ECOTOXICOLOGY

STUDY OF UPTAKE AND ELIMINATION OF DIOXINS, FURANS AND COPLANAR PCBs IN HENS

Catherine Pirard and Edwin De Pauw

Mass Spectrometry Laboratory, University of Liege, B6c Sart-Tilman, B-4000 Liege, Belgium. e-mail: Catherine.Pirard@ulg.ac.be

Introduction

A preliminary toxicokinetic study was conducted on young chicken, growing up in trading fashion. The initial aim was to investigate uptake, distribution between tissues, carryover and depuration rate of chlorinated compounds, especially dioxins, furans and PCBs. Only partial results will be presented here. Uptake and elimination via faeces of all polychlorinated dibenzodioxins and furans, included 2,3,7,8-substitued isomers, and non-*ortho* polychlorobiphenyls will be discuss.

Methods and Materials

Experimental design

A dozen twenty weeks old hens, purchased in a farm in a rural location, were housed individually in stainless steel cages allowing the collection of eggs and faeces separately. Four of them were fed with clean diet while the others were fed with a 30ng TEQ/kg voluntary contaminated feed. The contamination was carried out by mixing commercial feed with a steam cleaning oil from a Belgian sintering belt. All isomers of PCDDs and PCDFs were found in this oil and the pattern, characteristic of sintering process, was predominated by furans.

Feed and water were provided ad libitum. Urine and faeces were collected weekly at the same time, and eggs were picked up daily. After 12 weeks, two of the four "reference" hens and four of the eight "contaminated" animals were slaughtered and some organs were removed for analysis. Remaining animals were fed with clean diet for the second part of the experiment.

Analytical method

Faece samples were mixed with sodium sulphate and extracted using accelerated solvent extraction (ASE™ 200, Dionex, Sunnyvale, CA, USA) in toluene. Isotopic dilution labelled standards were added before extraction, directly on the sample in the extraction cell. Extract was evaporated and rediluted in hexane before acidic treatment. Further clean-up was carried out using the Power-Prep™ (FMS Inc., Waltham, MA, USA) system with multilayer silica, basic alumina and PX-21 carbon columns[1]. Dioxin fractions were analysed on a Agilent (Palo Alto, CA, USA) 6890 Series gas chromatograph coupled to an Autospec Ultima (Micromass, Manchester, UK) high resolution mass spectrometer. The column was a RTX-5SIL-MS (30m x 0.25mm I.D., 0.25μm film thickness) capillary column (Restek, Evry, France), excepted for analyses of all congeners for which column was a SP2331 (60m x 0.25mm I.D., 0.20μm film thickness) capillary column (Supelco, Bellefonte, USA) The mass spectrometer operated in the electron impact ionisation mode using selected ion monitoring (SIM) at a minimum resolution of 10.000 (10% valley). In addition to daily sensitivity and relative response factor (RRF) checks, the mean RRF were regularly re-evaluated for each congener.

Results and Discussion

Absorption of PCDD/Fs from the gastrointestinal tract has been studied in several species of

ECOTOXICOLOGY

animals[2,3,4,5,6,7,8] and even in human[9,10]. It's well known that absorption of these kind of compounds is vehicle-dependent and congener-specific, but seems to be interspecies independent[11]. Whereas McLachlan[3] found for lactating cow that absorption of persistent dioxins and furans is lower than the absorption of the non persistent ones in the same homologue group, analysis of all congeners of PCDD/Fs in faeces of chicken has demonstrated that there is no preferential absorption for 2,3,7,8-substitued compounds, as it can be seen on figure 1. This graph depicts the percentage for some of PCDFs found in faeces at the time of the sixth week with regard to the weekly estimated level ingested by hens (all isomers are not shown for more readability). A black star is distinguishing the 2,3,7,8-substitued congeners. Same results were found for dioxins (not shown).
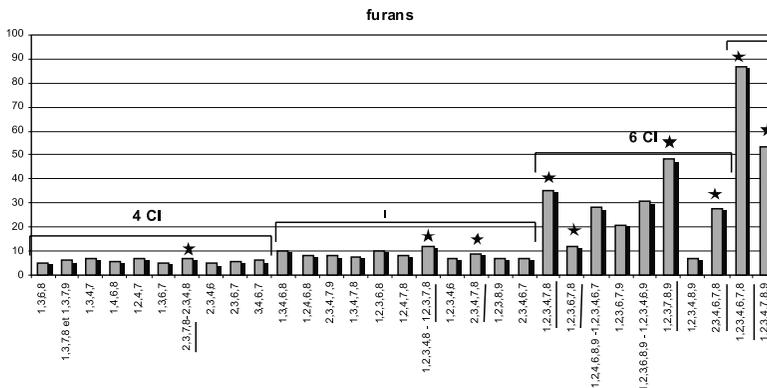
Figure 1. Percentage of PCDFs found in faeces after 6 weeks of feeding treatment.
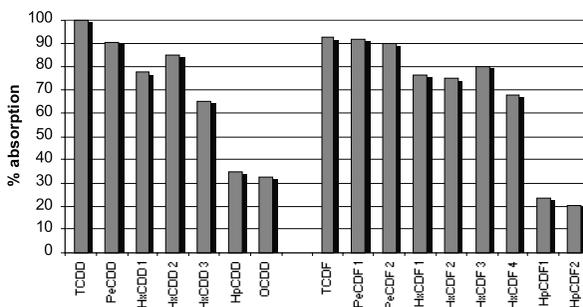

Figure 2. Percentage absorption of 2,3,7,8-substitued dioxins and furans

On the other hand, we found also found [2] that the absorption of congeners decreases when number of chlorine increases. It was expected knowing that passage of hydrophobic compounds across the intestinal wall is predominantly limited by molecular size and water solubility, and solubility decreases with increasing chlorination degree[2,11]. On the figure 2 which shows the % absorption of 2,3,7,8-substitued dioxins and furans, we can see that this trend is more pronounced for the hepta- and octachlorinated congeners. The pattern found is not consistent with the study of Stephens on White Leghorn chickens[12] in which absorption of tetrachlorinated is lower than pentachlorinated congeners, the other homolgues following a more classical pattern. This difference may be due to the vehicle

which is different in both studies. On the present, feed was contaminated via oil while Stephens used fly ashes as vehicle.

Correlation between n-octanol/water partition coefficient and bioconcentration factor (BCF) for persistent organic chemicals has been extensively studied in aquatic organisms[13,14,15,16,17,18]. Although bioconcentration process in fish is the result of a partitioning between organism's lipid and ambient water across the gill membranes, a non linear relationship is usually found for compounds which have log Kow >6. Dioxins and furans belong to this class of superhydrophobic chemicals. Conversely lung-breathing animals are exposed to these contaminants through the food chain. As previously mentioned, their absorption from the gastrointestinal tract depends on their transport from an aqueous environment through lipophilic cells of the intestinal wall. Therefore n-octanol/water partition coefficient can be an appropriate physicochemical properties to predict bioconcentration process. Graph on figure 3 presents the gastrointestinal absorption percentage versus log Kow for all isomers. It shows clearly a non-linear relationship. Although distinct relation has been observed for accumulation of dioxins and furans in fish[17] with regard to n-octanol/water partition coefficient, graph 3 doesn't show any significant difference excepted may be for hexachlorinated furans which are surrounded. For these ones, the absorption is a bit less than their dioxin's homologue.
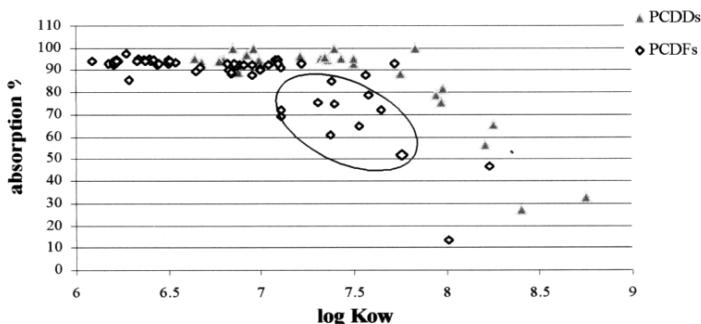
Figure 3. Gastrointestinal absorption versus Log Kow of PCDDs and PCDFs

Uptake of non ortho PCBs has also been studied. Absorption of these coplanar congeners in hens is nearly complete, with values up to 96 % and unlike dioxins, with no significant difference with regard to degree of chlorination as it was foreseen by Matthews[18].

Figure 4 shows level in faeces of the sum of the PCDDs and PCDFs 2,3,7,8-chlorosubstitued in wet weight basis versus time. There is an apparent steady-state from the fifth week. All these congeners follow the same kind of kinetic. Coplanar PCBs on figure 5 (where PCB 77 isn't included because concentrations were often lower than LOQ) are excreted in a different way. While PCB 81 and 169 seem to reach their steady-state after four or five weeks, concentration of PCB 126 continues to increase until the tenth one.

Conclusions

Understanding of gastrointestinal absorption is the first step in order to understand and predict halogenated environmental organic pollutant's behaviour in the food chain. Absorption of dioxins is mainly governed by number of chlorine and therefore by their molecular size and water solubility, although there was no apparent linear correlation between absorption and octanol/water partition coefficient. Dioxins and c-PCBs are excreted in a different way despite their structural similarities. All

ECOTOXICOLOGY
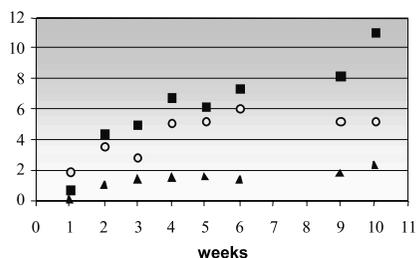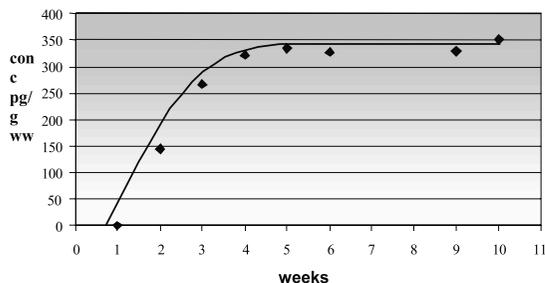
Figure 4. Concentration of the sum of PCDD/Fs on wet weight basis in faeces

Figure 5. Concentration of + PCB 126,tPCB 169 and ° PCB 81 on pg/g ww in faeces

these data have to be correlated with distribution and level in different tissues and the carryover of these contaminants through eggs.

References

1. Pirard C, Focant J-F and De Pauw E. (2002) Anal Bioanal Chem 372, 373–381
2. McLachlan M. and Richter W.(1998) J Agric Food Chem 46, 1166-1172
3. McLachlan M., Thoma H., Reissinger M; and Hutzinger O. (1990) Chemosphere 20, 1013-1020
4. Pipper W.N., Rose J.Q. and Gehring P.J. (1973) Advan Chem Ser 120, 85-91
5. Rose J.Q., Ramsey J.C., Wentzler T.H., Hummel R.A. and Gehring P.J.(1976) Toxicol Appl Pharmacol 36, 209-226
6. Fries G.F. and Marrow G.S.(1975) J Agric Food Chem 23, 265-269
7. Stephens R.D., Petreas M.X. and Hayward D.G. (1995) Sci Total Enviro 175, 253-273
8. Van den berg M., de Vroom E., van Greevenbroek M. and Olie K. (1985), Chemosphere 14, 865-869
9. Schrey P. Wittsiepe J., Mackrodt P. and Selenka F. (1998) Chemosphere 37, 1825-1831
10. Rohde S., Moser G.A., Päpke O., McLachlan M.S. (1999) Chemosphere 38, 3397-3410
11. Van den berg M., De Jongh J., Poiger H. and Olson J.R. (1994) Critical Review in Toxicology 24, 1-74
12. Stephens R.D., Petreas M.X., Hayward D.G. (1995) Science of the Total Environment 175, 253-273
13. Hawker D.W. and Connell D.W. (1989) Environ Sci Technol 23, 961-965
14. Chessels M.,Hawker D.W. and Connell D.W (1992) Ecotoxicology and Environmental Safety 23, 260-273
15. Devillers J., Bintein S. and Domine D.(1996) Chemosphere 33, 1047-1065
16. Govers H.A.J. and Krop H.B. (1998) Chemosphere 37, 2139-2152
17. Govers H.A.J, Loonen H. and Parsons J.R. (1996) SAT-R and QSAR in Environmental Research 5, 63-78
18. Mackay D. and Hughes A.I. (1984) Environ Sci Technol 18, 439-444
19. Matthews H.B. and Dedrick R.L. (1984) Ann. Rev Pharmacol. Toxicol. 24, 85-103