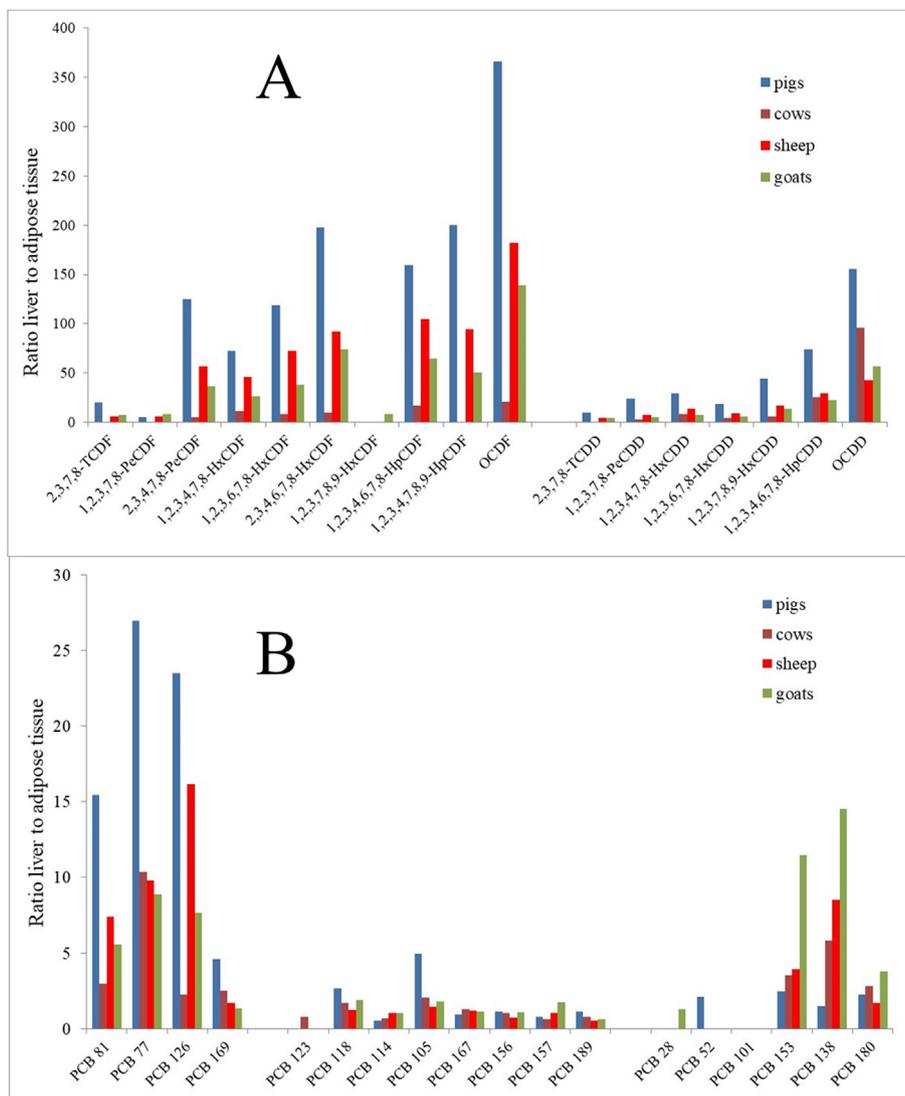
As shown in Fig. S5, this pattern was also the one observed in the cows with the highest PCDD/F-TEQ levels, with a median contribution of PeCDD of 47%, followed by 1,2,3,6,7,8-HxCDD (15%), 2,3,4,7,8-PeCDF (12%) and TCDD (9.9%). One sample, C3 showed a high contribution of dl-PCBs to the sum-TEQ (72%) and a high ndl-PCB level of 198 ng g<sup>-1</sup> fat. However, this sample showed a high contribution of PCDDs in addition to 2,3,4,7,8-PeCDF 19%, being more typical for burning (Fig. 7B). Also in cows, there might have been a shift in the relative contribution of PCDFs due to metabolism of TCDF and 1,2,3,7,8-PeCDF.

In sheep, the pattern pointed to burning as the most important source with the highest median contribution of 53% for PeCDD, followed by 1,2,3,6,7,8-HxCDD (12%), TCDD (8.5%) and 2,3,4,7,8-PeCDF (6.6%). This was e.g. the pattern in the one but highest contaminated sample (S13) (Fig. 7A). However, this was not the case for the sample with the highest level (S19) (Fig. 7B), which showed an unusual high contribution for 1,2,3,6,7,8-HxCDD (34%) and also HpCDD (15%). In sheep, several of the samples with high sum-TEQ levels also showed a high contribution of dl-PCBs, like samples S16 (71%), S17 (80%), S5 (71%) and S9 (59%). Sample S16

also showed by far the highest ndl-PCB level in sheep, being 281 ng g<sup>-1</sup> fat. However, the PCB contamination was not accompanied by the expected higher contribution of PCDFs to the PCDD/F-TEQ.

In adipose tissue of goats, the patterns were similar to those in cows and sheep with highest median contribution for PeCDD (56%), followed by TCDD (11%), 1,2,3,6,7,8-HxCDD (9.2%) and 2,3,4,7,8-PeCDF (6.4%), again pointing to burning as a general source. This pattern was also observed for the most contaminated samples, like sample G5 (Fig. 7A). The sample with the highest PCDD/F-TEQ level (G9) showed a slight shift with relatively high contributions of 1,2,3,6,7,8-HxCDD (23%) and HpCDD (10%), similar to the pattern observed for S19 (Fig. 7B). High contributions of higher chlorinated PCDDs could point to tetra- or pentachlorophenol as one of the sources. A number of the more contaminated goat samples showed an almost equal contribution of dl-PCBs to the sum-TEQ, like G10 (66%), G7 (50%), G13 (48%). Sample G10 was also the sample with the highest ndl-PCB level of 30 ng g<sup>-1</sup> fat. However, none of the goat samples showed a clear dominance of the PCBs.

To summarize, in some samples, the contamination is clearly caused by PCBs with in all samples the highest contribution of the higher chlorinated PCBs 138, 153 and 180. However, that pattern is likely to be different from the original source due to metabolism of the lower chlorinated congeners. In most samples, the PCDD/F pattern is similar to the general background observed in industrialized countries, which to a large extent may be due to the burning of waste. This pattern is commonly seen with accidental fires (Hoogenboom et al., 2012). It was also observed in milk of cows



**Fig. 6.** Mean ratios of lipid based levels of PCDD/Fs (A) and PCBs (B) in liver to adipose tissue in pigs (n = 10), cows (n = 4), sheep (n = 10) and goats (n = 10). Ratios were determined for individual animals and then averaged for those congeners detected. Zero levels are due to the fact that certain congeners could not be detected.

grazing in the vicinity of municipal waste incinerators (Traag et al., 1993) and in eggs from private owners in the Netherlands where the past small scale incineration of waste on the premises was thought to be the major source (Hoogenboom et al., 2016). In some cases, there was a shift to higher chlorinated compounds which could point in the direction of chlorophenols. It is unclear, to which extent PCBs and chlorophenols were actually used in Curaçao.

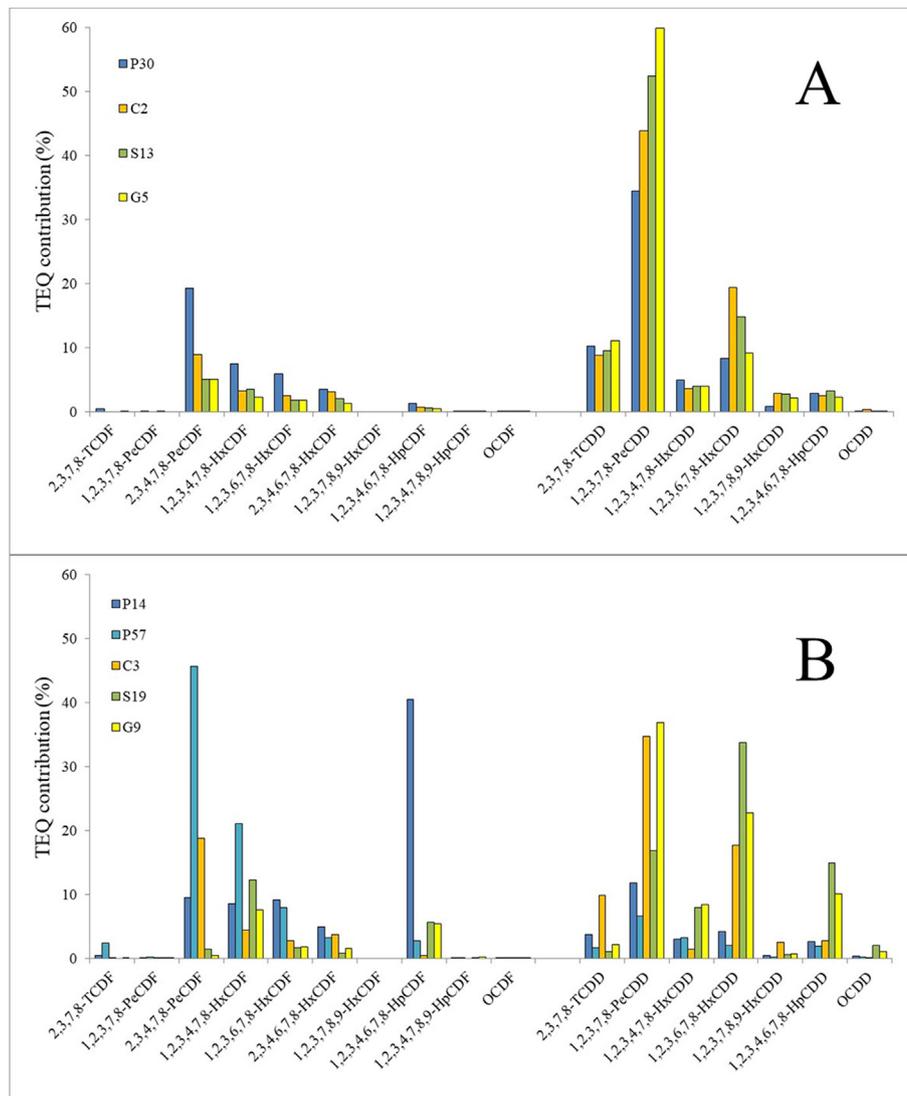
#### 4. Conclusions

The present study clearly shows that levels of PCDD/Fs and PCBs in fat of animals foraging outside can be rather high and would clearly exceed the current MLs set in the EU. Levels in meat of the examined species were quite similar to those in fat, indicating that fat is a good matrix for monitoring levels of PCDD/Fs and PCBs in animals. This does not apply to livers showing very high levels even when levels in fat and meat are relatively low. These alarming findings led to the implementation of targeted actions and measures by the Ministry of Health, Environment and Nature to minimize the burden of dioxins for the consumers. These actions not

only consisted of extensive public information and awareness programs for the farmers, but also financial support for the farmers to upgrade their animal husbandry.

#### Credit author statement

Ron L.A.P. Hoogenboom: was the lead author and responsible for finalizing and submitting the manuscript. Guillaume ten Dam: were responsible for the analysis and the compilation of the data and description of the methods applied. Stefan P.J. van Leeuwen: were responsible for the analysis and the compilation of the data and description of the methods applied. Harry van Egmond: was the project leader for the contact with Curaçao and the shipment of samples and reporting of the data. Jennyfer Nicolina: were representing the authorities of Curaçao and responsible for the project, and for the description of the sampling and conditions on the farms. Arnold J.S. Dwarkasing: were representing the authorities of Curaçao and responsible for the project, and for the description of the sampling and conditions on the farms



**Fig. 7.** Congener patterns representing the common pattern (A) and some exceptions (B) in adipose tissue of pigs (P), cows (C), sheep (S) and goats (G). Patterns based on contribution to the PCDD/F-TEQ level.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.chemosphere.2020.128057>.

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