An Experimental PCB-Contamination Incident: The Freeze Dryer and Its Dark Sides

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1 Introduction

Polychlorinated biphenyls (PCBs) are anthropogenic persistent organic pollutants (POPs) that are ubiquitous within the environment (*chm.pops.int*). The Western population is mainly exposed to PCBs by the consumption of bovine derived food products, accounting for more than 70% of the total dietary exposure[1]. Among various beef production systems, grass-based suckling beef husbandries have been found to be more prone in exceeding regulatory levels[2,3,4]. For that reason, a long-term transgenerational transfer study was performed by mimicking a grass-based suckling beef husbandry. Those results will be presented at the DIOXIN 22 symposium[5] and have been reported in detail earlier[6,7].

Among several biological samples analyzed along the study, grass silage was sampled regularly to quantify the PCB dynamics during a pregnancy-lactation cycle. However, the first grass silage samples analyzed during the trial showed unexpectedly high PCB concentrations compared to the harvested fresh grass analyzed before entering the silo tower. This resulted in the serendipitous discovery of a cross-contamination along the grass silage storing, desiling, sampling, and analyzing process (Figure 1). The aim of this report was to elucidate the source of grass silage PCB cross-contamination that occurred between grass harvesting and sample analysis.

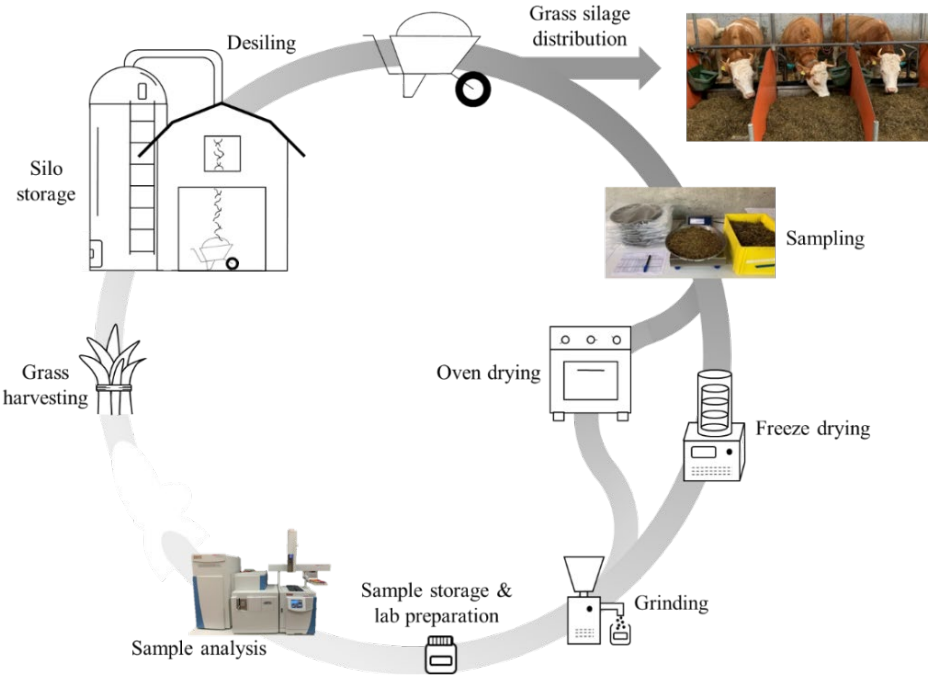

Figure 1: The grass processing cycle shows the different steps where a cross-contamination could have occurred.

2 Materials and Methods

To elucidate the source of cross-contamination, several samples at every step of the processing cycle were analyzed for PCBs, using a Q-Exactive Orbitrap gas chromatograph-high resolution mass spectrometer (GC-HRMS, ThermoFisher Scientific, MA, USA). Those samples were fresh grass at harvest before entering the silo tower, grass silage from the silo interior, grass silage after the desiling process, paints of the silo (HUBER Silobau & Kunststoffwerk AG, CH) and barn inner walls, swipe samples of the sample preparation materials used, including freeze dryers, corresponding pump oil (P3, Pfeiffer Vacuum GmbH, DE) and vacuum greases (Glisseal N of Borer Chemie AG, CH; high vacuum grease of Dow Corning Inc., MI, USA; high-vacuum silicone grease of Wacker Chemie AG, DE), oven, and grinder, as well as laboratory blanks.

Grass/Grass silage. The grass silage analyzed, was collected onto an aluminum plate and either dried using one of four freeze dryers (72 h; Alpha 2-4 LDplus and Delta 2-24 LSC, Martin Christ Gefriertrocknungsanlagen GmbH, DE) or an oven (60 °C for 72 h; FED720, BINDER GmbH, DE), followed by grinding (Brabender GmbH & Co. KG, DE) and sieving through a 1 mm grid. The dried and ground samples were Soxhlet extracted with toluene for 16 h, after which a known amount of $^{13}C_{12}$-labeled indicator PCB (iPCB) and dioxin-like PCB (dlPCB) internal standards (Cambridge Isotope Laboratories Inc., MA, USA) were added. This was followed by a multi-step clean-up, including a self-packed

multilayered silica column (from bottom to top: 5 g of neutral silica gel, 15 g of potassium silicate, 5 g of neutral silica gel, 45 g of acid silica gel, and 10 g of sodium sulfate) and an EZprep 123 system [Fluid Management Systems (FMS), MA, USA] with an Extra High Capacity Kit (EHCLS-KT05.0G, FMS, MA, USA). Before analysis, the two obtained fractions were concentrated to ~30 µl and spiked with the two recovery standards PCB70 and 1278TCDF (Cambridge Isotope Laboratories Inc.).

Soil. Ambient temperature-dried and ground soil was Soxhlet extracted with toluene for 16 h. An aliquot was spiked, similar to the grass silage, and purified using the EZprep 123 system with an Extra High Capacity Kit, followed by concentrating and spiking the sample with the recovery standards PCB70 and 1278TCDF.

Paint. A Soxhlet extraction of approximately 100 mg of paint was carried out with the use of toluene for 8 h. Afterwards, the volume was adjusted to 100 mL. An aliquot of 10 µl was spiked, similar to the grass silage, before being directly analyzed via the GC-HRMS.

Swipe samples. The swipe samples were Soxhlet extracted with the use of n-hexane for 8 h. Before being concentrated, the extract was spiked, similar to the grass silage. Purification was done using a self-packed mini silica column (from bottom to top: 1 g of acid silica gel and few mg of sodium sulfate), conditioned with 10 mL of n-hexane. The extract was eluted with 10 mL of n-hexane, after which it was re-concentrated, spiked with recovery standards, and analyzed.

Oil and greases. Oil and greases were spiked and treated with 1-2 mL Oleum (7%), which was then centrifuged at 1600 *g* for 10 min. The clear phase was purified using the self-packed mini silica column, described for the swipe samples, followed by spiking recovery standards and analysis.

For the analyses, the GC-HRMS was operated as previously described in Driesen *et al.* (2022)[6]. It used a resolution of 60,000 (full width half max), with an electron ionization of 70 eV. The GC was equipped with a 60 m DB-5 capillary column (0.25 mm diameter, 0.25 µm film thickness; Agilent Technologies, CA, USA) and helium as carrier gas, with a constant flow of 1.5 mL min^{-1}. For analysis, a 1 µl sample aliquot was injected in splitless mode at 260°C, whereas the oven temperature was programmed as follows: 100°C for 1 min, followed by an increase of 20°C min^{-1} up to 180°C and 5°C min^{-1} up to 300°C, where it was kept for 6 minutes. For quantification, the system operated in full scan mode from m/z 250 to 500, reading out the exact m/z of the two most abundant isotopes of the native and $^{13}C_{12}$-labeled molecular PCB ion.

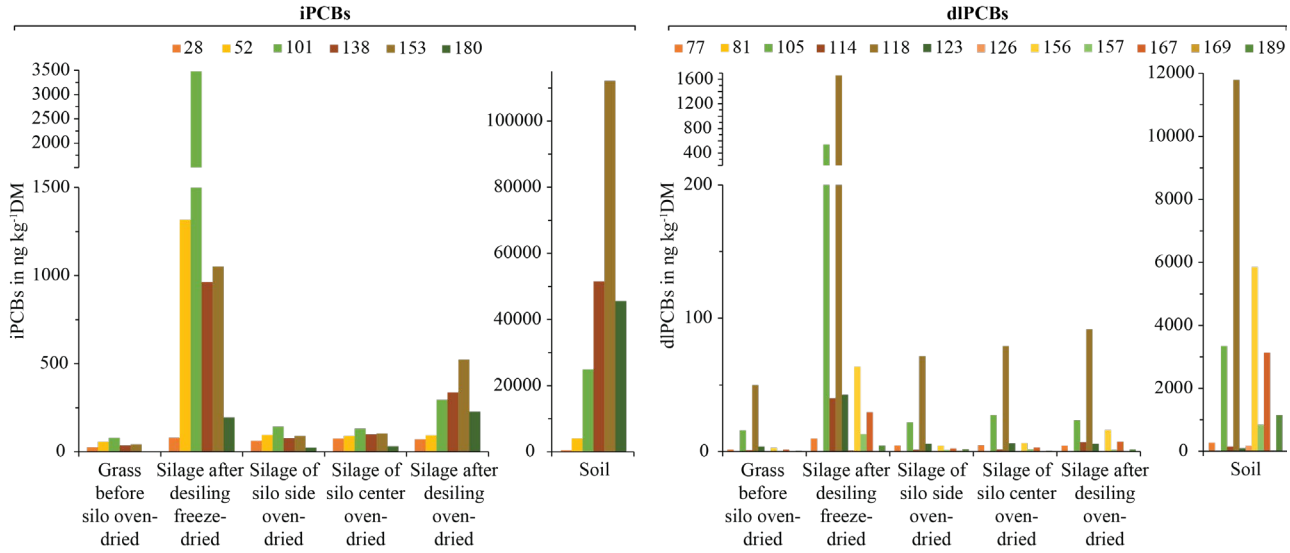

Figure 2: Indicator PCB (iPCB, left panels) and dioxin-like PCB (dlPCB, right panels) concentrations in harvested fresh grass, various grass silage samples from the same silo dried using a freeze dryer or oven, and soil used in the feeding experiment to generate the diet mixture for the exposed cows[6].

3 Results

Identification of a cross-contamination. Figure 2 represents iPCB and dlPCB concentrations in different grass and grass silage samples dried via a freeze dryer or oven as well as concentrations in environmentally PCB loaded soil used in the experiment. The lowest iPCB (245 ng kg^{-1} DM) and dlPCB (detected 77.7 ng kg^{-1} DM) concentrations were found in freshly harvested grass, followed by grass silage from inside the silo (average of 514 and 125 ng kg^{-1} DM, respectively, without remarkable differences when sampled from silo side or center), and grass silage sampled after desiling (1551 and 161 ng kg^{-1} DM, respectively), all dried using an oven. Grass silage after desiling and dried using one of the four freeze dryers showed higher concentrations with 7087 ng iPCBs and 2410 ng dlPCBs kg^{-1} DM. Additionally, the congener pattern differed among grass silage samples and in comparison with the loaded soil. Whereas for the dlPCBs the main contributing congeners were PCB118 and 105 whatever the sample, more variation was recognized for the iPCBs, especially between freeze-dried and oven-dried grass silage after desiling, and soil. The freeze-dried grass silage after desiling was characterized by PCB101 (49%), 52 (19%), 153 (15%), and 138 (14%), whereas oven-dried grass silage

after desiling (compared to grass silage from inside the silo) let to an increased contribution of higher chlorinated iPCBs [PCB153 (34%), 138 (22%), 101 (19%), 180 (15%)]. In any circumstance, the pattern was still different compared to the soil [PCB101 (25%), PCB153 (20%), PCB138 (19%), PCB52 (17%)].

Identification of the contamination source. To identify the source of contamination, paints of the silo and barn inner wall were analyzed. Whereas the PCB congeners of the silo paint were all below the limit of detection, the barn paint showed concentrations of 3155 ng iPCBs kg^{-1} and 261 ng dlPCBs kg^{-1} (lower bound, only PCB77, 105, 118, and 156 were detected). The PCB28 showed the highest contribution for the iPCBs and did not fit with the pattern found in the cross-contaminated grass silage. Next to that, swipe samples of the equipment used for drying and grinding the grass silage at Agroscope were analyzed for PCBs, as represented in the left part of figure 3. The freeze dryer swipe sample showed the highest levels with 707 ng iPCBs and 436 ng dlPCBs $(m^2)^{-1}$ compared to the grinder [202 and 88 ng $(m^2)^{-1}$, respectively] and oven [260 and 68 ng $(m^2)^{-1}$, respectively]. Besides, similar congener patterns were seen, except for a 10% higher PCB101 contribution for the freeze dryer swipe. As response, aliquots of a same batch of grass silage after desiling were dried via various freeze dryers, an oven and at ambient temperature (middle part of figure 3). This confirmed that the freeze-dried samples showed much higher levels, with concentrations between 20866 and 27637 ng iPCBs, and between 6116 and 18431 ng dlPCBs kg^{-1} DM, compared to oven- (801 and 259 ng kg^{-1} DM, respectively) and ambient temperature-dried grass silage (2232 and 428 ng kg^{-1} DM, respectively). However, not only a congener pattern difference between oven, ambient temperature, and freeze dryer was observed, also a pattern difference among freeze dryers was deciphered. To identify a potential source of contamination within the freeze dryer, corresponding pump oil and greases were analyzed and displayed in the right part of figure 3. Only one grease showed remarkably high concentrations (754406 ng iPCBs and 309647 ng dlPCBs $sample^{-1}$) with a related pattern as was seen for the grass silage dried with freeze dryer 4 and 5.

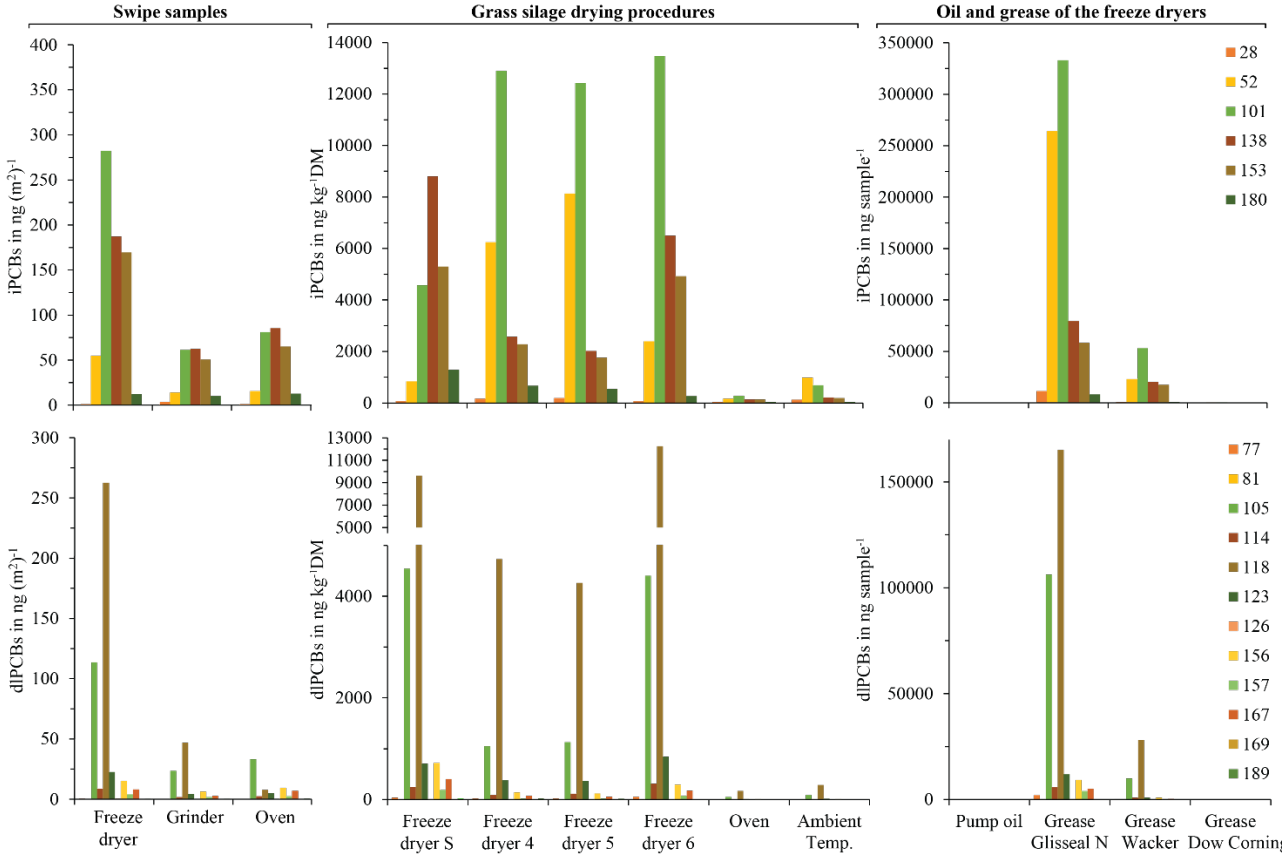

Figure 3: Indicator PCB (iPCB, upper panels) and dioxin-like PCB (dlPCB, lower panels) concentrations within swipe samples of the equipment used for drying and grinding the grass silage samples at Agroscope; concentrations in grass silage after desiling dried via one of four freeze dryers, oven, or at ambient temperature all collected on aluminum plates; and concentrations in oil and greases of the freeze dryer.

4 Discussion

Identification of a cross-contamination. The initially harvested fresh grass showed low iPCB and dlPCB concentrations before it entered the silo (Figure 2). Therefore, it was optimal for the use as control diet in the feeding experiment, as it corresponded to background contamination levels recorded for grass forages in Switzerland[8]. Conversely, the first grass silage samples of the trial, leaving the silo, showed on average 30-fold higher concentrations of iPCBs and dlPCBs compared to the initial fresh grass. Since environmentally loaded PCB soil was used within the feeding experiment, the first thought was that the soil was responsible for the cross-contamination. The soil was namely mixed to the grass silage

(2.5% DM basis) to generate the contaminated feed for the exposed treatment. A typical approach to identify potential contamination sources relies on the comparison of congener patterns[9]. However, the patterns were not alike, especially not for the iPCBs, so that in first instance a putative cross-contamination coming from the soil as well as a mix-up between control (grass silage) and exposed (grass silage-soil mixture) assigned feed could be ruled out.

Identification of the contamination source. To elucidate where the cross-contamination came from, every step of the sampling and preparation procedure between silo interior and POP analysis was investigated (Figure 1). One typical contamination can result from construction materials, such as PCB-containing paint that was allowed in open applications until 1972 in Switzerland[4]. Since the tower silo potentially dated back to the 1970s-80s, with an even older barn building and the estimation that 35% of the PCBs used in paints and joint sealants are still present in Swiss buildings[10], paint samples from inside the silo and barn were taken, analyzed, and further excluded as a source. Besides low concentrations, the barn-paint did not show typical PCB-containing paint patterns[3,4]. The exclusion of the silo inner wall was further strengthen based on no notable differences in concentration between grass silage samples taken from within the silo touching the silo inner wall and from the silo center (Figure 2). The next step in the processing cycle was linked to the desiling process. Desiling added some PCBs, with iPCBs 3.0-fold and dlPCBs 1.3-fold higher than within the silo, which might be related to the equipment and consumables used for desiling, such as hydraulic oil or joint sealant connecting the tubes. Nonetheless, the oven-dried grass silage sample after desiling was still clearly lower in concentration compared to the corresponding freeze-dried sample. Therefore, the main difference between the initial fresh grass sample and the grass silage after desiling, besides being in the silo for several months, had to be linked to the drying or grinding of the samples, the reason why in first instance swipe samples of the different tools were collected. As the highest iPCB and dlPCB concentrations within the swipe samples were found for the freeze dryer, a direct comparison of freeze- or oven-dried grass silage samples of the same lot was established (Figure 3).

The freeze dryer as source. Grass silage samples dried with a freeze dryer showed 26 up to 35-fold higher iPCB and 24 up to 71-fold higher dlPCB concentrations than samples dried within an oven (Figure 3). Besides, drying the same grass silage at ambient temperature resulted in slightly higher (2.8-fold for iPCBs and 1.6-fold for dlPCBs) levels than when using the oven, which in part might result from a potential air-grass depositing transfer of PCBs[11] and in part from a potential PCB evaporation, respectively. However, these results clearly showed that the cross-contamination was very likely linked to the freeze dryer. Also previous reports have identified freeze drying as a potential contamination source, mainly due to an increase of lower chlorinated PCBs[12,13].
One suggested PCB source was thought to be the pumping oil or vacuum grease used within the freeze dryer, as mineral greasy products sometimes contained PCBs in the past. Analysis of the oil showed that there were no relevant levels of PCBs in the oil (Figure 3) and also most of the greases were inconspicuous, except for one. The grease Glisseal N (only grease present in plastic tube) showed elevated PCB levels with a congener pattern matching two of the freeze dryers tested. However, not all four tested freeze dryers showed the same pattern, meaning there could be a different source of contamination, which was not identified here. An additional source could be the indoor air, as demonstrated by Söderström *et al*. (2005)[13]. They linked increased PCB levels after freeze drying in laboratories build before 1972 to airborne PCBs. Since the laboratory building dates back to 1970, it would be interesting to also have a measurement of the indoor air. However, the different patterns among freeze dryers and the elevation of not only lower chlorinated PCBs indicates that indoor air might be less likely in the present study.

5 Conclusions

Based on the present report, a clear cross-contamination was identified during grass silage sample preparation. The source of contamination was traced back to the freeze dryer, but a potential source within the freeze dryer could not be identified, with the putative exception of one of the vacuum greases. Since previous reports showed already similar problems with freeze drying and traced it back to a certain level of air contamination, it would be interesting to further measure the air within this context. Also the inclusion of more congeners within the pattern comparison might improve the validity of a potential source[9]. Besides, the incident highlighted how important it is to control the sample preparation procedure and that it might be safer to avoid freeze drying in the context of POP analyses. Especially for monitoring purposes, where feed and food contaminant concentrations have to be reported, quantification involves a preparation of the sample of interest as clean as feasible.

6 Acknowledgments

Funding was provided by the Federal Food Safety and Veterinary Office and the Federal Office for Agriculture of Switzerland (AgroPOP: n°4.17.06). The authors would like to acknowledge Siegenthaler R., Glauser W., and Aeby Y. and his team for organization and management of samples; Hermann J.M. for sample drying and grinding of Agroscope (Posieux, Switzerland); and Bongard L., Cavaliere D., Kälin O., and Perrone D. of Empa (Dübendorf, Switzerland) for POP analyses support.

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