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Combined *in vivo* proxies and *in silico* modelling for ensuring sheep meat chemical safety (ProxyPOP)

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Aim of the study
The aims of the ProxyPOP project were i) to quantify the accumulation, depuration kinetic and tissue distribution of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) in suckling ewes, ii) to set-up and calibrate physiologically-based toxicokinetic (PBTK) ewe and lamb models for PCDD/Fs, and derivate model-based recommendations for ensuring sheep meat chemical safety, and iii) to assess the reliability of target and non-target [i.e., profile in volatile organic compounds (VOCs)] analyses on non-invasive wool and cerumen sampling as proxies of adipose tissue contamination level.

Material and methods
Animal experimentation
Six suckling ewes from the sheep herd of the Lausanne municipality were first exposed over long-term to PCDD/F through feeding of pasture and hay harvested on the most polluted areas of Lausanne. They were further depurated (DEP) after being switched to a non-contaminated hay from 29 days of lactation. Four additional ewes served as control (CTL) and were fed continuously with non-contaminated hay. At the depuration days 0 (end of the exposure period), 32, 60 (weaning), 130 and 188 (slaughter), milk, blood, sternal adipose tissue, wool and cerumen were sampled, as well as *longissimus thoracis* muscle, liver and empty body homogenate at slaughter, for PCDD/F analysis by APGC-MS. Milk, wool and cerumen were also analyzed for VOC profile by SPME-GC-MS at the days 0, 60 and 188. Milk and adipose tissue biotransfer factors (BTFs), and mono-exponential depuration half-lives were determined for most 2378-chloro-substituted PCDD/F congeners. PCDD/F kinetic and tissue distribution data were analyzed by ANOVA, and VOC data by principal component analysis.
PBTK modeling
Two mechanist PBTK models describing the fate of PCDD/Fs in lactating ewe and growing lamb were developed. Both models consist of three coupled sub-models, one describing the PCDD/F absorption, distribution, metabolism and excretion (ADME), the second feed and soil ingestion and lipid digestion, and the last gestation and lactation (ewe) or growth (lamb) physiology. The rationale was to combine efficient mechanistic formalisms of contaminant adjective (e.g., blood-perfusion limited), diffusive (permeability-limited) and transformation (metabolism clearance) flows. The ewe PBTK model was calibrated for individual PCDD/F congeners against the *in vivo* observations gathered from the DEP ewes, whereas predictive capabilities were additionally assessed against the CTL ewe's dataset. For the lamb PBTK model, use of PCDD/F congener-specific parameters formerly calibrated for ewe was made. Models were further used according to prospective scenarios in order to derivate recommendations for management of the risk of meat contamination with PCDD/Fs in culled suckling ewe and growing lamb, with a specific focus on the case study of the Lausanne sheep herd.

Results and significance
Accumulation and depuration kinetic of PCDD/Fs in ewes

The ewe toxicokinetic experiment highlighted a relevant PCDD/F bioaccumulation potential (BTFs) from oral intake-to-milk and adipose tissue, especially for penta- and hexa-chlorinated congeners. It was feasible to depurate ewe adipose tissue under the Swiss regulatory maximum level (ML) for meat over a time period of 130 days, starting with an initial PCDD/F level around 10-fold the ML. During lactation, depuration presumably occurred mainly through excretion of PCDD/Fs *via* milk lipids. After weaning, excretion was limited to fecal output, and the dilution effect from increasing body lipid mass likely played a major role in the decline of adipose tissue concentration. Specific accumulation of PCDD/Fs was observed in ewe liver, especially for dibenzofurans.

PBTK modeling of PCDD/Fs fate in suckling ewes and lambs

Satisfactory performances of the ewe PBTK model were achieved for prediction of PCDD/F fate in milk and adipose tissue, against either the calibration (DEP ewes) or validation (CTL ewes) *in vivo* datasets. The use of PBTK models suggested various scenarios for obtaining sheep meat below the regulatory ML from 200 to 300 days after lambing. A first option would rely on constant exposure to PCDD/F along successive gestation-lactation cycles. Accordingly, the limit in the total oral intake (forage plus soil) to not exceed would be of 0.17 and 0.27 ng TEQ_{WHO-05} kg^{-1} DM for ewe and lamb, respectively. Depuration strategies were also explored, with initial constant exposure of 0.57 (regulatory feed action threshold) or 0.85 (feed ML) ng TEQ_{WHO-05} kg^{-1} DM, until the beginning or end of the third lactation, followed by a depuration phase (0.10 ng TEQ_{WHO-05} kg^{-1} DM). For ewes, a fattening strategy along depuration was also included from weaning, aiming at enhancing PCDD/F dilution through increase in empty body lipid mass. Thank to one or more of those scenarios, model simulations suggested that ewe and lamb meats would be lower than the ML from 250 to 300 days after lambing. Recommendations were finally set-up, defining which and when grasslands located in the specific areas of the Lausanne contamination mapping, can or cannot be used for grazing or harvesting forages, in order to ensure safe sheep meat production.

Wool and cerumen PCDD/F and VOC analyses as non-invasive proxies of adipose tissue contamination level

Due to the ease of their non-invasive sampling, the potential of wool sampling, and in a lesser extend of cerumen, as a monitoring tool for estimating the contamination status of sheep was confirmed. Especially the target analysis of PCDD/F in wool allowed a fair estimation of the corresponding contamination level in adipose tissue and milk at moderate and high PCDD/F concentrations (2-30 pg TEQ g^{-1} lipids). At lower ewe body PCDD/F level, external contamination of wool, presumably through air deposition, may explained poorer concordance between wool and adipose tissue concentrations. Besides, very low amount of cerumen collected from individual ewe, was not sufficient to perform reliable PCDD/F analysis, but allowed VOC analysis. Milk, cerumen and especially wool allowed distinguish exposed, depurated and non-exposed ewes from their volatolomics signatures.

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