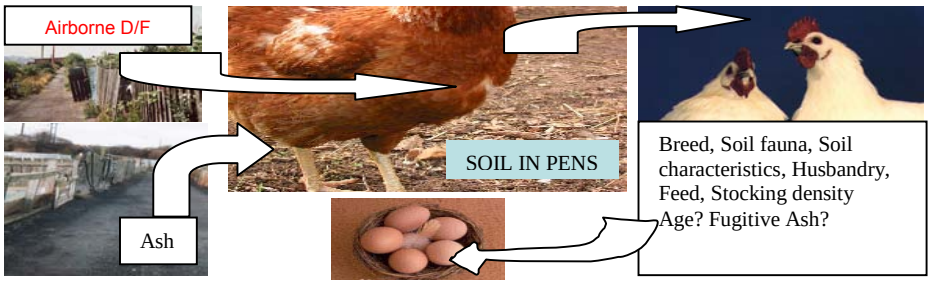
ENVIRONMENTAL NON-FEED CONTRIBUTORS TO PCDD/PCDF IN FREE-RANGE ALLOTMENT POULTRY EGGS: MANY QUESTIONS AND SOME ANSWERS

Vivienne Air[1], Tanja Pless-Mulloli[2], Bernd Schilling[3], Olaf Paepke[3]

[1] Public Health and Environmental Protection Department, Newcastle City Council, Newcastle upon Tyne, England
[2] School of Population and Health Sciences (Epidemiology and Public Health), Newcastle University, Newcastle upon Tyne, England
[3]Ergo Research Laboratory, Hamburg, Germany

Introduction

In 2001 and 2002 we reported studies investigating the link between the use of incinerator ash on allotment paths and in some cases in poultry pens, with levels of polychlorinated dibenzo-p-dioxins (PCDD) and dibenzofurans (furans) in eggs from poultry reared on the sites.[1, 2.] The data suggested that remediation by removal of all known ash from the sites may in fact not be the end of the story. Fig 1.



Falling short of a fully experimental study, this paper provides data from further follow-up observations that sought to explore potential contributors to contamination levels in free-range poultry. This took place in the context of the introduction of EC regulatory maximum permitted levels of PCDD/F in foods, [3ng/kg WHO-TEQ fat for eggs], for which the derogation for free-range and semi-intensive eggs is due to be removed in January 2004. As allotment eggs are generally for home consumption they would be exempt from statutory control. However, it would be difficult to defend exception of allotment eggs from a comparative regulatory standard which is based upon the application of the precautionary principle.

Methods

For the 2002 study, a total of 156 poultry eggs [hen n=139, bantam n=14, duck n=2, goose n=1] were collected from 11 allotments which had received incinerator ash, 1 control site and from an allotment which had not received incinerator ash but was in close proximity to the former incinerator plant. These eggs were either from poultry that had been on site at the time of the use of the incinerator ash and had been retained following remediation, or from poultry newly introduced to sites following remediation. We previously reported results from 55 eggs (19 analyses) from 9 of the ash allotments and control site, which were analysed using HRGC-HRMS (ERGO Laboratories, Hamburg, Germany). Here we report results of a further 31 of the egg

samples from allotments and a control site using CALUX assay based upon the biological response to dioxin like compounds (Lab. 1). Further eggs were used to investigate the role of possible remnants of fly ash in soils, variation in husbandry techniques, and the role of soil invertebrates as on-going, non-food contributors to the environmental burden of PCDD/F in the poultry eggs. Confirmatory analysis by HRGC-HRMS of 4 individual egg homogenates that had undergone CALUX assay was carried out; a further 4 HRGC-HRMS analyses were made of eggs from the site adjacent the former incinerator site (n=12), and of 11 further samples (n=16) from the retained store of composite homogenates from the ash allotments allowing consideration of the influence of flock characteristics. In addition, another 6 of the retained homogenised samples were analysed by Lab 2 using both CALUX assay and also PCDD/F and PCB analysis using HR-GCMS (n=7). Finally for comparative purposes, 6 samples of commercially produced eggs marketed as 'organic free-range', which were purchased anonymously from retail outlets in the City and were submitted as 6 pooled homogenised samples (n=36), to ERGO Laboratories for analysis for PCDD/F by HRGC-HRMS. Analysis was also carried out on soil and commercial feedstuff. Soil from 3 poultry pens on 2 allotments was collected to provide matched data for PCDD/F. A composite sample using 3-5 single samples, each at 0-300mm depth, from each poultry pen was collected and submitted for HRGC-HRMS analysis. Background soil data was also provided from a separate study being conducted in parallel identifying PCDD/F sources in soil in an industrial urban setting. HRGC-HRMS analysis was carried out on the sample of commercial feedstuff used extensively by the poultry holders. Finally, emission data for the incinerator plant was obtained and data collected from poultry holders at the time of initial harvesting of the eggs was reviewed to assess husbandry techniques. Table 1 shows a summary of all samples and analyses.

Table 1 Summary of all samples analysed

Matrix	Data Source	no. of HR-GCMS PCDD/F Tests (no. of eggs)	no. of HR-GCMS PCB Tests (no. of eggs)	no. of CALUX Tests (no. of eggs)
Eggs	2002 study (ERGO, Lab1)	19 (55)		31 (31) new
	Validation study (ERGO)	4 (4) new		
	2003 (ERGO)	11 (16) new		
	Lab2	6(7) new	6(7) new	6(7) new
	Allotment next to plant	4(12) new		
	Commercial	6(36) new		
Soil	Poultry pens	3 new		
	Background soils	82 new		

Data Analysis and Interpretation

HRGC-HRMS results are reported as ng/kg I-TEQ lipid basis in eggs and ng/kg I-TEQ dm in soils and 17 toxic congeners. CALUX results are reported as ng/kg CALUX –TEQ lipid basis for total dioxin-like chemical activity. The contribution of PCDD/F sources to the congener pattern in

HRGC-HRMS samples were examined. Previously, both the incinerator ash and the majority of eggs had shown a characteristic zigzag shaped congener pattern with TCDD<PCDD<HxCDD<HpCDD<OCDD and HxCDF >HpCDF > OCDF. Comparison was made between HRGC-HRMS and CALUX total TEQ's reported by Lab 2. Since CALUX would be responsive to PCBs as well as PCDD/F and other Ah receptor agonists would also contribute to the CALUX result, comparison of these results would help determine whether the CALUX result was predominantly attributable to PCDD/F. Where samples had undergone CALUX assay and HRGC-HRMS for PCDD/F and PCB contribution, direct comparison could be made between total PCDD/F and PCB contribution by both analytical methodologies and quantify actual PCB contribution in the samples. Comparison was made between levels at different allotment sites and also individual poultry holders on each site. Levels in the commercial eggs were compared against allotment eggs, and possible contribution to total levels from feed was considered. Where available, egg data was compared against matched soil data for total level and congener pattern. The plant PCDD/F emission congener pattern was compared against that known for ash and background soil congener patterns used as comparison data.

Results and Discussion

Table 2 shows a summary of the results. In the 6 samples where PCB levels were determined by HR-GCMS, mo-PCBs contributed only a minor extent to the TEQ level in the eggs. This was confirmed by good correlation in 5 of these 6 samples between CALUX assay and HR-GCMS and also in 3 out of the 4 homogenates that underwent HR-GCMS validation analysis following CALUX assay. The overall mean value of all allotment eggs analysed by HRGC-HRMS was 9.3 ng/kg I-TEQ lipid (n=92 min 0.2ng/kg I-TEQ lipid, max 31 ng/kg I-TEQ lipid) and for CALUX assay 11.0 ng/kg CALUX TEQ (n=37 min 0.9ng/kg CALUX TEQ, max 27ng/kg CALUX TEQ). The data series shows that whilst some poultry holders show consistently high or moderate levels of contamination others show great variation in their levels. The PCDD/F levels found in the commercial feedstuff showed low levels of contamination (mean 0.5 ng/kg I-TEQ lipid, min 0.1ng/kg I-TEQlipid, max 1.6ng/kg I-TEQ lipid) and no indication of a congener pattern resembling the one found in the majority of the egg samples. Altogether, the data suggest ***that time spent outdoors and picking*** which in turn may depend upon breed, environmental conditions, shelter etc, nature of material hens pick on (soil, vegetation, deep litter), extent of soil invertebrates and stocking density may be important considerations. Individual husbandry practises on the allotments vary and such practises will influence exposure to environmental factors. Whilst contamination levels found in soil can be a relevant contributor to PCDD/F levels in eggs, other environmental factors appear to contribute. Our soil-egg matched data showed variable PCDD/F levels in 2 poultry pens on the same site (40.5ng/kg I-TEQdm and 147.6ng/kg I-TEQdm), yet levels in eggs from the two pens to be similar (15.7ng/kg I-TEQ lipid, 18ng/kg I-TEQ lipid and 21ng/kg I-TEQ lipid (bantam)). A second site shows a soil pen level of 98.7ng/kg I-TEQdm and a matched egg level of 31ng/kg I-TEQlipid. The low PCDD/F levels in the commercially produced free range organic eggs sampled would appear to confirm that allotment birds may have more access to the environment than some commercial flocks and therefore more exposure to any environmental contamination, unlike those produced in the Netherlands reported by Traag et al.[3] From information on the age of the birds supplied by the poultry holders, no association was apparent between the age of the allotment poultry and PCDD/F levels on eggs. The incinerator pattern, previously described was still evident in many eggs and in the matched soils. The

background soil data collected in our parallel study and incinerator emission data indicated strong correlation between this pattern and the plant.

Conclusions

The results of this study suggest that eggs are efficient indicators of the *absence* of environmental contamination. However, the relationship between the environmental level and the level in the egg appear to be not directly correlated. We ask the question 'why do hens exposed to lower levels of contamination produce eggs with similar values as those exposed to higher concentrations?' Further research is required to identify the role of additional environmental contributing factors in order to identify the husbandry necessary to allow poultry continued access to the environment, whilst complying with the EC maximum permitted level.

Table 2. PCDD/F concentration in eggs (ng/kg I-TEQ lipid, ng/kg CALUX-TEQ lipid) and soils (ng/kg I-TEQ dm) by allotment and individual flocks

Allotment / Poultry Holder (*soil PCDD/F*)	HR-GCMS PCDD/F 2002/2003	CALUX 2002/2003	HR-GCMS PCBs 2003	Allotment / Poultry Holder (soil PCDD/F)	HR-GCMS 2003 (2002)	CALUX 2002/2003	HR-GCMS PCBs 2003
1 A	2.1			9 A	2.78	4, 8.2(D)	
B	13			10 A	26		
2 A	12.4, 13.4	14.8, 24.3, 27	1.6	B	7.8		
3 A *(147.6)*	18, 21(B)			C	6.7		
B *(40.5)*	15.7	4.8, 17.4		D	1.43	1.44	
4 A *(98.7)*	31(B)			E	12.6	7.9	
5 A	5.4			F	14.1, 16.0	17.48, 22	3.2
5 B	3.51, 2.43	3.6, 21(B)		G	15.99	12.04	
6 A	6.6			H		3.4	
B		5.7		11 A	3.3		
C	6.44	7.35		B		3.1	
D	6.04	4.98, 8,4		C	10.8, 13.0	16, 27	1.4
E	8.5	10.6, 20	2.2	D		3.4	
F		8.1		E	7.17	9.9	
7 A	11, 1.7			F		5.3	
B	6.7			G	1.84	0.9	
C	3.1, 8.7	3.9, 5.5,16	1.2	H		6.07	
8 A	17, 5.1			I		3.51	
B	5.2(B)			Adjac.plant A	11, 1.0(B)		
C	0.2			B	2.7		
D	13.1	12.5, 23		C	10.0(G)		

References

1. Pless-Mulloli T, Edwards R, Paepke O, Schilling B, Full technical report. PCDD/PCDF and heavy metals in soil and egg samples taken from Newcastle allotments: assessment of the role of the Byker incinerator. Newcastle upon Tyne: University of Newcastle, 2001b
2. Air V, Pless-Mulloli T, Schilling B, Paepke O, Foster K. Follow up assessment of PCDD/PCDF in eggs from allotments in Newcastle upon Tyne England. Organohalogen Compounds 2002:57
3. Traag W, Portier L, Bovee T, van der Weg G, Onstenk C, Elghouch N,et al. Residues of dioxins and coplanar PCBs in eggs of free range chickens. Organohalogen Compounds 2002:57