DEPLETION OF PCDD AND PCDF CONGENERS IN EGGS FOLLOWING EXPOSURE OF LAYING HENS TO PENTACHLOROPHENOL-CONTAMINATED WOOD SHAVINGS

Igor Fochi, Gianfranco Brambilla, Stefania P. De Filippis, Alessandro di Domenico

Department of the Environment and Primary Prevention, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy

Introduction

The improved legislation on animal welfare has prompted farmers to leave caged rearing systems toward free range ones.[1] This zootechnical change is currently highlighting the relevance of some non-feed contributions in determining unacceptable residue levels of polychlorinated dibenzodioxins (PCDDs) and dibenzofurans (PCDFs) — both also known as "dioxins" — in eggs.[2] Previous papers have already dealt with soil- and wood shaving-derived contaminations.[3,4] In the latter case, one of the main sources of dioxins was represented by the use of materials originating from wood treated with pentachlorophenol (PCP).[5] In this work we report upon a depletion study after an incurred exposure of a laying hen flock reared on a PCP-contaminated floor, with the aim to give useful information about appropriate management actions based on a deeper knowledge of the depletion of such toxicants in eggs.

Materials and methods

Six Brown Swiss laying hens of 1.5 kg body weight were randomly sampled from a 100-animal flock reared on PCP-treated wood shavings, whose dioxin content was found to be in the order of 40 pgWHO-TE/g. Such exposure had started more than two months earlier, and eggs were found to be not compliant with the regulatory limit for dioxins. The hens were then placed in an uncontaminated environment, under controlled conditions for feed and contact materials. The average egg production was approximately 0.7 egg/head per day; each egg weighed approximately 55 g. The depletion study lasted eight weeks, considering the first day of the experiment under controlled conditions as the initial Day 0. Observation points were planned every two weeks. Pools of six eggs were gently whipped until the mixture was homogeneous and then kept frozen at –20 °C until analyzed.

The analytical procedure was adapted from the US EPA Method 1613 and validated in-house.[6] Briefly, after fortification with ^{13}C-labelled congeners, the homogenate was allowed to rest for 24 hours at 4 °C and then subjected to extraction. Each test portion (50 g of egg) was mixed thoroughly with a double amount of distilled water by shaking vigorously. After addition of sodium oxalate, the mixture was extracted three times with a liquid-liquid extraction procedure involving methanol, diethyl ether, and *n*-hexane (50 mL of each solvent). The organic extract was purified by eluting through a column of Extrelut™ impregnated with 96% H_2SO_4 followed by an automated cleanup with a Power-Prep™ unit. In this unit, three sequential chromatographic steps take place on columns packed with silica gel, alumina, and graphitic carbon. PCDDs and PCDFs were quantitated by high resolution gas chromatography coupled with high resolution mass spectrometry (VG Autospec) used in the selected ion monitoring mode (SIM).

Congeners half-lives (HLs) were calculated by linear regression of log-transformed concentration data *vs*. time, according to the following canonical equation (Eqn. 1): $\ln(C) = \ln(C_0) - kt$. The regression line fitness was tested with the Fisher test ($P \leq 0.01$). The statistical work was performed by the Excel XP software.

Results and Discussion

The estimates obtained from Eqn. 1 linear regressions on sets of five (lnC, t) data are reported in Table 1. Congener depletion parameters of those congeners whose regression lines showed a correlation coefficient less than 0.878 (critical value with P = 95 %), such as 2,3,7,8-T_4CDD and 2,3,4,7,8-P_5CDF, have been highlighted. In the case of 2,3,7,8-T_4CDF, the regression equation resulted in a negative value of k, a finding already described in other studies that underlines the presence of possible non-fugacity-based factors in the toxicokinetics.[7,8] Nevertheless, it is worth noting that the cumulative contribution of the aforesaid congeners to

total TEQ levels was lower than 5 %. From the analytical profile derived from C_0 values, the PCP origin of the PCDD and PCDF contamination in egg, characterized by the predominant contribution of the H_7- and O_8CDD congeners (1300 and 5000 ng/kg fat, respectively), seems to be evident.[4]

The *F* test carried out on the linear regressions performed on the analytical values of each congener and on total TEQs indicates such model to be suitable to describe PCDD and PCDF depletion ($P_F \leq 0.01$). Only for 1,2,3,7,8-P_5CDD the P_F value falls within $0.01 < P < 0.05$. To this purpose, it is worth noting that previous papers dealing with PCDD and PCDF depletion in eggs reported total TEQ half-lives of approximately 1.5 weeks after one week of exposure to contaminated feed, and of around 7 weeks in a flock reared on naturally contaminated soil.[9,10] In our case, an overall TEQ half-life of 3.8 weeks was estimated, probably influenced by the following concomitant factors: (a) an exposure to wood shavings of more that 8 weeks, probably sufficient to reach a steady state;[11] (b) an exposure occurred in already egg-producing hens, thus minimizing possible body burdens contributions; (c) an egg production rate approximately 25 % higher than that reported by Le Bizec *et al*.,[9] thus allowing a more efficient depletion of such contaminants. It is worth noting that in the case of wood-based contaminated flooring systems, ways of exposure other than feed intake should not be excluded, such as dermal PCDD and PCDF absorption, and inhalation as a consequence of the dustiness and volatilization of such contaminants in 41-°C warmed blood animals.

Further work is in progress to calculate the bioaccumulation factors when such environmental animal welfare-driven contamination occurs and to study the hen physiological status that influences depletion.

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Table 1. Mean C_0, k, and half-life (HL) values and confidence intervals (P = 95%) of PCDD and PCDF congeners and total TEQs estimated from the depletion study. Values rounded off to two figures.

	C_0 (pg/g fat)		k (weeks^{-1})		HLs (weeks)		*R*	*F*	P_F
	Mean	**CI [95%]**	**Mean**	**CI [95%]**	**Mean**	**CI [95%]**			
2,3,7,8-T_4CDD	—	—	—	—	—	—	0.803	5	0.10
1,2,3,7,8-P_5CDD	**4.5**	3.4–6.0	**0.079**	0.14–0.023	**8.7**	5.1–31	0.932	20	0.02
1,2,3,4,7,8-H_6CDD	**17**	13–22	**0.15**	0.21–0.10	**4.5**	3.4–6.7	0.984	94	<0.01
1,2,3,6,7,8-H_6CDD	**110**	86–150	**0.14**	0.20–0.078	**5.1**	3.5–8.9	0.974	55	0.01
1,2,3,7,8,9-H_6CDD	**27**	22–32	**0.21**	0.25–0.17	**3.3**	2.8–4.1	0.995	296	<0.01
1,2,3,4,6,7,8-H_7CDD	**1300**	714–2500	**0.35**	0.48–0.22	**2.0**	1.4–3.1	0.981	75	<0.01
O_8CDD	**5800**	1100–30000	**0.55**	0.88–0.21	**1.3**	0.79–3.3	0.949	27	0.01
2,3,7,8-T_4CDF	—	—	—	—	—	—	0.914	15	0.03
1,2,3,7,8-P_5CDF	**2.1**	1.8–2.5	**0.072**	0.11–0.036	**9.7**	6.5–19	0.965	41	0.01
2,3,4,7,8-P_5CDF	—	—	—	—	—	—	0.713	3	0.18
1,2,3,4,7,8-H_6CDF	**50**	45–55	**0.17**	0.19–0.15	**4.0**	3.6–4.6	0.998	662	<0.01
1,2,3,6,7,8-H_6CDF	**14**	12–17	**0.18**	0.22–0.14	**3.9**	3.2–4.9	0.994	241	<0.01
1,2,3,7,8,9-H_6CDF	**0.83**	0.72–0.96	**0.14**	0.17–0.11	**5.0**	4.1–6.4	0.993	227	<0.01
2,3,4,6,7,8-H_6CDF	**14**	11–17	**0.25**	0.29–0.21	**2.8**	2.4–3.3	0.996	348	<0.01
1,2,3,4,6,7,8-H_7CDF	**180**	85–400	**0.39**	0.55–0.24	**1.8**	1.3–2.9	0.977	63	<0.01
1,2,3,4,7,8,9-H_7CDF	**36**	21–62	**0.33**	0.44–0.22	**2.1**	1.6–3.2	0.984	92	<0.01
O_8CDF	**310**	83–1100	**0.48**	0.75–0.22	**1.4**	0.92–3.2	0.958	34	0.01
Total WHO-TEQs	**46**	41–50	**0.18**	0.21–0.16	**3.8**	3.4–4.2	0.998	789	<0.01