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Persistent Organic Pollutants (POP) in the Swiss Agriculture: Assessment of the Current Status, Recommendations, Opportunities for Improvement and Added Value (AgroPOP)

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Key words
ADME, Bioaccumulation, Meat, Polychlorinated biphenyls, Polychlorinated dibenzo-*p*-dioxin/dibenzofurans, Ruminant, Transgenerational transfer

Aim of the study
The aims of this study were: i) to quantify the transgenerational accumulation and decontamination kinetics of polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-*p*-dioxins/dibenzofuran (PCDD/Fs) from grass silage and soil ingestion to blood serum, subcutaneous adipose tissue, and milk in suckling cow and calf, ii) to quantify the absorption, tissue distribution, metabolism and excretion (ADME) of PCBs and PCDD/Fs in suckling cow and calf, iii) to link PCB and PCDD/F fate to physiological changes that cow (body and milk lipid dynamics) and calf (body lipid dynamics) experience during gestation and lactation, and iv) to use these findings to improve chemical safety of most consumed beef-derived foods (i.e., meat and offal).

Material and methods
A toxicokinetic feeding experiment with 13 Simmental bovine females (six primiparous and six multiparous; referred to as cows) and their suckling calves took place at Agroscope Posieux. The experiment lasted 109 days prepartum until 288 days in milk (DIM). Nine of the cows were assigned to the exposed group, receiving grass silage mixed with 2.5% [dry matter (DM) basis] environmentally POP-loaded soil, whereas four control cows were fed a non-contaminated grass silage. Four of the exposed animals underwent a decontamination after DIM164 by switching to the control feed. After birth, calves were separated from cows and fed with the milk of their respective mother cow, which were milked twice daily. Calves received additionally non-contaminated hay, and concentrate after three months of age. Daily milk yield and individual intake were recorded for cow and calf, and body weight (BW) and body condition score were reported regularly. Throughout the study, grass silage, grass silage-soil mixture, hay, minerals, salts, concentrate, milk, and feces were sampled weekly and further pooled per lot (ffed) or over 4-6 months periods (milk and feces). Additionally, blood serum (DIM-109 for cow, 1, 29, 60 for calf, 89, 164, and 199, 227, 255 for decontaminated), subcutaneous adipose tissue (DIM-109 for cow, 1 for cow, 29 for cow, 89, 164), and milk (DIM1, 7, 14, 21, 29, 59, 89, 127, 164, 229, 288, and 167, 171, 178, 185, 199, 255 for decontaminated) were sampled at specific kinetic time points. After slaughter at DIM288, additional samples of blood serum, liver, kidney, subcutaneous, perirenal and intermuscular adipose tissues, muscles *Rectus abdominis* (*RA*) and *Longissimus thoracis* (*LT*), carcass, and empty body (without digesta and urine) were taken. Samples were analyzed for lipid, PCB and PCDD/F contents.
The PCB and PCDD/F analyses was based on Soxhlet extraction, except for blood serum and milk (liquid-liquid extraction), followed by purification with silica, alumina, and carbon column chromatography, and subsequent quantification by Q-Exactive Orbitrap GC-HRMS and APGC-Xevo TQ-XS Triple Quadrupole MS. Several calculations were performed to describe the transfer fate and detailed ADME of PCBs and PCDD/Fs:

- Transfer rate to milk (TR; %) = (compound concentration in milk × daily milk fat yield)/(compound concentration in feed × daily DM intake)

- Half-life in milk was estimated using a two-phased exponential decay model ($C = C_A \times e^{(-\alpha t)} + C_B \times e^{(-\beta t)}$; for decontaminated cows from DIM164-288)
- Metabolism (ng) = (Initial body burden + oral intake) – (final body burden + calf body burden$_{parturition}$ + fecal excretion + milk excretion)
- Absorption rate (AR; %) = (oral intake – fecal excretion)/oral intake × 100 (only for control and continuously exposed cows and calves)
- Assimilation efficiency (AE; %) = [final body burden – initial body burden) + milk excretion + calf body burden$_{parturition}$]/oral intake × 100 (only for control and continuously exposed cows and calves)

Where oral intake for the calf includes solid feed and milk, and where calf body burden$_{parturition}$ and milk excretion are only applicable for the cow.

Results and significance

It was shown that the apparent absorption of PCBs and PCDD/Fs was reduced when cows were exposed via the grass silage-soil mixture compared to grass silage alone. Physicochemical compound properties, such as a higher chlorinated degree and concomitantly higher lipophilicity (octanol:water partition coefficient, K_{OW}), were negatively correlated with the AR. Animal physiology affected the rate further with higher ARs in primi- vs. multiparous cows, presumably resulting from a higher lipid digestibility in the former. Accordingly, suckling calves showed the highest ARs via the ingestion of a highly digestible lipid-rich diet (milk) compared to cows, even without the reducing effect of the PCB chlorination degree, resulting in a transgenerational bioaccumulation. Calf blood serum and subcutaneous adipose tissue PCB and PCDD/F concentrations were therefore twice as high as in cows until DIM164. Transfer rates to milk and decontamination half-lives in milk were also affected by the physicochemical compound properties and the cow reproductive parity (encompasses differences in DM intake, body lipid mobilization and accretion dynamics, and milk yield). Increasing PCB chlorination resulted in a higher TR, with an inverse relationship for PCDD/F. Transfer rates were lower with decreasing milk yield (i.e., in primiparous cows), resulting further into longer half-lives in milk.

To quantify the PCB and PCDD/F fraction not transferred to milk, a transgenerational input-output mass balance was performed. The estimated metabolized fraction was negative correlated with the chlorinated degree, whereas young calves (3 months old) had a reduced estimated metabolism efficiency. Calves excreted only a small fraction via the feces, so that the main part ingested was accumulated within the body, especially for highly chlorinated PCBs. Still, calves were more efficient in reducing their PCB and PCDD/F body concentrations compared to cows, presumably due to a higher increase in body lipid mass over the course of lactation. In both cow and calf, a liver-specific PCB and PCDD/F enrichment was identified, with an additional enhanced accumulation in kidney and muscles for some congeners, especially lower chlorinated ones.

This study is so far, to the best of our knowledge, the largest and most comprehensive one about the fate of PCBs and PCDD/Fs in suckling cow husbandries. It delivered detailed insights into the transgenerational fate of PCBs and PCDD/Fs from grass silage and soil into suckling cow and calf. It was clearly shown that transfer assessment should consider the complex interplay between physicochemical compound properties and ruminant physiology on absorption, distribution, metabolism and excretion. Furthermore, it was shown that adipose tissue concentrations might underestimate up to 2-fold the levels in most consumed beef-tissues (i.e., muscle), which is important to take into account for monitoring purpose. In conclusion, these results help to implement new recommendations for agricultural practices to further reduce beef cattle PCB and PCDD/F exposure and accumulation, and subsequently improve bovine meat safety.

Besides, the project also encompassed other aspects, such as *in silico* applications to support risk assessment and specific practice oriented recommendations, which were detailed in previous annual reports and publications listed below.

Publications, posters and presentations (as of March 2022)

<u>*Peer-reviewed publications*</u>

Charlotte Driesen, Markus Zennegg, Isabelle Morel, Hans Dieter Hess, Bernd Nowack, Sylvain Lerch. 2021. Average transfer factors are not enough: The influence of growing cattle physiology on the transfer rate of polychlorinated biphenyls from feed to adipose. Chemosphere 270, 129698. https://doi.org/10.1016/j.chemosphere.2021.129698

Charlotte Driesen, Sylvain Lerch, Raphael Siegenthaler, Paolo Silacci, Hans Dieter Hess, Bernd Nowack, Markus Zennegg. 2022. Accumulation and decontamination kinetics of PCBs and PCDD/Fs from grass silage and

soil in a transgenerational cow-calf setting. Chemosphere. 296, 133951. https://doi.org/10.1016/j.chemosphere.2022.133951

Charlotte Driesen, Markus Zennegg, Myriam Rothacher, Sébastien Dubois, Ueli Wyss, Bernd Nowack, Sylvain Lerch. 2022. Transgenerational mass balance and tissue distribution of PCBs and PCDD/Fs from grass silage and soil into cow-calf continuum. In preparation for submission.

Non peer-reviewed publications

Charlotte Driesen, Markus Zennegg, Isabelle Morel, Hans Dieter Hess, Sylvain Lerch. 2019. The transfer rate of PCBs from feed to adipose tissue depends on body fatness in growing cattle. Organohalogen Compounds 81, 128-131.

John Albechaalany, Christelle Loncke, Charlotte Driesen, Philippe Schmidely, Isabelle Ortigues-Marty, Jacques Agabriel, Markus Zennegg, Daniel Sauvant, Sylvain Lerch. 2020. Mechanistic model of lipophilic contaminant transfer in growing cattle [In French]. 25^{éme} Rencontres autour des Recherches sur les Ruminants (3R), December 2.-3.

Charlotte Driesen, Markus Zennegg, Isabelle Morel, Hans Dieter Hess, Sylvain Lerch: The transfer rate of polychlorinated biphenyls (PCBs) from feed to adipose tissue depends on body fatness of growing beef cattle [In French]. 25^{éme} Rencontres autour des Recherches sur les Ruminants (3R), Virtual, France, December 2.-3. (2020)

Silvio Arpagaus, Andreas Buser, Charlotte Driesen, Andreas Ewy, Bettina Hitzfeld, Lucia Klauser, Sylvain Lerch, Valentin Luzi, Pascal Python, Markus Rombach, Gilles Rossier, Kurt Seiler, Mark Stauber, Sabine Vögeli, Markus Zennegg. 2021. PCB in der Tierhaltung Ursachen und Massnahmen [PCBs in livestock causes and measures]. Agridea, Product no. 3875

Sylvain Lerch, John Albechaalany, Charlotte Driesen, Philippe Schmidely, Isabelle Ortigues-Marty, Jacques Agabriel, Markus Zennegg, Daniel Sauvant, Christelle Loncke. 2022. Mechanistic physiologically-based toxicokinetic model of persistent organic pollutants transfer in growing cattle. Submitted to the 7th EAAP International Symposium on Energy and Protein Metabolism and Nutrition (ISEP 2022), September 12.-15.

Charlotte Driesen. 2022. Fate of PCBs and PCDD/Fs in beef-producing husbandries and the impact of animal physiology. Doctoral dissertation. ETHZ

Presentations

Charlotte Driesen, Christian Bogdal, Martin Scheringer, Hans Dieter Hess, Bernd Nowack, Markus Zennegg: Transgenerational fate modeling of polychlorinated biphenyls in cattle. Empa PhD Symposium 2018, Dübendorf, Switzerland, November 26. (2018)

Charlotte Driesen, Sylvain Lerch: Presenting AgroPOP to VetSuisse students. Agroscope Posieux, April 8. (2019)

Charlotte Driesen, Sylvain Lerch, Christian Bogdal, Raphael Siegenthaler, Ueli Wyss, Hans Dieter Hess, Martin Scheringer, Bernd Nowack, Markus Zennegg: AgroPOP – Toward a better understanding and mitigation of transgenerational transfer of polychlorinated biphenyls in cattle. CHanalysis 2019, Beatenberg, Switzerland, April 11.-12. (2019)

Charlotte Driesen: AgroPOP – Den generationsübergreifenden Transfer von persistent organischen Schadstoffen in Rindern besser verstehen und mindern. Swissbeef Mittelland, Posieux, Switzerland, June 14. (2019)

Charlotte Driesen, Markus Zennegg, Isabelle Morel, Hans Dieter Hess, Sylvain Lerch: The transfer rate of PCBs from feed to adipose tissue depends on body fatness in growing cattle. Pre-Dioxin 2019 Student Symposium, Kyoto, Japan, August 24. (2019)

Charlotte Driesen, Markus Zennegg, Isabelle Morel, Hans Dieter Hess, Sylvain Lerch: The transfer rate of PCBs from feed to adipose tissue depends on body fatness in growing cattle. 39th International Symposium on Halogenated Persistent Organic Pollutants, Kyoto, Japan, August 25.-30. (2019)

Charlotte Driesen, Sylvain Lerch: AgroPOP – Transfer von persistent organischen Schadstoffen in Fleisch produzierenden Rindern besser verstehen und mindern. Mutterkuh Schweiz, Brugg, Switzerland, December 13. (2019)

Charlotte Driesen, Markus Zennegg, Sylvain Lerch: The AgroPOP Project: Toward a better understanding and mitigation of POP transgenerational transfer in suckling cattle. 'COR' Group Germany, Virtual, June 12. (2020) – presented by Sylvain Lerch

Charlotte Driesen, Sylvain Lerch, Markus Zennegg: AgroPOP – Transgenerationalen Transfer von PCBs bei Rindern aus Mutterkuhhaltung besser verstehen und eindämmen. Federal working group on the implementation of measures to reduce PCBs in food of farm animals, Virtual, Switzerland, June 19. (2020)

Sylvain Lerch, Charlotte Driesen, Markus Zennegg: AgroPOP and ExpoCow: Improve risk assessment of persistent organic pollutant transfer from the environment to the bovine meat chain. Federal working group on the implementation of measures to reduce PCBs in food of farm animals, Virtual, Switzerland, October 21. (2020) – presented by Sylvain Lerch

Charlotte Driesen, Markus Zennegg, Isabelle Morel, Hans Dieter Hess, Bernd Nowack, Sylvain Lerch: The influence of growing cattle physiology on the transfer rate of polychlorinated biphenyls from feed to adipose. 'COR' Group Germany, Virtual, October 26. (2020) – presented by Sylvain Lerch

Charlotte Driesen, Markus Zennegg, Isabelle Morel, Hans Dieter Hess, Bernd Nowack, Sylvain Lerch: Does the physiology of growing cattle influence the transfer rate of PCBs from feed to adipose tissue? Agroscope PhD-PostDoc Symposium. Virtual, Switzerland, March 18. (2021)

Charlotte Driesen, Sylvain Lerch, Raphael Siegenthaler, Bernd Nowack, Markus Zennegg: How potent is the transfer of polychlorinated biphenyls from cow to calf? 13th IBP PhD Congress. Virtual, Switzerland, April 9. (2021)

Charlotte Driesen, Markus Zennegg, Sylvain Lerch: AgroPOP – Erkenntnisgewinnung zum generationsübergreifenden Transfer von Schadstoffen aus diffusen Quellen in Rindern. Agridea, Virtual, Switzerland, April 21. (2021)

John Albechaalany, Christelle Loncke, Charlotte Driesen, Philippe Schmidely, Isabelle Ortigues-Marty, Jacques Agabriel, Markus Zennegg, Daniel Sauvant, Sylvain Lerch: Mechanistic modeling of lipophilic contaminant transfer in growing cattle. 'COR' group Germany, Virtual, May 12. (2021) – presented by Sylvain Lerch

Charlotte Driesen, Sylvain Lerch, Bernd Nowack, Markus Zennegg: Polychlorinated biphenyls – once a high production volume chemical, now an omnipresent residue in our dairy and meat products. RFA ReP Energy Colloquim, Empa Akademie Dübendorf, Switzerland, September 6. (2021)

Charlotte Driesen, Markus Zennegg, Sylvain Lerch: AgroPOP – Erkenntnisgewinnung zum generationsübergreifenden Transfer von Schadstoffen aus diffusen Quellen in Rindern. Agridea, Virtual, Switzerland, October 5. (2021)

Charlotte Driesen, Markus Zennegg, Sylvain Lerch: AgroPOP – Erkenntnisgewinnung zum generationsübergreifenden Transfer von Schadstoffen aus diffusen Quellen in Rindern. 'COR group Germany, BMEL Bonn, Germany, October 12. (2021) – presented by Sylvain Lerch

Charlotte Driesen, Markus Zennegg, Raphael Siegenthaler, Myriam Rothacher, Bernd Nowack, Sylvain Lerch: AgroPOP – Transgenerationalen Transfer von PCBs bei Rindern aus Mutterkuhhaltung besser verstehen und eindämmen. Tagung Netzwerk Nutztiere 2021, Grangeneuve, Switzerland, November 11. (2021)

Charlotte Driesen, Sylvain Lerch, Bernd Nowack, Markus Zennegg: Mass spectrometry of polychlorinated biphenyls – once a high production volume chemical, now an omnipresent residue in our dairy and meat products. CEO Visit Empa, Virtual, Switzerland, November 29. (2021)

Posters

Charlotte Driesen, Christian Bogdal, Bernd Nowack, Martin Scheringer, Hans Dieter Hess, Markus Zennegg: Transgenerational fate modeling of polychlorinated biphenyls in cattle. IBP PhD Symposium 2018, Zürich, Switzerland, April 6. (2018)

Charlotte Driesen, Christian Bogdal, Bernd Nowack, Martin Scheringer, Hans Dieter Hess, Markus Zennegg: Transgenerational fate modeling of polychlorinated biphenyls in cattle. SCS Fall Meeting, Lausanne, Switzerland, September 7. (2018)

Charlotte Driesen, Markus Zennegg, Isabelle Morel, Hans Dieter Hess, Sylvain Lerch: Transfer of PCBs from feed to adipose tissue depends on body fatness in growing cattle. SCS Fall Meeting, Zürich, Switzerland, September 6. (2019)

Charlotte Driesen, Markus Zennegg, Isabelle Morel, Hans Dieter Hess, Sylvain Lerch: Body fatness of growing cattle influences the transfer factors of polychlorinated biphenyls. Empa PhD Symposium 2019, St. Gallen, Switzerland, November 28. (2019)

Charlotte Driesen, Sylvain Lerch, Raphael Siegenthaler, Bernd Nowack, Markus Zennegg: The transfer of polychlorinated biphenyls from cow to calf during gestation and lactation. Empa PhD Symposium 2020, Virtual, Switzerland, November 30. (2020)

Short videos

Charlotte Driesen, Sylvain Lerch, Raphael Siegenthaler, Markus Zennegg: Transgenerational fate of polychlorinated biphenyls from cow to calf during gestation and lactation. SCS Fall Meeting, Virtual, Switzerland, August 25. (2020)

Charlotte Driesen, Markus Zennegg, Isabelle Morel, Hans Dieter Hess, Sylvain Lerch: The transfer rate of polychlorinated biphenyls (PCBs) from feed to adipose tissue depends on body fatness of growing beef cattle [In French]. 25^{éme} Rencontres autour des Recherches sur les Ruminants (3R), Virtual, France, December 2.-3. (2020)

John Albechaalany, Christelle Loncke, Charlotte Driesen, Philippe Schmidely, Isabelle Ortigues-Marty, Jacques Agabriel, Markus Zennegg, Daniel Sauvant, Sylvain Lerch. 2020. Mechanistic model of lipophilic contaminant transfer in growing cattle [In French]. 25^{éme} Rencontres autour des Recherches sur les Ruminants (3R), December 2.-3.

Contributions listed in italic are projects that were conducted in combination with AgroPOP.

***Project** 4.17.b*

***Project duration** November 2017 – April 2022*