Adjusting serum concentrations of organochlorine compounds by lipids and symptoms: a causal framework for the association with K-ras mutations in pancreatic cancer.

Adjusting serum concentrations of organochlorine compounds by lipids and symptoms: A causal framework for the association with K-ras mutations in pancreatic cancer

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Abbreviations: CI, confidence interval; BMI, body mass index; DAG, causal directed acyclic graph; EPC, exocrine pancreatic cancer; HCB, hexachlorobenzene; HCH, hexachlorocyclohexane; ISE, interval from first symptom of EPC to blood extraction; OCs, organochlorine compounds; OR, odds ratio; PCBs, polychlorinated biphenyls; p,p′-DDE, dichlorodiphenyldichloroethene; p,p′-DDT, dichlorodiphenyltrichloroethane; TSL, total serum lipids.

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a b s t r a c t

In clinically aggressive diseases, patients experience pathophysiological changes that often alter concen-trations of lipids and environmental lipophilic factors; such changes are related to disease signs and symptoms. The aim of the study was to compare the effects of correcting for total serum lipids (TSL) and other clinical factors on the odds of mutations in the K-ras oncogene by organochlorine compounds (OCs), in logistic models, in 103 patients with exocrine pancreatic cancer (EPC) using a causal directed acyclic graph (DAG) framework. Results and likelihood of bias were discussed in the light of possible causal scenarios. The odds of K-ras mutated EPC was associated with some TSL-corrected OCs, including p,p′-DDT (p-value: 0.008) and polychlorinated biphenyl 138 (p-trend: 0.024). When OCs were not corrected by TSL, the OR of a K-ras mutation was significant for p,p′-DDT (p-trend: 0.035). Additionally adjusting for cholestatic syndrome increased the ORs of TSL-corrected OCs. When models were adjusted by the interval from first symptom to blood extraction (ISE), the ORs increased for both TSL-corrected and uncorrected OCs. Models with TSL-corrected OCs and adjusted for cholestatic syndrome or ISE yielded the highest ORs. We show that DAGs clarify the covariates necessary to minimize bias, and demonstrate sce-narios under which adjustment for TSL-corrected OCs and failure to adjust for symptoms or ISE may induce bias. Models with TSL-uncorrected OCs may be biased too, and adjusting by symptoms or ISE may not control such biases. Our findings may have implications as well for studying environmental causes of other clinically aggressive diseases.

1. Introduction

Organochlorine compounds (OCs) are highly lipophilic and resistant to degradation. Human contamination is common and exposure occurs primarily through consumption of fatty foods (Porta et al., 2008a; Reed et al., 2007). A substantial number of studies have shown associations between internal contamination by OCs and intermediate disorders (e.g., immunological) and severe diseases, including cancer (Centers for Disease Control and Prevention, 2009; Leijs et al., 2009; Porta, 2012), but it has often been difficult to rule out ‘‘disease progression bias’’ or ‘‘reverse causality’’ (Porta, 2008).

Indeed, there are still many unresolved methodological ques-tions related to the toxicokinetics of OCs in humans, particularly when they are sick. Correction of blood concentration of OCs by total serum lipids (TSL) is frequently used in studies, on the pre-mise that lipophilic substances are in a state of equilibrium across body compartments (blood, fat, organs) (Porta et al., 2009a). Thus, the lipid correction of OC blood levels improved precision, and it became common to express OCs on a lipid-basis. However, such conditions of equilibrium may not hold in overt disease, or even in the subclinical phases of disease, long before diagnosis (Porta, 2001; Schisterman et al., 2005; Wolff et al., 2007). It is also plausi-ble that some OCs affect lipid concentrations (e.g., by inducing lipid synthesis), thus, even regression adjustment by TSL might entail mis-adjustment by a variable in the causal pathway (Goncharov et al., 2008; Lee et al., 2011; Porta et al., 2009c).

Clinical, metabolic and toxicological factors may influence the accumulation of OCs and other lipophilic compounds in fatty tis-sues, their mobilization, the blood/fat ratio, and the blood/organ tissue ratio. Both subtle and abrupt changes in these processes are common in clinically aggressive diseases as cancer (Kritchevsky et al., 1991; Porta, 2001; Wolff et al., 2007). Little is known about the kinetics of lipophilic environmental pollutants during the prediagnostic phases of cancer and other disease peri-ods (Wolff et al., 2000). Statistical approaches to control lipid mobilization and the other pathophysiologic changes that com-monly take place during cancer progression are thus necessary to prevent biased estimates of associations between OCs and risk of cancer (Baris et al., 2000; Lee et al., 2011; Spinelli et al., 2007).

Disease signs and symptoms reflect underlying pathophysiolog-ical processes (Porta et al., 2003, 2009c, 2012). In this paper we will consider where such symptoms fit on the causal spectrum in deter-mining whether characteristics of the disease influence associa-tions between the occurrence of disease-related genetic alterations and environmental exposures, keeping in mind that the associations may also be reflective of disease-related lipid alterations.

We wish to estimate the possible causal effect of OC levels on K-ras mutations in exocrine pancreatic cancer (EPC) without bias. To this end, the use of causal directed acyclic graphs (DAGs) can be helpful. DAGs can clarify issues relating to adjustment and bias (Greenland et al., 1999; Hernán et al., 2002). Using DAGs, Schisterman et al. (2005) showed that lipid adjustment may lead to bias, depending on the underlying causal relationship in ques-tion. As many other types of cancer, EPC itself affects lipid levels and, therefore, cannot be considered in the context of this previous work. In particular, patients with a highly aggressive disease as EPC experience pathophysiological changes (e.g., part of the constitu-tional or the cholestatic syndrome) that profoundly alter TSL levels. TSL thus vary in different stages of the disease (Porta et al., 2008b). It is important to note that in EPC K-ras mutations do not affect lipid metabolism, BMI or the prognosis of the disease. Therefore, utilizing a causal understanding of the relationships

between K-ras mutations, OC exposure levels, lipid correction, age, sex, and cancer symptoms, we sought to clarify understanding of appropriate adjustment for confounding factors to minimize bias in a clinical series of EPC patients.

The aim of the present study was to compare alterations to the odds of having a K-ras mutated EPC vs. a K-ras wild-type EPC, given a certain concentration of an OC, from additional adjustment for TSL, disease symptoms and other clinical factors in patients with EPC.

2. Material and methods

Methods and strategies of analysis of the PANKRAS II study have been previously described in detail (Crous-Bou, 2009; Porta et al., 2008b). Briefly, subject recruitment took place between 1992 and 1995 at five general hospitals in the Mediterranean part of Spain, where 185 incident cases of EPC were prospectively identified. The present report is based on 103 EPC patients with known K-ras status and with analysis of OCs.

2.1. Clinicopathological information and personal interviews

Patients were interviewed face-to-face by trained monitors dur-ing hospital stay, close to the time of diagnosis. Over 40% of the blood draws took place in the same day of the interview, and over 94% of the interviews took place in the 2 weeks before or after the blood extraction. Interviews included questions about occupation, lifestyle, and past clinical history (Crous-Bou, 2009; Porta et al., 2009b). On the basis of previous research (Porta et al., 2003), detailed information on signs and symptoms was obtained from two sources: medical records and patient interviews. The chole-static syndrome involved jaundice, hypocholia and choluria, while the constitutional syndrome comprised asthenia, anorexia and weight loss (Porta et al., 2008b).

Blood was drawn in the early morning and with patients fasting (Porta et al., 2007). Each subject had one measure of serum concen-trations of organochlorines. The median time between the first symptom of cancer and blood extraction (ISE) was 73 days.

2.2. Laboratory analysis

K-ras mutations were analyzed by nested polymerase chain reaction (PCR) and direct DNA sequencing as described previously (Crous-Bou, 2009; Gasull et al., 2010). It should be noted that the occurrence of K-ras mutations is not affected by lipid mobilization, EPC symptoms, or any clinical markers of disease emergence or progression.

Details of laboratory protocols of OCs have also been described elsewhere (Gasull et al., 2013; Porta et al., 2009b). Briefly, gas chro-matography analyses were performed and selected samples were analyzed by negative-ion chemical ionization gas chromatogra-phy–mass spectrometry. Statistical analyses were limited to com-pounds that were detected above the detection limit >85% of subjects. When a sample had an OC concentration below the detec-tion 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.

Total cholesterol and triglycerides were determined enzymati-cally, using serum obtained at the same time as the serum used for the OC analyses. TSL were calculated by the traditional or stan-dard formula 2 proposed by Phillips et al. (1989) which uses total cholesterol and triglycerides. Among cases, mean (standard devia-tion) serum concentrations of total cholesterol, triglycerides and TSL were, respectively, 209 (102), 178 (94) and 714 (293) mg dL^{1}.

The main variables in this study were TSL-corrected OC, organo-chlorine concentrations individually corrected by TSL and expressed in ng g^{1} lipid, the lipid correction is performed by sim-ply dividing the serum OC value by the serum TL value and TSL-uncorrected OC, organochlorine concentrations uncorrected by TSL and expressed in ng mL^{1} (i.e. TSL-uncorrected p,p^{0}-DDE).

2.3. Statistical analyses

We use multivariate-adjusted odds ratios (OR) and their corre-sponding 95% confidence intervals (CI), calculated by uncondi-tional logistic regression. We categorized the OCs (corrected and uncorrected by TSL) in tertiles, the cutoff points of the tertiles are shown in Table A.1. Categorical ordinal variables were analyzed for a linear dose-response relation through the multivariate ana-logue of Mantel's extension test, when a linear trend was not apparent, Wald's test was used. Age and sex were assessed in all models as potential confounders. Final models were chosen coher-ently with the study objectives and the nature of the variables, i.e, variable selection was primarily based on coherence with the explicit DAGs. In final models age, sex, ISE and cholestatic and con-stitutional syndrome were not associated with K-ras mutational status (both in models with OCs corrected and uncorrected by TSL). The level of statistical significance was set at 0.05, and all tests are two-tailed. Analyses were performed using SPSS, version 12.0.

We used the DAG framework (Greenland et al., 1999; Hernán et al., 2002) to express our assumptions regarding the underlying causal structure of the variables. DAGs serve as a graphical method to facilitate causal inference in epidemiology and other fields of research. We provide greater detail regarding DAG methodology in the Appendix A.

We used heuristic diagrams to assess different scenarios based on results from previous analyses (Porta et al., 2009c). The heuris-tic diagrams in Fig. A.1 are useful to construct DAGs. The DAG in the Fig. 1 includes time-dependent variables (namely OCs1, OCs2, OCs3, lipids1, lipids2, lipids3, EPC1 and EPC2), they are actually variables related to disease progression. OCs1 and lipids1 represent concentrations of OCs and lipids before development of EPC, OCs2 and lipids2 represent concentrations when EPC was in the initial subclinical phase (EPC1), OCs3 and lipids3 represent concentra-tions when EPC was symptomatic (EPC2). The PANKRAS II study obtained measures only of OCs3. The DAG in Fig. 1 also shows that: OCs1 influence the occurrence of K-ras mutations, OCs1, K-ras mutations, and other effects (U) are associated with development of EPC1, EPC1

causes signs and symptoms (including cholestatic syndrome or the constitutional syndrome), and affects lipids2 and OCs2. In the next disease stage, EPC2 affects OCs3 and lipids3, while the pathophysiological processes underlying some signs and symptoms (like the cholestatic syndrome or the constitutional syn-drome) affect concentrations of lipids3. Therefore, some EPC signs and symptoms are associated with blood concentrations of some OCs (certainly, through lipids, perhaps directly as well) measured after the symptomatic diagnosis of EPC (Porta et al., 2009c). It fol-lows that adjustment for certain signs and symptoms may be nec-essary to estimate the causal association between OC and K-ras mutations. Variables we adjusted for in our analyses are depicted with boxes around them, consistent with DAG notation.

3. Results

In models adjusted for age and sex, we compared the OR of a K-ras mutation in relation to OC levels both correcting for TSL and not correcting for TSL. Overall, we found that several OCs in tertiles were statistically significantly associated with the odds of K-ras mutation, but that additional adjustment for TSL strengthened the point estimates for p,p^0 -DDE, PCB 138 and PCB 153. TSL-corrected concentrations of p,p^0-DDT, PCB 138 and PCB 153 were significantly associated with an increased odds of a K-ras mutated EPC (Table 1). When OCs in tertiles were uncorrected by TSL, only p,p^0-DDT and HCB were statistically significantly associated with the odds of a K-ras mutation, with increasing levels of HCB associated with a decreased odds of a K-ras mutation. The ORs of K-ras mutation for TSL-corrected p,p^0-DDE were slightly higher than those of TSL-uncorrected p,p^0-DDE for the middle and upper tertiles compared with the lowest, respectively (1.61 and 2.35 vs. 1.16 and 1.74, respectively). For TSL-corrected PCBs 138 and 153, the ORs in the mid-tertile were lower than the ORs for TSL-uncorrected PCBs 138 and 153, however, this was not so for the ORs in the upper tertile. Patients in the upper tertile of TSL-corrected PCBs 138 and 153 were over 4 and 5 times more likely to have a K-ras mutated tumor than patients in the lower tertile, whereas the ORs were only 2.7 and 2.2 in the upper tertile of TSL-uncorrected PCBs 138 and 153, respec-tively. There was a statistically significant linear trend for these PCBs in the models with the TSL-corrected OCs in tertiles (p for trend 0.024 and 0.017, respectively), while the p-values for these TSL-uncorrected PCBs were not statistically significant. Only for HCB the p-value was smaller in the TSL-uncorrected model (p-value 0.044) than in the model for TSL-corrected HCB (p-value 0.175) (Table 1). When we considered the OCs as continuous variables (log-transformed), the relations of K-ras with OCs corrected and uncor-rected by lipids were very similar (similar ORs and p-values).

Additional adjustment for cholestatic syndrome, while lipid correcting, strengthened the associations between the following OCs in tertiles and the odds of a K-ras mutation: p,p^0-DDT, PCB 138, and PCB 153. The pattern shown in models adjusted by age and sex was similar when the models were further adjusted by the cholestatic syndrome (Table 1). Most ORs from the models for TSL-corrected OCs in tertiles increased slightly when adjusting by cholestatic syndrome (as compared to models adjuested by age and sex), by contrast, the ORs for the TSL-uncorrected OCs in ter-tiles were not affected. Adjustement for constitutional syndrome resulted in slight changes in the ORs of TSL-corrected p,p^0 -DDT and b-HCH (Table A.2). ORs for the rest of the OCs, TSL-corrected and TSL-uncorrected, were not affected when the models were adjusted by the constitutional syndrome (i.e., parameters they were similar to those shown in Table 1, models adjusted by age and sex). The ORs in models adjusted by the interval from first symptom to blood extraction (ISE) increased slightly (both in the models corrected and uncorrected by TSL) compared with the models adjusted only by age and sex (Table A.3).

The direction of the change in the ORs of the OCs (TSL-corrected and TSL-uncorrected) when adjusted by signs, symptoms, syn-dromes and ISE is summarized in Table A.4. Adjustment for chole-static syndrome and ISE together altered all 7 of the OCs we evaluated when additionally TSL corrected. Adjustment for ISE and pruritus altered levels of 6 of the 7 OCs when additionally TSL corrected. TSL correction was associated with greater percent change in estimates compared with not correcting for TSL. When the models were adjusted by constitutional syndrome, only the ORs of p,p^0-DDT (TSL-corrected) and b-HCH (TSL-corrected and TSL-uncorrected) increased (changes between 10 and 20%) com-pared with the models without constitutional syndrome. There were no relevant changes when the models were adjusted by asthenia. When weight loss was included in the models, the odds of a K-ras mutation increased for TSL-corrected p,p^0 -DDT and decreased for TSL-corrected b-HCH. However, when the models were adjusted by cachexia, the most important change was observed in the lowering of the ORs of TSL-uncorrected HCB.

4. Discussion

Serum concentrations of TSL-corrected p,p^0-DDT, PCB 138 and PCB 153 were associated with K-ras mutations in EPC patients (Porta et al., 2009b). Two OCs were associated with K-ras muta-tions when they were not TSL-corrected: p,p^0-DDT and HCB. With the exception of HCB, the associations were stronger after TSL correction. In healthy populations, under the assumption that body concentrations of OCs are in a state of equilibrium (between blood and fat tissue), models corrected by TSL are deemed preferable than models uncorrected by TSL (Phillips et al., 1989). This assumption may not hold in populations with significant changes in lipid concentrations, such as EPC patients. The change in the OR after adjustment suggests that TSL confounded the association between p,p^0-DDT, PCB 138 and PCB 153 and K-ras.

Therefore, TSL and EPC symptoms could be considered as con-founders among EPC patients, and we provide DAGs to demon-strate these associations (Fig. 2a). Adjustment and modeling strategies alone cannot prove that TSL-corrected models are better than TSL-uncorrected models, because modeling strategies alone are inadequate to determine the impact of unmeasured confound-ing, time varying confounding, and additional bias, and demand an explicit causal framework to support these strategies. Residual confounding may persist due to the large variation in lipid concen-trations and EPC related symptoms in patients with EPC. Dimin-ished bias following adjustment for EPC symptoms would be expected if an unmeasured variable was associated with K-ras mutations and symptoms, but was not affected by OC levels. In Fig. 2a we summarize this causal structure, and based upon our structured analysis including and excluding likely confounding fac-tors, the DAG that best exemplifies our findings is demonstrated in Fig. 2a.

OCs3 was the only measured OCs in our study, and it is the only OCs that can be obtained in case-control and case-case studies. However, OCs3 is a proxy for OCs1 and all our comments here on the association between OCs3 and K-ras mutations refer to the pos-sible causal effect of OCs1 on K-ras mutations.

The results show that signs and symptoms affected the TSL-cor-rected OCs more than they affected TSL-uncorrected OCs, changes in most ORs were higher in TSL-corrected models than in TSL-uncorrected models (compared with models adjusted for age and sex). Cholestatic syndrome, constitutional syndrome, and vomiting reflect a range of severity that may not be fully captured by adjust-ment for either having such symptoms or

not. Therefore, some degree of residual confounding may be present, even in cases where adjustment for EPC symptoms was appropriate. Inclusion of signs and symptoms in TSL-uncorrected models increased the ORs only slightly, indicating that signs and symptoms did not neg-atively bias the associations between K-ras mutation status and OCs.

Another important factor to consider is the disease stage (e.g., tumoral stage) when biological samples are obtained. In the case of pancreatic cancer, tumor stage may not be a powerful discrimi natory variable, because most patients are diagnosed at stage IV (Porta, 2001; Porta et al., 2012). By contrast, the interval between first symptom and blood extraction (ISE) may be one of the possi-ble accurate measures of duration of disease until blood draw. In principle, ISE is not a better proxy of disease progression than tumor stage because diseases do not progress uniformly as time increases. Thus, adjusting for ISE may be appropriate both when analyzing TSL-corrected and TSL-uncorrected OCs, but might not be sufficient. An aggressive disease process further implies that patients begin to develop different signs and symptoms (e.g., weight loss, jaundice, hypocholia).

Fig. 2a illustrates that adjustment for cholestatic syndrome and TSL at time 3 (when EPC was symptomatic), together with adjust-ment for age and sex are sufficient to control for confounding in the association between OCs at time 3 and K-ras mutation. Chole-static syndrome and TSL together are a consequence of an unmea-sured confounding factor, represented by a U on the DAG (Fig. 2a). The best candidates for U are the pathophysiological processes inherent to EPC. As control for an unmeasured confounding factor is impossible, measuring a variable that is affected by such a factor is one way to control for it. Fig. 2b illustrates the causal structure under which adjustment for EPC-related symptoms and TSL is not appropriate. In this case, neither variable may be considered a confounding factor in the association between K-ras mutation and OCs at time 3. Therefore, adjustment for TSL or EPC symptoms would not diminish bias. However, as it is not possible to detect the underlying causal structure by comparing the magnitude of ORs adjusted and not adjusted for particular factors, the proposed causal structure must be determined a priori. Addressing such issues through a causal DAG approach is a good solution, as relying on change in estimate criteria –though commonly done– cannot reliably differentiate between true confounding factors and factors that introduce bias if they are conditioned on.

Additional criteria support the inclusion of TSL when evaluating the association between OCs and K-ras mutations. Den Hond et al. (2009), conclude that in terms of adjusted R^2, the regression model with TSL-corrected OCs was superior to models with TSL-uncor-rected OCs. In previous articles we concluded that correcting OCs by TSL may be inappropriate in patients with severe diseases as pancreatic cancer (Porta et al., 2009a). Schisterman et al. (2005) and Gaskins and Schisterman (2009) reported that lipid standard-ization, or the division of serum concentrations of PCBs by serum lipids (in a simulation study of patients with breast cancer) was highly prone to bias, but that simple lipid adjustment did not induce significant bias in similar settings. The reason for bias with TSL-standardized OCs may be that the formula to calculate TSL (Phillips et al., 1989) stemmed from a study in healthy patients, in whom an equilibrium exists between serum lipids and lipids in adipose tissue; as mentioned, such an equilibrium is rarely pres-ent among individuals with cancer (Porta et al., 2009c). Alterna-tively, lipids may not be true confounders of the relationship of interest, as suggested by Fig. 2b. Other formulas to calculate TSL are based on the same principle (Bergonzi et al., 2009).

Our work has some limitations. The study size was small. Fur-thermore, this is the largest study on OCs, genetic alterations and clinical information in pancreatic cancer, and one of the largest with analyses on OCs and tumor DNA in any type of cancer (Fryzek et al., 2006; Slebos et al., 2000). Also, a high response rate was achieved and a high proportion of cases were interviewed face-to-face and had blood drawn around the time of diagnosis. Our objective was to compare the alterations to the odds of a K-ras mutation in relation to OC exposure from additional adjust-ment for lipid, disease symptoms and other clinical factors, in patients with EPC. As adjustment for TSL strengethened associa-tions of interest, and based on a priori knowledge, in our view Fig. 2a best exemplifies that TSL and EPC symptoms are descen-dents of a common cause between the exposure and outcome of interest. Therefore, adjustment for these factors diminished con-founding bias. If the study measures OCs prior to the onset of symptoms and lipid mobilization, correction for lipids and symp-toms may not be necessary based upon our proposed DAGs. If OCs are measured after disease onset, then correcting by lipids may bias estimates, and one may have no alternative: crude OC concentrations will also be biased (Porta et al., 2009c).

It is important to note how different these two situations are:
(1) OCs affect lipids, and (2) the disease process affects OCs and lip-ids. The present paper deals with (2) and in this situation, adjusting by symptoms of the disease (which reflect pathophysiologic pro-cesses that causally influence lipids) while simultaneously adjust-ing for TSL would minimize bias, with the additional advantage that cancer symptoms are seldom or never instantaneous, i.e., they have a temporal dimension. There is also another situation (3): lipid correction or adjustment is a valid option (it is probably required) to deal with lack of information on fasting status, and when we are not in situation (1) (i.e., OCs do not affect lipids), nor are we in situation (2) (the disease has not affected OCs). In other words, correction of OCs by TSL is a valid option when the bias that may cause ignorance of fasting status is potentially much higher than the bias that could cause the influence of OCs on lipids.

If OCs alter lipid concentrations, profiles or patterns over a long time and subtlely (which is likely) (Goncharov et al., 2008; Lee et al., 2011): (a) it will be scientifically relevant to focus on such changes as an intermediate outcome (e.g., influence of OCs on lip-ids as a main study aim); (b) it will be important to have longitu-dinal data with a long time between measurement(s) of OCs and measurement(s) of lipids; and (c) measurements of metabolic bio-markers and other intermediate biomarkers will also be of interest (Leijs et al., 2009; Porta, 2012).

In aggressive diseases that may alter lipid profiles, correcting OCs by lipids and adjusting the models for variables such as the cholestatic syndrome or the ISE may generate less bias or palliate some bias. Our findings might have implications as well for study-ing environmental causes of other clinically aggressive diseases. Nonetheless, further research is needed to refute or to confirm these results before methodological implications are considered. The rationale for the current practice of correcting OCs and other lipophilic biomarkers of exposure in research on aggressive dis-eases is yet unclear.

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Table 1
Odds of mutation in the K-ras oncogene by concentrations of organochlorine compounds in cases of EPC.

	OCs total lipids corrected[a] (N = 103)			OCs total lipids uncorrected[a] (N = 103)			OCs total lipids corrected[b] (N = 103)			OCs total lipids uncorrected[b] (N = 103)		
Tertiles of OC	OR	(95% CI)	P	OR	(95% CI)	P	OR	(95% CI)	P	OR	(95% CI)	P
p,p^0 -DDT												
Low tertile	1.00		0.008[c]	1.00		0.035[d]	1.00		0.009[c]	1.00		0.046[d]
Mid tertile	26.8	(3.06–234.5)		3.50	(1.08–11.30)		31.1	(3.33–290.6)		3.66	(1.06–12.62)	
Upper tertile	2.61	(0.80–8.52)		3.47	(0.94–12.85)		2.17	(0.64–7.37)		3.36	(0.89–12.69)	
p,p^0 -DDE												
Low tertile	1.00		0.176[d]	1.00		0.393[d]	1.00		0.245[d]	1.00		0.566[d]
Mid tertile	1.61	(0.53–4.85)		1.16	(0.38–3.48)		1.47	(0.47–4.54)		1.09	(0.35–3.42)	
Upper tertile	2.35	(0.67–8.25)		1.74	(0.50–6.10)		2.13	(0.58–7.73)		1.47	(0.41–5.28)	
PCB 138												
Low tertile	1.00		0.024[d]	1.00		0.083[d]	1.00		0.014[d]	1.00		0.075[d]
Mid tertile	2.08	(0.62–6.96)		2.40	(0.74–7.83)		2.00	(0.58–6.87)		2.37	(0.72–7.84)	
Upper tertile	4.02	(1.16–13.85)		2.68	(0.83–8.67)		4.84	(1.35–17.36)		2.81	(0.85–9.26)	
PCB 153												
Low tertile	1.00		0.017[d]	1.00		0.157[d]	1.00		0.012[d]	1.00		0.158[d]
Mid tertile	1.66	(0.55–5.00)		2.37	(0.69–8.13)		1.56	(0.50–4.83)		2.31	(0.67–7.90)	
Upper tertile	5.17	(1.30–20.47)		2.24	(0.72–7.00)		6.03	(1.47–24.77)		2.25	(0.71–7.12)	
PCB 180												
Low tertile	1.00		0.161[c]	1.00		0.203[d]	1.00		0.171[c]	1.00		0.219[d]
Mid tertile	0.41	(0.13–1.33)		1.51	(0.49–4.70)		0.43	(0.13–1.38)		1.38	(0.44–4.37)	
Upper tertile	1.15	(0.30–4.33)		2.20	(0.65–7.50)		1.21	(0.31–4.72)		2.17	(0.63–7.44)	
HCB												
Low tertile	1.00		0.175[d]	1.00		0.044[c]	1.00		0.388[c]	1.00		0.048[c]
Mid tertile	0.94	(0.27–3.22)		1.81	(0.48–6.77)		1.14	(0.32–4.01)		1.82	(0.47–7.03)	
Upper tertile	0.34	(0.08–1.44)		0.28	(0.07–1.15)		0.44	(0.10–1.93)		0.28	(0.06–1.23)	
b-HCH												
Low tertile	1.00		0.133[c]	1.00		0.143[c]	1.00		0.193[c]	1.00		0.139[c]
Mid tertile	3.63	(1.03–12.77)		3.06	(0.95–9.82)		3.28	(0.91–11.81)		3.04	(0.93–9.90)	
Upper tertile	1.44	(0.44–4.70)		2.19	(0.59–8.06)		1.55	(0.46–5.28)		2.43	(0.64–9.22)	

[a] OR adjusted by age and sex.
[b] OR adjusted by age, sex and cholestatic syndrome.
[c] p-Value derived from Wald test.
[d] Test for linear trend (multivariate analogue of Mantel's extension test).