# DETERMINATION OF PCBs AND DDTs IN SMALL SIZE CHAMELEON EGGS (Chamaeleo chamaeleon), USING A MINIATURISED SAMPLE PREPARATION METHOD FOLLOWED BY GC-MICRO-ECD.

Belen Gomara[1], Maria Jose Gonzalez[1], Juan Jose Ramos[1], Lurdes Ramos[1]

[1]Institute of Organic Chemistry, CSIC. Madrid, Spain

## Introduction

**Organochlorine compounds such as polychlorinated biphenyls (PCBs) and dichlorodiphenyl ethanes (DDTs) are well known as toxic and persistent contaminants that accumulate in the trophic levels of food chains, apparently associated with the habitat and dietary habits. In recent years there has been an increasing concern for these chemicals mainly due to their estrogenic properties. In many oviparous species, PCB pollution can produce severe impacts on eggs leading to embryo death or abnormal embryonic development. It is reported that pollutants can severely affect reptiles and could be contributing to their global decline (1,2)**

The analytical determination of these toxic compounds using conventional methodologies (3,4), involves tedious multisteps cleanup and time consuming methodologies, which usually require high amount of sample and volumes of solvents. Besides, these successive treatments of the samples are often carried out off-line, and much manual handling of the extracts is usually required (5). In the case of small amount of samples, such in the case of chameleon's eggs (<1 g of fresh weight), the development of new methods, coupling at-line or on-line the different steps required for sample preparation is a valuable analytical alternative to the quote above procedures. Several examples of on-line clean-up procedures have been described in the literature for PCBs analysis in biological samples, but they do not included the extraction step. Regarding this latter aspect, miniaturisation of extraction step and, if at all possible, no additional clean-up requirements could be considered as key factors when developing complete coupled methodologies. Recently, we have developed a new miniaturised method for fast determination of PCBs in solid fatty foodstuffs, which allowed the exhaustive extraction of the analytes from the sample and the clean up of the extract in a single step with a minimum consumption of solvents and sorbents (6).

In this paper, that miniaturised method has been tested for the determination of PCBs and DDTs in small size environmental samples. The method was applied to the determination of the levels of 20 PCBs and 3 DDTs in chameleon eggs (*Chamaeleo chamaeleon)* from the South Western Spain. Chameleon is a protected species in Spain from 1973 (R.D. 2573/1973 October 5$^{th}$ ), which usually

lives near the coast in highly urbanized areas. Data will be compared with that found in that particular species from the same area in 1997.

## *Material and Methods*

Sample collection:

36 eggs were collected from various chameleon nests at 3 different locations in Cadiz (South-Western of Spain): four nests were located near Rota (point 1), three near Puerto de Santa Maria (point 2), and two in Puerto Real (point 3), in 2001. Four eggs from each nest were pooled and analysed together.

Chemicals:

All solvents used were pestipur quality (SDS, Peypin, France), except hexane that was of Unisolv quality (Merck, Darmstadt, Germany). The following organochlorines were analysed: *p,p'*-DDT, *p,p'*-DDE, *p,p'*-TDE; and PCBs # 28, 52 , 95, 101, 123, 149, 118, 114, 153, 132, 105, 138, 183, 167, 156, 157, 180, 170, 189 and 194 (Ehrenstorfer, Augsburg, Germany). 1,2,3,4, tetrachloronaftalene (TCN) and PCB 209 were used as injection standards for PCBs and DDTs quantification.

Sample preparation:

A miniaturised one-step method previously validated for the analysis of PCBs in fatty foodstuffs (6) is now tested for the determination of PCBs and DDTs in chameleon eggs. The original method, involving 0.1 g of sample, has been adapted to the amount of sample available for each egg nests. Briefly, the methodology involved the dispersion of the freeze-dried egg samples (between 0.4 and 1.0 g), on similar amount of $Na_2SO_4$ and silica modified with 44 % (w/w) sulphuric acid ($SiO_2$-$H_2SO_4$). After blending and homogenized the sample, the mixture was packed in a glass disposable extraction column on top of 1.5 times of sample amount of neutral silica and 3 times of sample amount of $SiO_2$-$H_2SO_4$. A maximum of 50 ml of hexane was used as extracted solvent. After two 10 min static extractions, some fresh solvent was eluted through the column to ensure proper purging of the sample and clean-up of the sorbent. Procedure blanks were prepared following the same as for eggs but without sample. No background interferences were detected by the analytical instrumentation used for the final analysis determination.

Analytical procedure

The analysis was performed by GC-micro-ECD (Agilent 6890 Series II, PA, California, USA) as was described elsewhere (7). Samples were injected in the hot splitless mode (1 µl, 270 ºC, splitless time 1.0 min) in a capillary DB-5 column (J&W Scientific, USA; 60 m, 0.25 mm i.d., 0.25 µm film thickness). The column temperature was programmed from 80ºC (2 min) to 185ºC (3 min) at a rate of 30 ºC/min, then to 230ºC (10 min) at 1.5 ºC /min and then to 270ºC (10 min) at 5 ºC/min. Nitrogen was used as carrier gas (constant flow, 1.5 ml/min) and as make up gas 30 ml/min. The detector temperature was set at 300 ºC. The relative standard deviations (RSDs, n =3) were always

lower than 10 %. The detection limits (LODs) were between 0.009 and 0.1 ng/g freeze-dried sample.

## Results and discussions

The practicability of a simple and fast method providing a quantitative extraction of the most environmentally relevant and toxic PCBs from fatty foodstuffs with simultaneous clean-up of the extracts (6) for chameleon egg samples of different sizes has been demonstrated. The performance of the analytical method was found to be the same irrespective of the initial sample size in the investigated range of 0.4-1.0 g and no overpressure was observed under these experimental conditions.

The sum of the 20 PCB congeners analysed (on a freeze-dried weight basis, f.d.w.) did not show significant variations among the sampling sites 1 and 2. Sampling area 3, which highest industry and inhabitants in their nearby, exhibited the highest PCBs values and showed significant differences with those found in the other two sites (1 and 2). They ranged between 176 and 341 ng/g f.d.w (Table 1). The highest value was found in sampling site 3 and the lowest in sampling site 1. These values were higher than those found in eggs from the same species in the same area in 1997, which ranged from 16.2 to 185 ng/g f.d.w. (8) The increase of PCB concentrations in chameleon's eggs could be due to the increase of industrial and touristy activities during the last years in the study area.

PCBs # 28, 52, 95 and 101 were the most abundant among PCB congeners investigated, accounting for 15 to 26 % of the total PCBs investigated, followed by PCBs 153 (7-11 %), 138 (3-6 %) and 180 (1-4 %), except for eggs collected in sampling site 3. In this location PCBs 153 and 180 were the most abundant (Table 1).

Regarding DDTs, the concentration levels were similar in all sampling sites, ranging from 3.62 to 12.3 ng/g d.f.w. (Table 1). These values were similar to those found in chameleon's eggs collected in 1997 in the same area, in the range 0.68 to 9.38 ng/g f.d.w. (8). *p,p'*-DDT and it main metabolite *p,p'*-DDE were detected in all eggs samples analysed while the metabolite *p,p'*-TDE was only detected in one egg sample from point 2. The *p,p'*-DDE levels indicated that *p,p'*-DDT was used in the studied area after being banned in Spain (in the 70's). However the ratio *p,p'*-DDE/*p,p'*-DDT (> 1) proved that *p,p'*-DDT has not recently used.

The practicability of a fast MSPD-based procedure for the determination of PCBs in small size egg samples has been demonstrated. The method allowed complete sample preparation with a maximum consumption of 50 ml of hexane and 6 g of sorbent in one step. Complete sample preparation can be accomplished in about 1 hour and up to 9 samples can be simultaneously processed.

The application of the proposed methodology to chameleon's eggs from southern in Spain showed that DDTs levels were similar and PCBs levels were higher than those obtained in 1997, probably due to the increase of industries and inhabitants of the studied area.

**Acknowledgement:** The authors would like to thank Junta de Andalucía and CSIC for financial support, and EBD (A. Marco and C. Diaz-Paniagua) for providing the egg samples. B. Gómara thanks CSIC for a predoctoral grant.

**References:**

1. Gibbons J.W., Scott D.E., Ryan T.J., Buhlmann K.A., Tuberville T.D., Metts B.S., Greene J.L., Mills T., Leiden Y., Poppy, S. and Winne C.T. (2000) Bioscience 50, 653.
2. Kleinow K., Baker J., Nichols J., Gobas F., Parkerton T., Muir D., Monteverdi G., and Mastrodone P. (1999) Exposures, uptake, and disposition of chemicals in reproductive and developmental stages of selected oviparous vertebrate. In: Reproductive and developmental effects of contaminants in oviparous vertebrates (Di Giulio, R.T. and Tillit, T. eds), SETAC Press, Pensacola FL, 9-112.
3. Liem A.K.D. (1999) Trends Anal. Chem. 18, 429.
4.Hess P., de Boer J., Cofino W.P., Leonards P.E.G., and Wells D.E. (1995) J. Chromatogr. 703, 417.
5. Lou X., Jassen H.G. and Cramers C.A. (1977) Anal. Chem. 69, 1598.
6. Ramos J.J., Gonzalez M.J. and Ramos L. (2003) Dioxin 2003 Boston, MA. Organohalogen Compounds, 60, 61.
7.Gómara B., Ramos L. and González M.J.(2002) J. Chromatogr B 766, 279.
8.Diaz –Paniagua C., Marco A., Fernández M., and Hernández L.M. (2002) Fresenius Environ,. Bull. 11, 631.
9.Ballschmitter K., and Zell M., (1980) Fresenius Z. Anal. Chem. 302, 20.

Table 1. PCBs and DDTs range concentrations (ng/g on freeze-dried weight) on eggs of chameleon clutches from Cadiz (South-Western of Spain) collected in 2001.

| Congeners (9) | Sampling point 1 | Sampling point 2 | Sampling point 3 | 1997 Sampling campaign (8) |
|---|---|---|---|---|
| 28 | 30.0-34.6 | 34.4-31.9 | 34.4-36.0 | |
| 52 | 45.8-50.0 | 43.8-79.5 | 48.5-79.5 | |
| 95 | 32.8-35.2 | 31.6-47.0 | 34.8-61.1 | |
| 101 | 29.8-33.2 | 28.6-45.4 | 31.9-61.6 | |
| 123+149 | 4.76-5.59 | 5.02-7.23 | 5.15-11.1 | |
| 118 | 3.41-3.98 | 4.09-9.63 | 6.11-8.70 | |
| 114 | 0.03-0.18 | 0.12-0.28 | 0.21 | |
| 153 | 13.5-15.7 | 14.8-22.7 | 36.4-41 | |
| 132 | 1.24-1.45 | 1.07-1 .78 | 0.91-3 | |
| 105 | 0.85-1.10 | 1.37-1.54 | 1.05-3.58 | |
| 138 | 6.41-7.84 | 5.93-9.22 | 12.4-18.6 | |
| 183 | 0.35-0.46 | 0.30-0.59 | 0.89-0.90 | |
| 167 | 0.01-0.05 | ND-0.18 | 0.11-0.39 | |
| 156 | 0.13-0.16 | 0.14-0.28 | 0.53-0.59 | |
| 157 | ND | ND-0.03 | ND-0.10 | |
| 180 | 2.49-6.21 | 3.09-7.50 | 9.46-20.9 | |
| 170 | 1.42-2.12 | 1.62-3.04 | 5.42-5.57 | |
| 189 | 0.15-0.24 | 0.21-0.29 | 0.45-0.51 | |
| 194 | 1.07-2.82 | 1.50-2.82 | 3.77-6.26 | |
| Total PCBs | 176-184 | 183-238 | 251-341 | 16.2-185 |
| DDE | 3.09-3.65 | 3.81-3.83 | 3.45-9.74 | |
| TDE | ND | ND-0.05 | ND | |
| DDT | 0.99-1.30 | 1.04-1.41 | 1.01-2.53 | |
| Total DDTs | 4.08-4.95 | 3.62-5.25 | 4.46-12.3 | 0.68-9.38 |