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CAMOUFLAGE NET CAUSED SERIOUS DIOXIN CONTAMINATION OF PIGS

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Introduction

The annual number of finishing pigs slaughtered in Denmark is currently around 20 million (1) and the high quality of the meat is of uttermost importance for the export of pork to foreign markets.
During the Danish official chemical control program for food a random collected free range sow was analyzed for content of dioxins and PCBs in August 2015. The dioxin content was approximately ten times higher than compared to the level of dioxins normally observed in samples from Danish free range sows. The content of WHO-PCDD/F-TEQ was 4.0 pg TEQ/g of fat and exceeded the European residue limit, which is 1.0 pg TEQ/g fat (2). We have never observed that high dioxin content in a Danish pig before. A dioxin incident would be an economic catastrophe for the Danish farmers therefore all actions were taken to identify the source and to reduce the extent of the incident.
The Veterinary Officer at the slaughterhouse traced the contaminated meat to the Danish and the German market and the authorities in other countries were informed through the EU Rapid Alert system for Food and Feed – RASFF (Rapid Alert no. 2015.1122).
The busiest period in the history of the lab followed!
The District Veterinary Officer visited the farm several times to look for possible contamination sites. A thorough examination of the photos from the visit finally gave the clue to investigate the strange cover of one of the shelters at the back of the farm. It was an old camouflage net with greenish and moldy pieces of fabric sewn to the grid. The sow and its piglets probably found it interesting using it as "Chewing Gum" and toy to play with.

Materials and methods

All samples were spiked with 13C-labeled internal standards used for quantification. Fat from pigs was sampled. Approximately 3 g of the fat was dissolved in hexane and cleanup and fractionation was performed on a Power Prep system (FMS, USA). The procedure was based on the method by Focant et al. (3). Two fractions were collected: A fraction containing the 17 2378-substituted PCDD/Fs and the 4 non-ortho PCBs, and a fraction containing the 8 mono-ortho PCBs, the ICES-6 PCBs, and the PCB170. A gas chromatograph coupled to a high-resolution sector mass spectrometer (Trace GC ultra and Finnigan MAT95) with electron ionisation and a resolution of at least 10000 was used for detection. The TargetQuan software (ThermoFinnigan, Germany) was used for the quantification. TEQ values were calculated with TEF-2005 values (2).
GC column: 60m DB5MS 0.25mm, 0.25 µm with 10 m guard column. GC program for the dioxin fraction: 140ºC; 2 min hold; 15ºC/min to 240ºC; 1ºC/min to 255ºC; 10 min hold; 10°C/min to 325ºC; 7 min hold. Flow 1.0 ml/min.

Results and discussion:

The presence of a non-compliant pig in Denmark, where the export of pork is extremely important, gave the Danish dioxin alert group some busy weeks in the autumn of 2015.
In table 1, the content of dioxin and PCB congeners in the contaminated sow is shown and compared to the average content found in Danish sows. The 1234678-heptachloro substituted dioxin and furan dominates together with HxCDF which is normal for pigs, but the peaks are enormous and unknown peaks appear in the chromatogram for both HxCDF and HpCDD/F. The very high amounts together with the strange congener profile gave fear about a new dioxin crisis. During the first weeks after the discovery, we analyzed 4 feed samples, 15 finishing pigs and 8 sows from the farm. All samples had low WHO-PCDD/F-TEQ values, see table 2.

It was a big relief to exclude the feed as source for the contamination, because contaminated feed could lead to a large scale crisis like the German dioxin crisis in 2011. The 15 finishing pigs only contained traces of dioxins. Sows are of course older than finishing pigs and dioxins are accumulated during their

lifetime. As a result the 8 sows contained higher amounts although much lower than the contaminated sow, see table 2. This indicated that not the complete farm was affected, but more likely one or more hot spots somewhere on the farm. However, the same special congener profile, with higher values for 1234678-HpCDF and 1234678-HpCDD were observed in all of the 8 samples from sows.

On the photo documentation it was seen that every shelter on the field was surrounded by an electric fence preventing individual sows access to the complete area. This restriction explained why one sow could get a quite high dioxin contamination, while other sows only contained traces of dioxins. The presence of the camouflage net on one hut in the corner of the farm used for shelter for hunters, gave us a hint to collect a sample and analyze it for dioxins. We did not have a method for dioxins in materials like this, but we used a qualitative test where the sample was shaken with pentane/acetone (88/12), filtered, evaporated and measured ……and BANG!! The source was found and the whole lab was contaminated with HxCDF and HpCDD/F congeners!
The most obvious explanation for the presence of dioxins in such amounts must be that pentachlorphenol (PCP) has been used to impregnate the camouflage net. PCP and its derivatives have been widely used as fungicides and preservative for robes and tarpaulin in Europe (4). PCP is also well known for its varying amounts of dioxins originating from the manufacturing process. The camouflage net, which had a military origin, contained extremely high concentrations of especially HxCDF, HpCDD and HpCDF congeners. The congener profile is seen in figure 2.
The profile is dominated by HxCDF-32.34 ("32.34" refers to the retention time of the non-2378-substituded congener), 1234678-HpCDF, HpCDF-40.06, HpCDD-39.89 and 1234678-HpCDD. Often the OCDF and OCDD congeners are dominant in cases with PCP contaminations (4), but in our analysis the OCDF and OCDD congeners are nearly absent. This could be due to the quick extraction procedure used or the solvent mixture without toluene.

In Figure 3, the congener profile for some of the congeners found in the camouflage net is compared to the congeners found in the sow. The scale on the left-hand side gives an indication of the enormous amount of dioxins in the camouflage net. The numbers are the Quan mass area of the chromatographic peak, showing areas 10000 times larger for the camouflage net compared to the sow fat. The dominant peaks from the camouflage net can also be seen in the sow, but in different ratios. The 2378-congengeners are still dominant, but the non-2378-congeners are not accumulated in the body at the same extent. Further, non-ortho-PCBs and especially OCDD and OCDF are found in the sow, but not in the camouflage net.
A survey in our analytical database showed a sow analyzed in 2013 and yet another one in 2016 from the same farm which also showed the characteristic congener profile and a slightly increased level of dioxins compared to the normal level in Danish pigs (see values in Table 2).This indicates that the contamination has taken place over years and probably contaminated many sows.
The content of dioxins in the camouflage net is enormous if we can trust our semi-quantitative measurement. Humans and animals in contact with a camouflage net like this must be subject to large exposure of dioxins. The camouflage net from this farm has been destroyed, but many more may still be in use for all kind of purposes.

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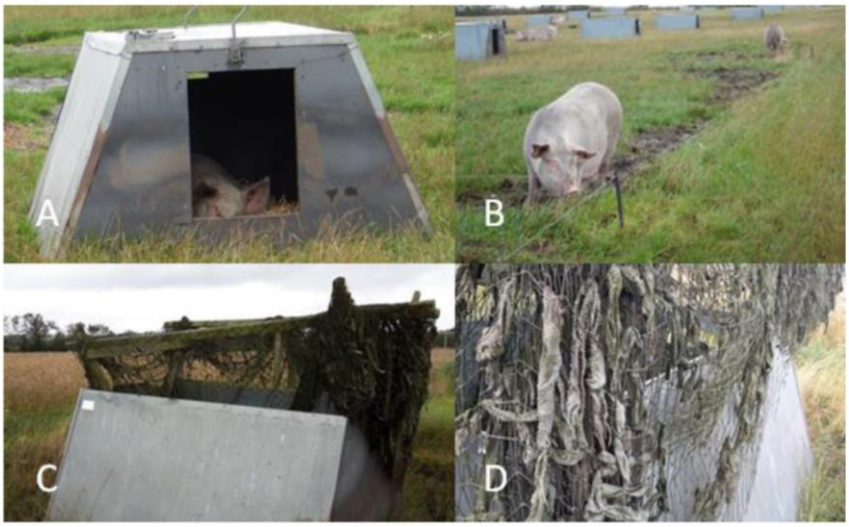

Figure 1. Photos from the farm showing: A) Shelter B) Sow, shelters and electric fence. C) Shelter with camouflage net. D) Close-up of the camouflage net.

Table 1. Concentration of dioxins and PCB's in the contaminated sow and for comparison average for Danish sows sampled in 2015 (n=16). Numbers in italic are upper bound values for non-detect congeners.

Congener	Sow pg/g fat	Average pg/g fat
2378-TCDF	*0,029*	0,028
12378-PeCDF	*0,058*	0,023
23478-PeCDF	0,72	0,050
123478-HxCDF	4,0	0,052
123678-HxCDF	7,1	0,048
234678-HxCDF	4,6	0,032
123789-HxCDF	*0,20*	0,028
1234678-HpCDF	58	0,25
1234789-HpCDF	2,4	0,083
OCDF	6,0	0,30
2378-TCDD	*0,015*	0,019
12378-PeCDD	*0,12*	0,17
123478-HxCDD	0,93	0,046
123678-HxCDD	5,8	0,026
123789-HxCDD	0,93	0,024
1234678-HpCDD	66	0,34
OCDD	72	1,8
PCB-77	1,6	0,59
PCB-81	0,13	0,10
PCB-126	3,6	0,52
PCB-169	1,4	0,24

Congener	Sow ng/g fat	Average ng/g fat
PCB-28	0,11	0,041
PCB-52	0,26	0,075
PCB-101	0,60	0,11
PCB-105	0,051	0,017
PCB-114	*0,011*	0,0078
PCB-118	0,36	0,083
PCB-123	*0,013*	0,0087
PCB-138	1,6	0,33
PCB-153	2,0	0,47
PCB-156	0,13	0,021
PCB-157	*0,0081*	0,0028
PCB-167	0,036	0,0083
PCB-170	0,50	0,11
PCB-180	0,91	0,19
PCB-189	*0,0076*	0,0076

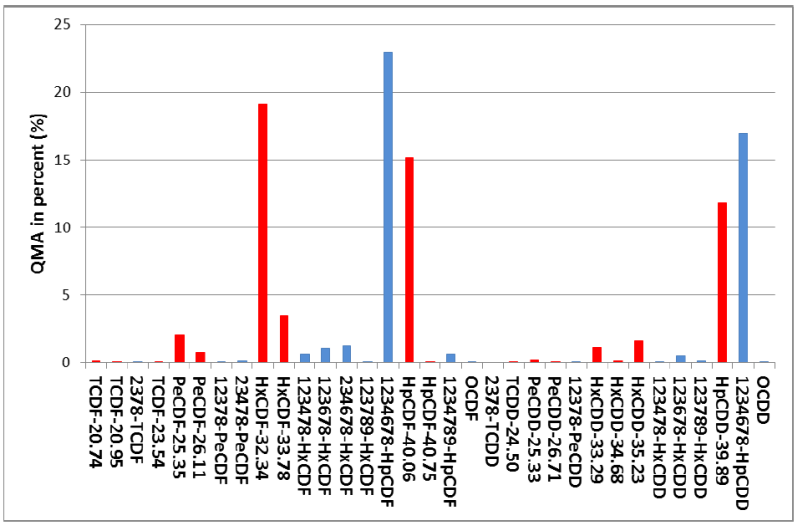

Figure 2. The congener profile obtained from the camouflage net based on the Quan mass area of the chromatographic peaks. The red bars are the non-2378-substituted dioxins. The blue bars are the 2378-substituted congeners. As the non-2378-substituted congeners are unknown to us, we have named them by the group to which they belong and the retention time in our analysis.

Table 2. Levels of dioxins in pigs from the incident farm and from the Danish monitoring program. All samples are of Danish origin.

Origin of sample	Year	Sample	pg WHO-PCDD/F-TEQ per gram of fat
Incident farm	2015	The contaminated sow	4.0
		The 8 sows (min and max)	0.3 – 0.9
		The 16 finishing pigs (min and max)	0.1 – 0.4
	2013	One sow	0.55
	2016	One sow	0.22
Random sampling	2015	Danish sows (n=16)	0.1 – 0.4
Random sampling	2015	Danish pigs (n=54)	0.04 – 0.4
EU maximum level for pigs (2)			1.0

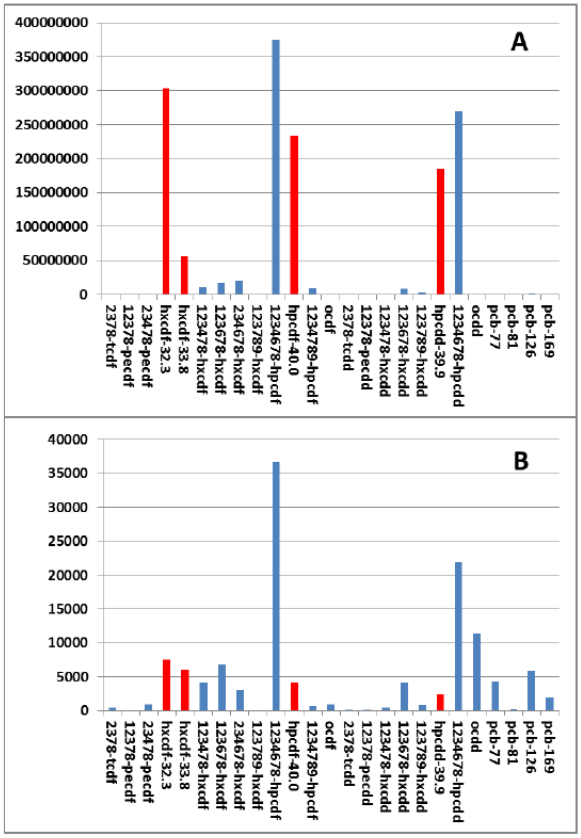

Figure 3. Comparison between the congener profiles found in the camouflage net (A) and in fat from the sow (B). See Figure 2 for further explanations concerning colors and names.