



The influence of lipid and lifestyle factors upon correlations between highly prevalent organochlorine compounds in patients with exocrine pancreatic cancer

Miquel Porta^{a,b,*}, Joan O. Grimalt^c, Manuel Jarod^{a,d}, Laura Ruiz^a, Esther Marco^c, Tomàs López^a, Núria Malats^a, Elisa Puigdomènech^a, Ekhine Zumeta^a for the PANKRAS II Study Group¹

^a *Institut Municipal d'Investigació Mèdica, Barcelona, and CIBER en Epidemiologia y Salud Pública (CIBERESP), Spain*

^b *School of Medicine, Universitat Autònoma de Barcelona, Spain*

^c *Department of Environmental Chemistry, Institute of Chemical and Environmental Research (IQAB-CSIC), Spain*

^d *Universitat Rovira i Virgili, Tarragona-Reus, Spain*

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Abstract

We aimed to analyse the influence of cholesterol and triglycerides, and of tobacco, coffee and alcohol consumption upon correlations between serum concentrations of organochlorine compounds (OCs) in patients with exocrine pancreatic cancer (EPC). Incident cases of EPC diagnosed in eastern Spain were prospectively identified ($N=144$). OCs were analysed by high-resolution gas chromatography with electron-capture detection. A strong correlation was observed between hexachlorobenzene (HCB) and β -hexachlorocyclohexane (β -HCH) (Spearman's $\rho=0.758$). β -HCH showed $\rho>0.4$ with p,p' -DDT, p,p' -DDE, PCB138 and PCB153 (all $p<0.001$). Some correlations among compounds were slightly affected by tobacco, coffee or alcohol consumption. We observed a striking diversity of correlation patterns by strata of cholesterol and triglycerides. Most correlations were higher in the lowest category of triglycerides than in the lowest category of cholesterol. Most coefficients above 0.7 were seen in the lowest category of triglycerides (e.g., OC pairs p,p' -DDT and HCB, p,p' -DDT and β -HCH, p,p' -DDE and β -HCH, or HCB and β -HCH). Correlations among OCs may be stronger when concentrations of triglycerides are low than when they are high. This is compatible with a dilution in the early phases of cancer and with a concentration effect as triglycerides become lower in the advanced phases of the disease.

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1. Introduction

Organochlorine compounds (OCs) such as p,p' -DDT, p,p' -DDE, polychlorinated biphenyls, hexachlorobenzene and the hexachlorocyclohexanes are of concern because of their persistence, prevalence in humans and potential relationship with increased risks of severe diseases, including cancer (Porta et al., 1999a; Institute of Medicine, 2003; Department of Health and Human Services et al., 2005; Porta and Zumeta, 2002; De Roos et al., 2005; Howsam et al., 2004; Wolff et al., 2005; Porta, 2001; Olea and Fernandez, 2007; Porta, 2006). Some OCs may be involved in the etiopathogenesis of pancreatic cancer (Porta et al., 1999a, 2003; Porta, 2001; Hoppin et al., 2000; Fryzek

Abbreviations: DDT, dichlorodiphenyltrichloroethane; DDE, dichlorodiphenyldichloroethane; PCBs, polychlorinated biphenyls; HCB, hexachlorobenzene; HCH, hexachlorocyclohexane; ρ , Spearman's coefficient of correlation; r , Pearson's correlation coefficient.

* Corresponding author. Institut Municipal d'Investigació Mèdica (IMIM), Universitat Autònoma de Barcelona, Carrer del Dr. Aiguader 88, E-08003 Barcelona, Spain. Tel.: +34 93 316 0700, +34 93 316 0790; fax: +34 93 316 04 10.

E-mail address: mporta@imim.es (M. Porta).

¹ Members of the Multicentre Prospective Study on the Role of the *K-ras* and other Genetic Alterations in the Diagnosis, Prognosis and Etiology of Pancreatic and Biliary Diseases (PANKRAS II) Study Group are mentioned in previous publications (Porta et al., 1999a).

et al., 1997; Garabrant et al., 1993; Li et al., 2005), although findings are tentative and further studies are underway (Fryzek et al., 1997, 2005).

Reports on correlations between OCs in the environment are abundant; however, as a result of their physico-chemical interactions and metabolism in the human body, relationships between compounds may differ in humans from those observed in air, water, soil or food. In humans relationships among OCs are only partly understood (DeVoto et al., 1997; Glynn et al., 2001, 2000; Moysich et al., 1999; Gladen et al., 1999, 2003; Masuda et al., 2005), and limitations of the available evidence are particularly clear in highly lethal diseases as pancreas cancer (Porta, 2001; Hoppin et al., 2000).

Studies on correlation patterns among concentrations of specific compounds are important, among other reasons, because they help identify the actual OC mixtures that bioaccumulate in humans following environmental or metabolic alteration; some bioaccumulated mixtures appear to be more toxic than commercial mixtures (Cogliano, 1998). Correlation analyses may also help identify common sources of exposure. Even when sources differ, correlation analyses can improve understanding of collinearity, a process that may bias risk estimates (DeVoto et al., 1997; Gladen et al., 1999). Characterizing patterns of correlations among OCs is hence important to interpret toxicological and epidemiological studies on the effects of individual compounds and mixtures of compounds (DeVoto et al., 1997; Glynn et al., 2001, 2000; Moysich et al., 1999; Gladen et al., 1999, 2003; Masuda et al., 2005; Cogliano, 1998).

The aims of this study were: first, to analyse correlations between serum concentrations of highly prevalent OC in patients with exocrine pancreatic cancer; second, to analyse the influence upon OC correlations of age, sex, tobacco, coffee and alcohol consumption; and third, to analyse how patterns of OC correlations vary by serum concentrations of cholesterol and triglycerides.

2. Subjects and methods

Methods of the PANKRAS II study have been described in detail (Porta et al., 1999a,b, 2000, 2002a,b, 2005; Alguacil et al., 2002; Crous-Bou et al., 2007; Morales et al., 2007; Mendez et al., 2006). Briefly, subject recruitment took place between 1992 and 1995 at five general hospitals in the eastern Mediterranean part of Spain, where 185 incident cases of exocrine pancreatic cancer (EPC) were prospectively identified. A structured form was used to collect clinicopathological information from medical records, including details on diagnostic procedures, laboratory results and follow-up. Over 88% of patients were interviewed face-to-face by trained monitors during hospital stay, close to the time of diagnosis. All diagnoses were reviewed by a panel of experts and by the study reference pathologists.

2.1. Analysis of serum concentrations of organochlorine compounds

2.1.1. Materials

Detailed accounts of laboratory methods have also been previously published (Porta et al., 1999a; To-Figueras et al., 1997; Otero et al., 1997; Chaler et al., 1998; Sunyer et al., 2005; Carrizo et al., 2006). In summary, 15 mL screw-capped Pyrex centrifuge tubes capped with Teflon septa (ref. Pyrex SVL 611/54; Afora, Barcelona, Catalonia, Spain) were used to keep and digest the samples. These tubes, Pasteur pipettes and vials for the chromatographic analysis were heated at 400 °C for 12 h before use. After analysis the Pyrex tubes and the Pasteur pipettes were disinfected by immersion in a commercial lye solution for 24 h. Then the tubes were re-

cycled by ultrasonic cleaning in Extran AP 13 (Merck, Darmstadt, Germany) solution for 10 min and rinsed with Milli-Q water, acetone and *n*-hexane. Residue analysis *n*-hexane (ref. 1.04371), *iso*-octane (ref. 1.15440), concentrated sulphuric acid 95–97% (ref.1.00731) and acetone (ref. 1.00012) were from Merck. The potassium hydroxide pellets (Panreac, Barcelona, Catalonia, Spain) were cleaned by sonication in *n*-hexane for 10 min. The procedure was repeated three times replacing the *n*-hexane in each case. They were then dissolved in Milli-Q water to obtain a 5 M solution. This solution was re-cleaned by three successive liquid–liquid extraction steps with *n*-hexane. The purity of the solvents and reagents was checked by analysis of 2 mL of Milli-Q water following the same procedures and the dilution factors as for the samples. *p,p'*-DDE and 1,2,4,5-tetrabromobenzene (TBB) were from Aldrich-Chemie (Steinheim, Germany). All PCB congeners were from Promochem (Wesel, Germany). β -HCH, *p,p'*-DDT, pentachlorobenzene (PeCB) and hexachlorobenzene (HCB) were from Dr. Ehrenstorfer (Augsburg, Germany). All standard mixtures were prepared in *iso*-octane.

2.1.2. Extraction and clean-up

50 μ L of the surrogate solution (0.36 μ g/L of TBB and 0.52 μ g/L of PCB 209) was added to 2 mL aliquots of serum in the same Pyrex centrifuge tubes where the samples were stored. The mixture was vortex stirred for 30 s at 2000 rpm. Acid digestion of the mixture was performed by addition of 3 mL of *n*-hexane and 2 mL of concentrated sulphuric acid (drop by drop). Then, the tube was locked and again vortex stirred for 30 s. The tube was let to cool at room temperature and then five drops of acetone were added to help phase separation. The supernatant *n*-hexane phase was removed and the remaining sulphuric acid solution was re-extracted two more times with 2 mL of *n*-hexane (acetone was added again and the mixture stirred like in the first step). All extracts were collected together and the resulting 7 mL of *n*-hexane were purified by vortex stirring (2000 rpm) with 2 mL of sulphuric acid for 3 min. Then, the *n*-hexane phase was concentrated to nearby dryness under a nitrogen stream and rediluted to 500 μ L with *iso*-octane.

2.1.3. Instrumental analysis

GC analyses were performed with a Hewlett-Packard model 5890A provided with an ECD and a 30 m \times 0.25 mm i.d. DB-5 column (J & W Scientific, Folsom, CA, USA; film thickness 0.25 μ m). A fused silica precolumn of 2 m \times 0.32 mm i.d. was used and renewed every 30 samples. The DB-5 column was heated from 80 °C (holding time 2 min) to 300 °C at 6 °C/min, keeping the final temperature for 10 min. The injector and detector temperatures were 270 °C and 310 °C, respectively. Injection was performed in split/splitless mode (hot needle technique), keeping the split valve closed for 35 s. Helium (50 cm/s) and nitrogen (60 mL/min) were the carrier and the make up gases, respectively. Selected samples were analysed by NICI GC-MS with a Fisons MD 800 for confirmation of the qualitative and quantitative results. The samples were injected in split/splitless mode (48 s) at 280 °C and data acquisition started after a solvent delay of 4 min. Source temperature was 150 °C. Ammonia was used as reagent gas. The chromatographic conditions were the same as described above. Ion source pressure (currently 1.6 Torr) was adjusted to maximise the perfluorotributylamine ions (*m/z* 312, 452, 633 and 671). Ion repeller was 1.5 V. Data were scanned from *m/z* 50 to 450 at 1 s per decade.

2.1.4. Quantitation

The linear range of the detector was determined from injection of standard mixtures. Calibration lines were performed for all OC mentioned above. These compounds were then quantitated in the samples by the external standard method after replicate analysis. The concentrations of HCB and β -HCH were corrected for volatility losses using TBB as internal standard. The recoveries of TBB and PCB 209 were 102 \pm 4.2 and 93 \pm 1.8, respectively. The recoveries of spiked serum samples were 74–86% for PeCB and HCB, 92–93% for DDTs, 81–95% for HCHs and 77–110% for PCBs. When a sample had an OC concentration below the detection threshold, it was assigned the mid-value of this limit; when an OC was detected but under the quantification threshold, the mid-value between detection and quantification limits was assigned. Samples were analysed in two periods, 1997 (phase I) and 2001 (phase II) (Porta et al., 1999a; Fryzek et al., 1997; Garabrant et al., 1993). In phase I limits of detection and quantification (ng/mL) were: 0.09 and 0.3 for *p,p'*-DDT, 0.6 and 2 for *p,p'*-DDE, 0.1 and 0.33 for PCB 138, 0.24 and 0.79

for PCB 153, 0.4 and 1.3 for PCB 180, 0.23 and 0.76 for hexachlorobenzene (HCB) and 0.6 and 2.1 for β -HCH. In phase II, they were 0.26 and 0.39 for p,p' -DDT, 0.09 and 0.14 for p,p' -DDE, 0.11 and 0.16 for PCB 138, 0.12 and 0.18 for PCB 153, 0.10 and 0.16 for PCB 180, 0.03 and 0.05 for HCB, and 0.15 and 0.22 for β -HCH (Fryzek et al., 1997; Garabrant et al., 1993). In phase I, serum samples from 51 cases with EPC and 27 controls were analysed. In phase II analyses included: a) 93 new cases of EPC, not analysed in phase I, and b) for quality control purposes, repeated analyses of 6 cases of EPC and 23 controls that had been part of phase I. In statistical analyses the OC concentrations from phase II were used for all subjects for whom they were available. A conversion factor was obtained by linear regression of paired results from the 29 subjects with quantified values in the two phases, and it was applied to samples from phase I not reanalysed in phase II. Thus, the final group of EPC cases ($n=144$) was made up of 45 cases with OC concentrations analysed in phase I and corrected, and 99 cases with concentrations determined in phase II.

2.2. Analysis of serum concentrations of lipids

Total cholesterol and triglycerides levels were determined enzymatically (CHOD-PAP and GPO-PAP methods, respectively, Roche Diagnostics, Basle, Switzerland), and measured in a Cobas Mira Plus analyser (Roche, Basle, Switzerland), using serum obtained at the same time than the serum used for the organochlorine analyses. The methods were standardised with the World Health Organisation Lipid Quality Control Program (QCP) and the Monitrol QCP (Baxter Diagnostics, Düringen, Switzerland) (Marrugat et al., 1996). Interassay coefficients of variation were 2.57% and 2.90% for total cholesterol and triglycerides, respectively. The mid range of values for cholesterol was set at 100 to 250 mg/dL, and for triglycerides, at 80 to 250 mg/dL (Wallach, 2000). Total serum lipids were calculated by the standard formula of Phillips et al. (1989). Organochlorine concentrations were individually-corrected for total lipids (Porta et al., 1999a).

Table 1

Sociodemographic characteristics and organochlorine concentrations in 144 subjects with exocrine pancreatic cancer by levels of cholesterol and triglycerides

| | Total | | Cholesterol | | | | | Triglycerides | | | | | | | | |
|---|-------|---------|------------------|--------|------|--------|------------------|---------------|--------------------|-------|---------|------|--------|------|--------|--------------------|
| | | | Low ^a | Mid | High | p^b | Low ^c | Mid | High | P^b | | | | | | |
| Total | 144 | (100.0) | 13 | (9.0) | 85 | (59.0) | 46 | (32.0) | | 11 | (7.6) | 105 | (72.9) | 28 | (19.5) | |
| <i>Gender</i> | | | | | | | | | | | | | | | | |
| Male | 85 | (59.0) | 11 | (84.6) | 49 | (57.6) | 25 | (54.3) | 0.137 | 7 | (63.6) | 57 | (54.3) | 21 | (75.0) | 0.146 |
| Female | 59 | (41.0) | 2 | (15.4) | 36 | (42.4) | 21 | (45.7) | | 4 | (36.4) | 48 | (45.7) | 7 | (25.0) | |
| Age (years) | 66.4 | (67.7) | 66.7 | (67.1) | 67.1 | (67.8) | 65.0 | (67.1) | 0.778 ^d | 69.3 | (71.0) | 66.5 | (67.6) | 64.9 | (66.5) | 0.627 ^d |
| <i>Social class</i> | | | | | | | | | | | | | | | | |
| I–II | 14 | (10.4) | 2 | (16.7) | 7 | (9.0) | 5 | (11.4) | 0.978 | 1 | (10.0) | 10 | (10.4) | 3 | (10.7) | 0.993 |
| III | 32 | (23.9) | 2 | (16.7) | 20 | (25.6) | 10 | (22.7) | | 3 | (30.0) | 22 | (22.9) | 7 | (25.0) | |
| IV | 75 | (56.0) | 7 | (58.3) | 43 | (55.1) | 25 | (56.8) | | 5 | (50.0) | 55 | (57.3) | 15 | (53.6) | |
| V | 13 | (9.7) | 1 | (8.3) | 8 | (10.3) | 4 | (9.1) | | 1 | (10.0) | 9 | (9.4) | 3 | (10.7) | |
| <i>Education</i> | | | | | | | | | | | | | | | | |
| Illiterate | 17 | (11.8) | 0 | (0.0) | 9 | (11.5) | 8 | (17.8) | 0.215 | 2 | (20.0) | 11 | (11.3) | 4 | (14.3) | 0.153 |
| Read & write | 33 | (22.9) | 4 | (33.3) | 14 | (17.9) | 15 | (33.3) | | 1 | (10.0) | 23 | (23.7) | 9 | (32.1) | |
| ≤10 years | 74 | (51.4) | 7 | (58.3) | 48 | (61.5) | 19 | (42.2) | | 7 | (70.0) | 57 | (58.8) | 10 | (35.7) | |
| >10 years | 11 | (7.6) | 1 | (8.3) | 7 | (9.0) | 3 | (6.7) | | 0 | (0.0) | 6 | (6.2) | 5 | (17.9) | |
| <i>Tobacco (ever/never)</i> | | | | | | | | | | | | | | | | |
| Yes | 76 | (52.8) | 8 | (66.7) | 43 | (54.4) | 25 | (55.6) | 0.754 | 7 | (70.0) | 47 | (48.0) | 22 | (78.6) | 0.011 |
| No | 60 | (41.7) | 4 | (33.3) | 36 | (45.6) | 20 | (44.4) | | 3 | (30.0) | 51 | (52.0) | 6 | (21.4) | |
| <i>Coffee (regular drinker)</i> | | | | | | | | | | | | | | | | |
| Yes | 115 | (79.9) | 10 | (83.3) | 67 | (85.9) | 38 | (84.4) | 0.876 | 9 | (90.0) | 83 | (85.6) | 23 | (82.1) | 0.916 |
| No | 20 | (13.9) | 2 | (16.7) | 11 | (14.1) | 7 | (15.6) | | 1 | (10.0) | 14 | (14.4) | 5 | (17.9) | |
| <i>Alcohol (regular drinker)</i> | | | | | | | | | | | | | | | | |
| Yes | 96 | (66.7) | 9 | (75.0) | 55 | (70.5) | 32 | (71.1) | 1.000 | 10 | (100.0) | 66 | (68.0) | 20 | (71.4) | 0.096 |
| No | 39 | (27.1) | 3 | (25.0) | 23 | (29.5) | 13 | (28.9) | | 0 | (0.0) | 31 | (32.0) | 8 | (28.6) | |
| <i>Concentrations (ng/mL)^e</i> | | | | | | | | | | | | | | | | |
| p,p' -DDT | 4.6 | [2.9] | 3.3 | [1.8] | 4.9 | [2.9] | 4.4 | [3.1] | 0.194 | 4.6 | [2.2] | 4.9 | [2.9] | 3.5 | [2.7] | 0.999 |
| p,p' -DDE | 27.1 | [19.5] | 26.3 | [22.9] | 25.8 | [18.9] | 29.8 | [18.9] | 0.862 | 24.2 | [12.1] | 27.9 | [19.6] | 25.3 | [20.7] | 0.763 |
| PCB 138 | 1.8 | [1.6] | 1.1 | [1.1] | 1.7 | [1.5] | 2.2 | [1.9] | 0.003 | 1.9 | [1.3] | 1.7 | [1.5] | 2.1 | [1.9] | 0.154 |
| PCB 153 | 2.1 | [1.7] | 1.2 | [0.9] | 2.0 | [1.6] | 2.6 | [2.1] | 0.002 | 2.1 | [1.4] | 2.0 | [1.6] | 2.4 | [2.4] | 0.048 |
| PCB 180 | 2.3 | [1.7] | 1.4 | [1.0] | 2.1 | [1.5] | 2.9 | [2.2] | 0.001 | 2.1 | [1.2] | 2.2 | [1.5] | 2.5 | [2.4] | 0.011 |
| HCB | 13.2 | [10.3] | 5.9 | [4.0] | 12.3 | [10.5] | 17.0 | [12.9] | 0.002 | 10.9 | [7.4] | 13.7 | [11.2] | 12.4 | [11.3] | 0.478 |
| β -HCH | 7.4 | [6.2] | 4.7 | [2.4] | 7.4 | [5.9] | 8.3 | [8.1] | 0.038 | 7.8 | [6.5] | 7.2 | [5.9] | 8.2 | [8.1] | 0.224 |

Unless otherwise stated, figures refer to the number of subjects (figures within parentheses are the corresponding percentages).

^a Cholesterol: low, ≤100 mg/dL; mid, >100 to <250 mg/dL; high, ≥250 mg/dL.

^b Except where otherwise noted, Fisher's exact test (two tail).

^c Triglycerides: low, ≤80 g/dL; mid, >80 to <250 mg/dL; high, ≥250 mg/dL.

^d Kruskal–Wallis test.

^e Figures refer to the mean [and median]. All p -values in this section of the table are derived from the Kruskal–Wallis test.

2.3. Statistical analysis

Univariate statistics were computed as customary (Armitage et al., 2002). In contingency tables, comparison of two qualitative or categorical variables was performed with Fisher's exact test (two tail). For quantitative variables we used the Kruskal–Wallis test. Correlation coefficients were used to evaluate correlations between pairs or couples of organochlorine compounds. Specifically, Spearman's rank correlation coefficient (ρ) was used on crude concentrations, not corrected by lipids, while Pearson's correlation coefficient (r) was used on values individually-corrected by total lipids and log-transformed. Serum concentrations of the compounds were also log-transformed and partial correlations were calculated to describe the relationship between two different compounds while controlling for the effect of other variables, such as age or total lipids (Porta et al., 1999a,b, 2000, 2002a,b, 2005; Alguacil et al., 2002; Armitage et al., 2002). The level of statistical significance was set at 0.05, and all tests are two-tailed. Analyses were performed with SPSS, version 12.0 (SPSS Inc, Chicago, IL).

3. Results

Sociodemographic characteristics and OC concentrations for the 144 cases of EPC are shown in Table 1 according to the three categories of cholesterol and triglyceride concentrations. About 32% and 20% of cases had concentrations of cholesterol and triglycerides, respectively, above the normal limit. No statistically significant differences in lipid levels were apparent by age and sex, nor by tobacco, coffee and alcohol consumption, except that there were more smokers in the lowest and highest categories of triglycerides ($p=0.011$) (Table 1).

Organochlorine compounds with highest serum concentrations were p,p' -DDE (median, 19.5 ng/mL) and HCB (10.3 ng/mL) (Table 1). Concentrations of PCB congeners 138, 153 and 180, and of HCB and β -HCH increased significantly with increasing cholesterol (all $p<0.04$). With triglycerides this association was present only for the three PCBs (statistically significant for congeners 153 and 180).

Table 2
Correlations among pairs of organochlorine compounds by sex and age*

| Pairs of compounds | Total | Men | Women | <60 years | ≥ 60 years | |
|--------------------------|--------|--------------------|--------------------|--------------------|--------------------|--------------------|
| Number of subjects | 144 | 85 | 59 | 44 | 100 | |
| p,p' -DDT p,p' -DDE | ρ | 0.576 | 0.552 | 0.617 | 0.476 | 0.603 |
| | r | 0.540 | 0.507 | 0.608 | 0.436 [†] | 0.570 |
| p,p' -DDT PCB 138 | ρ | 0.297 | 0.358 | 0.219 [§] | 0.511 | 0.253 [†] |
| | r | 0.257 [†] | 0.356 | 0.171 [§] | 0.486 | 0.238 [†] |
| p,p' -DDE PCB 138 | ρ | 0.341 | 0.361 | 0.292 [†] | 0.460 [†] | 0.301 [†] |
| | r | 0.204 [†] | 0.244 [†] | 0.166 [§] | 0.369 [†] | 0.179 [§] |
| p,p' -DDT HCB | ρ | 0.302 | 0.381 | 0.060 [§] | 0.438 [†] | 0.156 [§] |
| | r | 0.290 | 0.338 [†] | 0.028 [§] | 0.321 [†] | 0.220 [†] |
| p,p' -DDE HCB | ρ | 0.284 | 0.332 [†] | 0.256 [†] | 0.332 [†] | 0.202 [†] |
| | r | 0.319 | 0.353 | 0.317 [†] | 0.321 [†] | 0.308 [†] |
| p,p' -DDT β -HCH | ρ | 0.428 | 0.422 | 0.410 | 0.511 | 0.338 |
| | r | 0.436 | 0.432 | 0.380 [†] | 0.485 | 0.377 |
| p,p' -DDE β -HCH | ρ | 0.485 | 0.523 | 0.463 | 0.454 [†] | 0.450 |
| | r | 0.513 | 0.541 | 0.486 | 0.468 | 0.526 |
| PCB138 PCB153 | ρ | 0.840 | 0.872 | 0.803 | 0.922 | 0.806 |
| | r | 0.644 | 0.711 | 0.571 | 0.873 | 0.587 |
| PCB138 PCB180 | ρ | 0.660 | 0.642 | 0.678 | 0.794 | 0.612 |
| | r | 0.506 | 0.503 | 0.503 | 0.669 | 0.467 |
| PCB153 PCB180 | ρ | 0.811 | 0.773 | 0.855 | 0.888 | 0.781 |
| | r | 0.819 | 0.789 | 0.848 | 0.852 | 0.806 |
| HCB β -HCH | ρ | 0.758 | 0.763 | 0.705 | 0.746 | 0.718 |
| | r | 0.771 | 0.787 | 0.727 | 0.664 | 0.783 |

*All $p \leq 0.001$ except [†] $p \leq 0.05$ and [§] $p > 0.05$.

ρ : Spearman's rank correlation coefficient on crude values, not corrected by lipids.
 r : Pearson's correlation coefficient on values individually-corrected by total lipids and log-transformed.

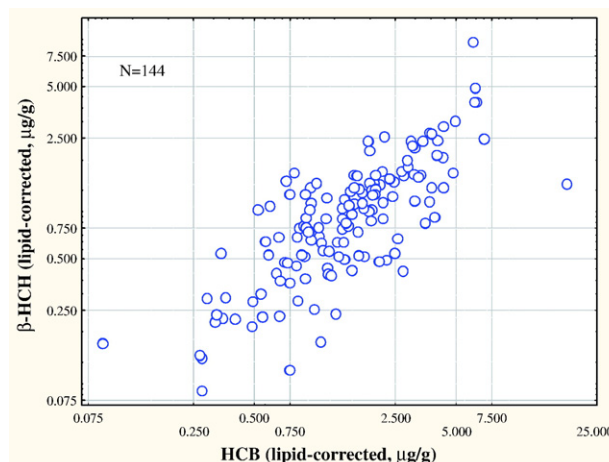


Fig. 1. Correlation between serum concentrations of hexachlorobenzene (HCB) and β -hexachlorocyclohexane (β -HCH) (individually-corrected by total lipids, log-scale).

Table 2 shows the 6 pairs of organochlorines that were more strongly correlated, along with other relevant pairs. As expected, high correlations were seen for pairs of PCBs. There was also a high correlation between HCB and β -HCH ($\rho=0.758$) (Fig. 1); this correlation was stronger than the correlation between p,p' -DDT and p,p' -DDE in all subgroups (Table 2). In fact, the correlation between HCB and β -HCH was weaker only than those between two pairs of PCBs. After being individually-corrected by total lipids, log-transformed and further adjusted by age, the correlation between HCB and β -HCH was 0.734 ($p<0.001$). The correlation between p,p' -DDT and p,p' -DDE was slightly stronger among cases older than 60 years than in younger cases. For all other pairs the correlation was slightly or substantially stronger among younger than among older patients; this was always so for Spearman's coefficients and almost always so for Pearson's. The other coefficients not shown in Table 2 were below 0.5. β -HCH had ρ coefficients of 0.405 with PCB 138, 0.447 with congener 153, and 0.259 with congener 180 (all $p<0.002$). Correlations with β -HCH were similar in women and men. In younger subjects we also observed moderate correlations between PCB 138 and p,p' -DDT ($\rho=0.511$), PCB 138 and p,p' -DDE ($\rho=0.460$), PCB 153 and p,p' -DDT ($\rho=0.446$), and between PCB 153 and p,p' -DDE ($\rho=0.411$) (all $p<0.01$).

A minority of correlations among compounds appeared to be affected by tobacco, coffee or alcohol consumption (Table 3). The correlation between p,p' -DDT and p,p' -DDE was slightly stronger in non-smokers, in non-coffee drinkers and in non-alcohol drinkers; the opposite pattern was observed for several of the other pairs of OCs (e.g., for tobacco, between p,p' -DDT and PCB 138, and between p,p' -DDT and HCB; for alcohol, between the latter two pairs, and between p,p' -DDE and PCB 138).

As compared to Spearman's rank correlation coefficients computed on crude OC concentrations and not corrected by lipids, a slight majority of Pearson's correlation coefficients computed for concentrations individually-corrected by total lipids and log-transformed were moderately weaker, but a remarkable minority were stronger (Tables 2 and 3). Pearson's correlation coefficients computed for concentrations individually-corrected by total lipids and log-transformed were virtually unaffected when further adjusted by age (not shown in tables).

We observed a striking variety of correlation patterns among pairs of organochlorine compounds across strata of cholesterol and triglycerides. Table 4 shows totally crude, unadjusted results. They do not differ

Table 3
Correlations among pairs of organochlorine compounds by tobacco, coffee and alcohol consumption*

| Pairs of compounds | | | Tobacco ^a | | Coffee ^b | | Alcohol ^c | |
|--------------------|------------------|----------|----------------------|--------------------|---------------------|--------------------|----------------------|--------------------|
| | | | No | Yes | No | Yes | No | Yes |
| Number of subjects | | | 60 | 76 | 20 | 115 | 39 | 96 |
| <i>p,p'</i> -DDT | <i>p,p'</i> -DDE | ρ | 0.610 | 0.593 | 0.661 | 0.594 | 0.649 | 0.585 |
| | | <i>r</i> | 0.604 | 0.521 | 0.625 [†] | 0.561 | 0.587 | 0.549 |
| <i>p,p'</i> -DDT | PCB 138 | ρ | 0.191 [§] | 0.324 [†] | 0.537 [†] | 0.209 [†] | -0.022 [§] | 0.381 |
| | | <i>r</i> | 0.109 [§] | 0.361 | 0.660 [†] | 0.093 [§] | -0.084 [§] | 0.402 |
| <i>p,p'</i> -DDE | PCB 138 | ρ | 0.352 [†] | 0.328 [†] | 0.585 [†] | 0.298 | -0.037 [§] | 0.495 |
| | | <i>r</i> | 0.168 [§] | 0.233 [†] | 0.616 [†] | 0.118 [§] | -0.066 [§] | 0.365 |
| <i>p,p'</i> -DDT | HCB | ρ | 0.070 [§] | 0.368 | 0.199 [§] | 0.334 | 0.074 [§] | 0.398 |
| | | <i>r</i> | 0.086 [§] | 0.362 | 0.341 [§] | 0.316 | -0.001 [§] | 0.385 |
| <i>p,p'</i> -DDE | HCB | ρ | 0.244 [§] | 0.304 [†] | 0.128 [§] | 0.315 | 0.167 [§] | 0.318 [†] |
| | | <i>r</i> | 0.292 [†] | 0.355 [†] | 0.168 [§] | 0.355 | 0.266 [§] | 0.362 |
| <i>p,p'</i> -DDT | β -HCH | ρ | 0.389 [†] | 0.438 | 0.424 [§] | 0.436 | 0.211 [§] | 0.546 |
| | | <i>r</i> | 0.381 [†] | 0.464 | 0.511 [†] | 0.451 | 0.142 [§] | 0.553 |
| <i>p,p'</i> -DDE | β -HCH | ρ | 0.520 | 0.433 | 0.505 [†] | 0.481 | 0.216 [§] | 0.573 |
| | | <i>r</i> | 0.535 | 0.487 | 0.409 [§] | 0.533 | 0.329 [†] | 0.591 |
| PCB138 | PCB153 | ρ | 0.809 | 0.872 | 0.905 | 0.826 | 0.805 | 0.856 |
| | | <i>r</i> | 0.577 | 0.704 | 0.733 | 0.622 | 0.527 | 0.710 |
| PCB138 | PCB180 | ρ | 0.661 | 0.653 | 0.771 | 0.650 | 0.657 | 0.670 |
| | | <i>r</i> | 0.511 | 0.500 | 0.527 [†] | 0.512 | 0.413 [†] | 0.558 |
| PCB153 | PCB180 | ρ | 0.841 | 0.779 | 0.881 | 0.803 | 0.826 | 0.823 |
| | | <i>r</i> | 0.849 | 0.813 | 0.882 | 0.822 | 0.793 | 0.848 |
| HCB | β -HCH | ρ | 0.718 | 0.742 | 0.624 [†] | 0.777 | 0.840 | 0.699 |
| | | <i>r</i> | 0.715 | 0.813 | 0.644 [†] | 0.795 | 0.869 | 0.738 |

*All $p \leq 0.001$ except [†] $p \leq 0.05$ and [§] $p > 0.05$.

ρ : Spearman's rank correlation coefficient on crude values, not corrected by lipids.

r: Pearson's correlation coefficient on values individually-corrected by total lipids and log-transformed.

^a Never smokers vs. ever smokers.

^b Non-regular coffee drinkers vs. regular coffee drinkers.

^c Non-regular alcohol drinkers vs. regular alcohol drinkers.

substantially from results based on OC concentrations individually-corrected by total lipids, log-transformed and further adjusted by age (Table 5 and Fig. 2): Only slightly more than half of the coefficients based on crude values are higher than the coefficients based on adjusted values.

Most correlations between pairs of compounds were higher in the lowest category of triglycerides than in the lowest category of cholesterol. Most of the coefficients above 0.7 were observed in the

analyses by triglycerides; seven coefficients above 0.7 were seen in the lowest category of triglycerides (OC pairs *p,p'*-DDT and HCB, *p,p'*-DDT and β -HCH, *p,p'*-DDE and β -HCH, PCB 138 and PCB 153, PCB 138 and PCB 180, PCB 153 and PCB 180, HCB and β -HCH) (Tables 4 and 5). Only coefficients between *p,p'*-DDT and PCB 138

Table 4
Correlations among pairs of organochlorine compounds (crude values, not corrected by lipids), according to cholesterol and triglyceride concentrations*

| Pairs of compounds | | | Cholesterol | | | Triglycerides | | |
|--------------------|------------------|----------|---------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| | | | Low ^a | Mid | High | Low ^b | Mid | High |
| <i>p,p'</i> -DDT | <i>p,p'</i> -DDE | ρ | 0.624 [†] | 0.589 | 0.594 | 0.755 [†] | 0.593 | 0.394 [†] |
| | | <i>r</i> | -0.124 [§] | 0.267 [†] | 0.312 [†] | 0.418 [§] | 0.232 [†] | 0.481 [†] |
| <i>p,p'</i> -DDT | PCB 138 | ρ | -0.011 [§] | 0.311 [†] | 0.503 | 0.300 [§] | 0.301 [†] | 0.419 [†] |
| | | <i>r</i> | 0.462 [§] | 0.328 [†] | 0.115 [§] | 0.645 [†] | 0.255 [†] | 0.350 [§] |
| <i>p,p'</i> -DDE | HCB | ρ | 0.412 [§] | 0.304 [†] | 0.260 [§] | 0.418 [§] | 0.259 [†] | 0.258 [§] |
| | | <i>r</i> | 0.636 [†] | 0.464 | 0.264 [§] | 0.855 | 0.411 | 0.282 [§] |
| <i>p,p'</i> -DDT | β -HCH | ρ | 0.536 [§] | 0.540 | 0.383 [†] | 0.764 [†] | 0.519 | 0.180 [§] |
| | | <i>r</i> | 0.856 | 0.767 | 0.923 | 0.964 | 0.816 | 0.859 |
| PCB138 | PCB153 | ρ | 0.509 [§] | 0.602 | 0.704 | 0.909 | 0.639 | 0.565 [†] |
| | | <i>r</i> | 0.603 [†] | 0.835 | 0.751 | 0.909 | 0.832 | 0.533 [†] |
| HCB | β -HCH | ρ | 0.820 | 0.698 | 0.791 | 0.800 | 0.785 | 0.645 |

*All $p \leq 0.001$ except [†] $p \leq 0.05$ and [§] $p > 0.05$.

Figures in the table correspond to Spearman's rank correlation coefficient.

^a Cholesterol: low, ≤ 100 mg/dL; mid, > 100 to < 250 mg/dL; high, ≥ 250 mg/dL.

^b Triglycerides: low, ≤ 80 ; mid, > 80 to < 250 mg/dL; high, ≥ 250 mg/dL.

Table 5
Partial correlations among serum concentrations of pairs of organochlorine compounds (individually-corrected by total lipids, log-transformed and adjusted by age), according to cholesterol and triglyceride concentrations*

| Pairs of compounds | | | Cholesterol | | | Triglycerides | | |
|--------------------|------------------|----------|---------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| | | | Low ^a | Mid | High | Low ^b | Mid | High |
| <i>p,p'</i> -DDT | <i>p,p'</i> -DDE | ρ | 0.472 [§] | 0.541 | 0.579 | 0.675 [†] | 0.537 | 0.349 [§] |
| | | <i>r</i> | -0.107 [§] | 0.279 [†] | 0.444 [†] | 0.023 [§] | 0.183 [§] | 0.873 |
| <i>p,p'</i> -DDT | PCB 138 | ρ | -0.247 [§] | 0.179 [§] | 0.503 | 0.287 [§] | 0.171 [§] | 0.319 [§] |
| | | <i>r</i> | 0.313 [§] | 0.257 [†] | 0.142 [§] | 0.735 [†] | 0.186 [§] | 0.120 [§] |
| <i>p,p'</i> -DDE | HCB | ρ | 0.417 [§] | 0.287 [†] | 0.295 [†] | 0.679 [†] | 0.278 [†] | 0.079 [§] |
| | | <i>r</i> | 0.556 [§] | 0.381 | 0.324 [†] | 0.874 | 0.375 | 0.155 [§] |
| <i>p,p'</i> -DDT | β -HCH | ρ | 0.461 [§] | 0.531 | 0.464 | 0.864 | 0.526 | 0.219 [§] |
| | | <i>r</i> | 0.741 [†] | 0.597 | 0.887 | 0.970 | 0.611 | 0.647 |
| PCB138 | PCB153 | ρ | 0.415 [§] | 0.485 | 0.674 | 0.915 | 0.483 | 0.388 [†] |
| | | <i>r</i> | 0.632 [†] | 0.833 | 0.816 | 0.848 [†] | 0.827 | 0.716 |
| HCB | β -HCH | ρ | 0.768 [†] | 0.717 | 0.771 | 0.913 | 0.735 | 0.669 |

*All $p \leq 0.001$ except [†] $p \leq 0.05$ and [§] $p > 0.05$.

Figures in the table correspond to Pearson's correlation coefficient.

^a Cholesterol: low, ≤ 100 mg/dL; mid, > 100 to < 250 mg/dL; high, ≥ 250 mg/dL.

^b Triglycerides: low, ≤ 80 ; mid, > 80 to < 250 mg/dL; high, ≥ 250 mg/dL.

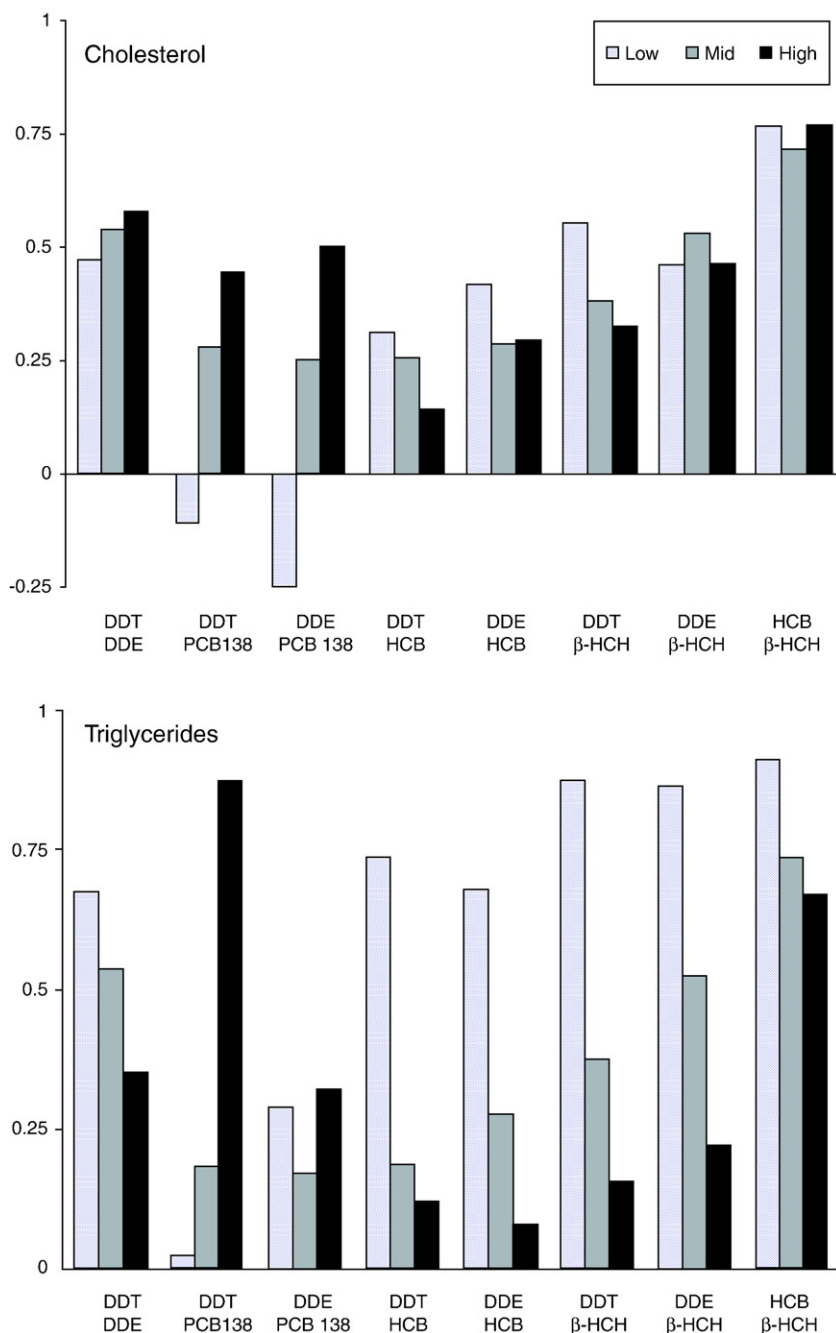


Fig. 2. Partial correlations among serum concentrations of pairs of organochlorine compounds (individually-corrected by total lipids, log-transformed and adjusted by age), according to cholesterol and triglyceride concentrations (low, mid or high). The height of the bar represents Pearson's correlation coefficient.

increased with increasing concentrations of triglycerides (Table 5 and Fig. 2, bottom).

Correlations between p,p' -DDT and PCB 138, and between p,p' -DDE and PCB 138 were inverse or null in the lowest category of cholesterol, and tended to increase with increasing cholesterol (Fig. 2, top). Furthermore, for a given pair of organochlorine compounds the correlation pattern could be quite different in the categories of cholesterol and of triglycerides. Thus, stratification by cholesterol showed a virtually "flat" pattern (similar values) of correlations between p,p' -DDE and β -HCH, while the same correlations decreased substantially with increasing triglycerides; a similar contrast applies to the pairs p,p' -DDT and p,p' -DDE, and HCB and β -HCH. Six of the 8 comparisons shown in Fig. 2 (bottom) present a monotonic decrease with increasing

triglycerides. Overall, in 9 of the 11 comparisons shown in Table 5 the correlations are higher when triglycerides are lower than when they are higher.

4. Discussion

We observed a diversity of correlation patterns among pairs of OCs in different strata of cholesterol and triglycerides. To our knowledge, no studies have previously analysed associations between lipid content and OC correlations; this is somewhat surprising, given the well-known lipophilic nature of many OCs (Wolff et al., 2005). The frequency of such diversity of patterns

in other cancers and chronic diseases needs to be assessed by independent studies.

Most correlations among OC were stronger when concentrations of triglycerides were low than when they were high. This is compatible with a dilution in the early phases of cancer, when triglycerides often increase, and with a concentration effect as triglycerides become low in the advanced phases of the disease (Tisdale, 2002).

This is the largest and most detailed analysis of correlations among serum concentrations of OCs in pancreatic cancer. The only other available study (Hoppin et al., 2000) reported similarly high OC correlations among cases of pancreatic cancer ($N=108$) and among healthy controls ($N=82$). The correlation they observed between HCB and p,p' -DDE (Spearman's $\rho=0.29$ for cases and controls combined) (Hoppin et al., 2000) was very similar to the one we found ($\rho=0.30$) (Porta et al., 2002b). In that study (Hoppin et al., 2000), the sum of 11 PCB congeners (“total PCBs”) was moderately correlated with HCB ($\rho=0.45$) and with DDE ($\rho=0.58$) (again, for cases and controls combined). Knowledge on these patterns is essential to understand the etiopathogenic significance, if any, of the associations between OC concentrations and pancreatic cancer risk (Porta et al., 1999a; Porta, 2001; Li et al., 2005). Extending the analyses to other diseases will be relevant to assess the relationship between OC and chronic metabolic diseases like diabetes mellitus, which OC might contribute to cause (Porta, 2006; Glynn et al., 2003; Lee et al., 2007).

The relationships among several OCs tended to change with the age of patients in two different directions. On the one hand, the correlation of p,p' -DDT and p,p' -DDE was slightly stronger among older cases. This suggests that younger subjects incorporated the two compounds in a more independent way than older subjects; hence, in the elderly a larger proportion of the p,p' -DDE body burden would stem from degradation of p,p' -DDT, whilst the predominant source of p,p' -DDE in younger subjects would be background dietary exposure. On the other hand, for most other pairs the correlation was slightly or substantially stronger among younger than among older patients. This is compatible with at least two explanations. First, exposure to a higher number of compounds or mixtures of compounds may have been more frequent among ‘younger’ subjects (those below 60 years, i.e., born after 1932–1935). Second, differences in elimination kinetics among compounds may have had more time to operate in older subjects. The observations stress the need to conduct age-cohort-period analyses that disentangle the specific contributions of the three factors (biological age, birth cohort and calendar period) to human concentrations of POPs (Porta, 2006).

A moderate association between HCB and β -HCH has been reported by studies in Sweden, Canada and Japan (Glynn et al., 2001; Moysich et al., 1999; Masuda et al., 2005), which suggests the existence of a fairly global phenomenon. Reasons for the association may lie in the similar commercial origin, properties and behaviour in humans of the two compounds; however, to our best knowledge comprehensive explanations are lacking. The range of correlations among the three PCBs is

well within values reported by other studies (DeVoto et al., 1997; Glynn et al., 2001, 2000; Moysich et al., 1999; Gladen et al., 1999, 2003). The strength of some other associations was somewhat different from those reported in other papers; e.g., in our patients p,p' -DDT and p,p' -DDE each tended to be less correlated with PCBs. This could be due to the pancreatic cancer that all our cases suffered, or perhaps also to historical exposure processes specific to Spain. The literature shows a suggestive diversity of correlations among concentrations of OCs; the extent to which this might partly reflect historical differences in exposure among geographical areas deserves scrutiny. A similar point can be made about the influence of age on patterns of correlations.

The fact that concentrations of two OCs are not independent is generally known to bear important implications for mechanistic studies aiming to elucidate the etiopathogenic role of each compound (Glynn et al., 2001, 2000; Moysich et al., 1999; Gladen et al., 1999, 2003; Masuda et al., 2005; Cogliano, 1998). The specific implications, nonetheless, are often less clear, particularly when the influence of lipids is considered. In this respect our results, if replicated, would suggest some practical conclusions. For instance, the need to adjust a given ‘OC 1’ by another ‘OC 2’ could generally be stronger in strata of lowest concentrations of triglycerides, and weaker when concentrations of triglycerides are higher. Also, analyses could need to consider the following possibility: while the causal effect of a given ‘OC 1’ (e.g., the disease risk associated to certain ‘OC 1’ concentrations) may appear to vary according to the concentrations of cholesterol and of triglycerides, the effect of ‘OC 1’ might in fact be modified by ‘OC 2’ (Gladen et al., 1999). And vice versa: the risk of ‘OC1’ may be modified by lipids. Though logical, this second possibility may be less common than the first, since it is also clear that correlations of OCs with cholesterol and triglycerides are often weaker than correlations among OCs themselves (Porta et al., 2002b; Moysich et al., 2002; Laden et al., 1999); this fact needs to be considered when discussing the most appropriate methods to adjust for lipids (Rylander et al., 2006; Covaci et al., 2006). Some correlations in the study were slightly affected by tobacco, coffee or alcohol consumption; this suggests that these common exposures might contribute to confound or modify risk estimates for OCs. Naturally, we did not expect tobacco, coffee or alcohol to chemically interfere with correlations between OCs, but the possibility of confounding has seldom been addressed.

To our knowledge no information has been published on correlations of OCs in the Spanish general population. While suffering a severe disease, our patients did not come from “hot spots” with particularly high exposure to pesticides or industrial organochlorines. Some reasons for correlation patterns among OCs are still ignored in many populations worldwide, both healthy and sick. Perhaps this is partly because studies have often been, to a large extent, descriptive; we are confident that when studies become more analytical, further reasons for the correlations will be unveiled. Such progress would contribute to advance knowledge on the toxicology and epidemiology of organochlorine compounds.

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