Early report

Serum concentrations of organochlorine compounds and K-*ras* mutations in exocrine pancreatic cancer

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Summary

Background Organochlorine compounds such as 1,1,1-trichloro-2,2-bis(p-chlorophenyl)-ethane (*p,p′*-DDT), 1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene (*p,p′*-DDE), and some polychlorinated biphenyls (PCBs) are carcinogenic to animals and possibly also to human beings. Occupational exposure to DDT may increase the risk of pancreas cancer. The high frequency of K-*ras* mutations in pancreatic cancer remains unexplained. We analysed the relation between serum concentrations of selected organochlorine compounds and mutations in codon 12 of the K-*ras* gene in patients with exocrine pancreatic cancer.

Methods Cases were prospectively identified in five hospitals. Mutations in K-*ras* were analysed by PCR and artificial restriction fragment length polymorphism. Cases of pancreatic cancer with wild-type K-*ras* (n=17) were frequency matched for age and sex to cases of pancreatic cancer with a K-*ras* mutation (n=34, case-case study). These 51 cases were further compared with 26 hospital controls (case-control comparison). Serum organochlorine concentrations were measured by high-resolution gas chromatography with electron-capture detection and negative ion chemical ionisation mass spectrometry.

Findings Serum concentrations of *p,p′*-DDT were significantly higher in pancreatic cancer cases with a K-*ras* mutation than in cases without a mutation (odds ratio for upper tertile 8·7 [95% CI 1·6–48·5], p for trend=0·005). For *p,p′*-DDE the corresponding figures were 5·3 (1·1–25·2, p for trend=0·031). These estimates held after adjusting for total lipids, other covariates, and total PCBs. A specific association was observed between a glycine to valine substitution at codon 12 and both *p,p′*-DDT and *p,p′*-DDE concentrations (odds ratio 15·9, p=0·044 and odds ratio 24·1, p=0·028; respectively). A similar pattern was shown for the major di-ortho-chlorinated PCBs (congeners 138, 153, and 180), even after adjustment for *p,p′*-DDE, but without a specific association with spectrum. Concentrations of *p,p′*-DDT and *p,p′*-DDE were similar among wild-type cases and controls, but significantly higher for K-*ras* mutated cases than for controls (p<0·01).

Interpretation Organochlorine compounds such as *p,p′*-DDT, *p,p′*-DDE, and some PCBs could play a part in the pathogenesis of exocrine pancreatic cancer through modulation of K-*ras* activation. The results require replication, but they suggest new roles for organochlorines in the development of several cancers in human beings.

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Introduction

The relation between exposure to organochlorine compounds and the risk of several major cancers is receiving abundant attention,[1,2] but there are no data for pancreatic cancer. 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)-ethane (*p,p′*-DDT), its main metabolite and environmental degradation product 1,1-dichloro-2,2-bis(*p*-chlorophenyl)-ethylene (*p,p′*-DDE), and some polychlorinated biphenyls (PCBs) are ubiquitous in the environment, lipophilic, resistance to excretion, and stored in many human tissues.[3–5]

DDT and PCBs have been judged as "possibly" and "probably" carcinogenic to human beings, respectively,[4,6] and as "reasonably anticipated to be human carcinogens".[7] Several organochlorine compounds can act as carcinogens and tumour promoters.[3–8] Some modulate the expression of oncogenes, including *ras* genes.[9,10] DDT and some PBCs have endocrine effects.[1,2,11,12] Although presumably weak, such effects may be enhanced by environmental biodegradation, the long half-lives of the compounds (about 10 years for DDE, 30 years or more for some PCBs), and their concentrations in target tissues (100-fold to 350-fold higher in adipose tissue than in blood).[1,5,6]

The aetiology of exocrine pancreatic cancer remains poorly understood. Smoking is the only firmly established environmental risk factor.[8,13] Some epidemiological studies showed that exposure to DDT, PCBs, and other organochlorine compounds increased the risk of pancreatic cancer.[1,4,8,13,14] In a study of chemical workers in Philadelphia, USA, relative risks of pancreatic cancer of 15 and higher were associated with exposure to DDT and related compounds.[14] However, epidemiological studies have generally found weak or no association.[1,4,6,8,13]

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Pancreatic cancer has a high prevalence of K-*ras* mutations in codon 12, but whether this is partly due to environmental or other factors is not known. Carcinomas of the pancreas with wild-type (non-mutated) K-*ras* may arise through a genetic pathway distinct from carcinomas with K-*ras* mutations.[15] The *ras* genes are critical DNA targets for chemical carcinogens.[16–18]

In 1991, we designed a multicentre prospective study, one of whose primary aims was to assess interactions between specific genetic alterations (notably K-*ras* mutations) and environmental, occupational, and lifestyle factors. In this report we analyse the relation between serum concentrations of organochlorine compounds and mutations in codon 12 of the K-*ras* gene in patients with exocrine pancreatic cancer. We also compare serum organochlorine concentrations in patients with pancreatic cancer with concentrations in a hospital control group.

Patients and methods

Selection of patients

The PANKRAS II study took place in 1992–95 at five general hospitals in eastern Spain.[19] The longest distance between any two of the hospitals is 420 km. Incident cases of exocrine pancreatic cancer were prospectively identified and patients were interviewed during their hospital stay on preceding symptoms, and on tobacco, coffee, and alcohol consumption for each period of life.

Blood and other biological samples were collected before any treatment was given. We took blood from cubital veins and serum was separated within 3 h. 1 mL samples were frozen and stored at −80°C. Among 185 patients with exocrine pancreatic cancer, mutations in codon 12 of the K-*ras* gene were tested for in 121, of whom 94 (77·7%) had mutations, and 27 did not. For our study, we selected all 17 cases with wild-type K-*ras* tumours from whom at least 3 mL serum were available. These wild-type cases were frequency matched (1:2 ratio) to cases with a K-*ras* mutated tumour (n=34) for age and sex. The mean age of the 51 cases was 65·9 years (SD 11·9), 55% were men, and 57% were ever smokers. There were no major differences between the 51 selected cases and the other cases in terms of a broad range of variables. There were no major differences between mutated and wild-type cases, except that mutated cases smoked slightly less and drank somewhat more coffee and alcohol than wild-type cases.[19] There were also no significant differences in the sociodemographic, ethnic, and clinical characteristics of cases, nor in K-*ras* mutation rate between hospitals. The 51 cases were also compared with 26 conventional hospital controls recruited in one of the study hospitals from patients admitted for benign, non-digestive disorders. Controls were older and had smoked less than cases. The study protocol was approved by the ethics committee of the participating hospitals, and patients gave informed consent.

Laboratory studies

We studied K-*ras* codon 12 mutations in DNA extracted from paraffin-embedded tumour tissue.[20] DNA was amplified in two steps by nested PCR. An artificial BstNI restriction endonuclease site was introduced to discriminate between wild type and mutated K-*ras* codon 12 sequences. Products were analysed by acrylamide gel electrophoresis and ethidium bromide staining. This technique can detect one homozygous mutated cell among 100 normal cells. To characterise the nucleotide substitution in codon 12, all mutated samples were then analysed by a similar approach based on restriction fragment length polymorphism. Interpretation of electrophoresis results from digestion products was done independently by two investigators (NM and FXR).[20]

All serum samples (500–800 μL volumes) were analysed in random order under masked conditions after addition of a surrogate solution (0·36 μg/L tetrabromobenzene).[21] The mixture was acid digested in *n*-hexane plus concentrated sulphuric acid, and we extracted the organochlorine compounds in *n*-hexane solution after vortex stirring and centrifugation. The *n*-hexane extracts were further purified with additional sulphuric acid oxidation. Compound identification and quantification used electron capture gas chromatography (Hewlett-Packard model 5890A, Palo Alto, CA, USA) and a DB-5 column (film thickness 0·25 μm). Helium and nitrogen were the carrier and the make-up gases, respectively. Selected samples were analysed by negative ion chemical ionisation gas chromatography coupled to mass spectrometry for confirmation of the qualitative and quantitative results. Ammonia was used as reagent gas.[21]

	All cases (n=51)	K-*ras*		Unadjusted odds ratio (95% CI)	p
		Mutated (n=34)	Wild-type (n=17)		
***p,p′*-DDT**					
Mean (SD)*	1·35 (1·40)	1·72 (1·46)	0·61 (0·95)		0·014†
Median*	1·20	1·37	0·20		..
Tertiles					
Nd+DNq	21 (41·2%)	9 (26·5%)	12 (70·6%)	1·0	..
⩽1·87	15 (29·4%)	12 (35·3%)	3 (17·6%)	5·3 (1·2–24·7)	..
>1·87	15 (29·4%)	13 (38·2%)	2 (11·8%)	8·7 (1·6–48·5)	0·005‡
***p,p′*-DDE**					
Mean (SD)*	18·80 (19·65)	22·45 (22·23)	11·51 (10·08)		0·067†
Median*	12·49	14·79	6·82		..
Tertiles					
⩽7·38	17 (33·3%)	8 (23·5%)	9 (52·9%)	1·0	..
7·39–20·77	17 (33·3%)	12 (35·3%)	5 (29·4%)	2·7 (0·7–11·1)	..
>20·77	17 (33·3%)	14 (41·2%)	3 (17·6%)	5·3 (1·1–25·2)	0·031‡
PCB 138					
Mean (SD)*	1·45 (1·29)	1·70 (1·24)	0·94 (1·28)		0·047†
Median*	1·45	1·72	0·22		..
Tertiles					
Nd+DNq	18 (35·3%)	8 (23·5%)	10 (58·8%)	1·0	..
⩽1·87	17 (33·3%)	12 (35·3%)	5 (29·4%)	3·0 (0·7–12·1)	..
>1·87	16 (31·4%)	14 (41·2%)	2 (11·8%)	8·8 (1·5–50·3)	0·008‡
PCB 153					
Mean (SD)*	1·59 (1·14)	1·87 (1·24)	1·04 (0·63)		0·014†
Median*	1·31	1·45	0·82		..
Tertiles					
DNq	14 (27·5%)	6 (17·6%)	8 (47·1%)	1·0	..
⩽1·87	19 (37·3%)	14 (41·2%)	5 (29·4%)	3·7 (0·9–16·3)	..
>1·87	18 (35·3%)	14 (41·2%)	4 (23·5%)	4·7 (1·0–21·7)	0·047‡
PCB 180					
Mean (SD)*	2·01 (1·49)	2·33 (1·67)	1·35 (0·72)		0·017†
Median*	1·56	2·00	0·85		..
Tertiles					
DNq	19 (37·3%)	9 (26·5%)	10 (58·8%)	1·0	..
⩽1·87	16 (31·4%)	11 (32·4%)	5 (29·4%)	2·4 (0·6–9·8)	..
>1·87	16 (31·4%)	14 (41·2%)	2 (11·8%)	7·8 (1·4–44·0)	0·013‡

Data are number of cases (%) except * ng/mL. †Mann-Whitney *U* test. ‡Test for linear trend. Nd=not detected. DNq=detected, not quantifiable. The lower tertile is the reference category (odds ratio=1·00).

Table 1: **Serum concentrations of *p,p′*-DDT, *p,p′*-DDE, and predominant PCBs in cases of exocrine pancreatic cancer with and without a mutation in the K-*ras* gene**

We determined the linear range of the detector from injection of standard mixtures. Calibration lines were calculated for all organochlorine compounds. These compounds were then quantified in the samples by the external standard method after replicate analysis. The concentrations of pentachlorobenzene, hexachlorobenzene, and hexachlorocyclohexanes were corrected for volatility losses, with tetrabromobenzene used as internal standard. Limits of detection and quantification were 0·1 and 0·3 ng/mL, respectively.[21] When we detected concentrations of organochlorine compound that were lower than our quantification threshold, the mid-value between detection and quantification limits was assigned. The total PCB value was the arithmetic sum of the concentrations of all PCB congeners. Concentrations did not vary significantly by study centre, tumour stage, or by preceding signs and symptoms such as cachexia and weight loss.

Total cholesterol and triglycerides concentrations were determined enzymatically by use of CHOD-PAP and GPO-PAP methods, respectively (Roche Diagnostics, Basle, Switzerland), and measured in a Cobas Mira Plus analyser (Roche) in a sample from the same serum volume that was used for the organochlorine analyses. Interassay coefficients of variation were 2·5% and 3·2% for total cholesterol and triglycerides, respectively. To adjust

Tertiles (μg/g lipid)	Adjusted by lipids and covariates*		Adjusted by lipids, covariates* and total PCBs† or DDE‡	
	Odds ratio (95% CI)	p for trend	Odds ratio (95% CI)	p for trend
***p,p′*-DDT**				
Nd+DNq	1·0		1·0	
≤0·25	11·9 (1·1–134)		12·3 (1·0–147)	
>0·25	17·6 (1·0–320)	0·027	17·9 (1·0–331)	0·031
***p,p′*-DDE**				
≤1·05	1·0		1·0	
1·053–2·56	1·0 (0·2–5·6)		1·1 (0·2–5·8)	
>2·56	6·6 (1·1–40·1)	0·043	7·4 (1·1–51·6)	0·049
PCB 138				
ND+DNq	1·0		1·0	
≤0·27	2·9 (0·5–17·2)		4·0 (0·5–30·7)	
>0·27	6·8 (1·1–41·5)	0·034	6·6 (0·9–47·4)	0·050
PCB 153				
DNq	1·0		1·0	
≤0·23	1·8 (0·4–7·6)		1·7 (0·3–8·3)	
>0·23	7·2 (1·1–45·6)	0·035	6·0 (0·9–41·2)	0·069
PCB 180				
DNq	1·0		1·0	
≤0·28	2·8 (0·6–14·3)		5·3 (0·7–40·9)	
>0·28	6·3 (1·0–38·8)	0·038	9·6 (1·1–83·7)	0·028

*Estimates are derived from values individually corrected by total lipid; in addition to age and sex (matching criteria), estimates are adjusted by tobacco, alcohol, and coffee consumption. †Estimates for *p,p′*-DDT and *p,p′*-DDE further adjusted by total PCBs. ‡Estimates for the 3 PCB congeners further adjusted by *p,p′*-DDE.
Nd=not detected. DNq=detected, not quantifiable.

Table 2: **Results of multivariate conditional logistic regression analysis**

organochlorine concentrations individually for total lipids we used a standard formula.[22] There were no significant differences between the 34 K-*ras* mutated cases and the 17 wild-type cases in serum cholesterol concentration (mean 214·1 mg/100 mL [SD 110·7]) and triglycerides (179·9 [101·7]). The two measurements were only slightly lower among the 26 controls (cholesterol 203·6 mg/100 mL [52·8]; triglycerides 159·8 [151·2]; p=0·577 and p=0·545, respectively, for differences from the 51 cases).

Statistical analysis

Our case-case analysis compared the 34 cases of pancreatic cancer that had a codon 12 K-*ras* mutation with the 17 cases of pancreatic cancer that did not have such mutation. Our case-control analyses compared all 51 cases of pancreatic cancer with the 26 hospital controls. In contingency tables, for ordered categorical variables we used the Mantel-Haenszel χ^2 test for linear trend. We estimated multivariate-adjusted odds ratios and their 95% CIs by unconditional and conditional logistic regression (SAS, version 6.12). We analysed categorical ordinal variables for a linear dose-response relation through the multivariate analogue of Mantel's extension test. Significance was set at 0·05, and all tests were two-tailed.

Results

All 51 cases of exocrine pancreatic cancer had detectable concentrations of *p,p′*-DDE, and 36 (71%) cases had detectable concentrations of *p,p′*-DDT. Cases of pancreatic cancer with a K-*ras* mutation had significantly higher concentrations of *p,p′*-DDT and *p,p′*-DDE than cases with wild-type K-*ras* (table 1). Tumours of patients in the mid and upper *p,p′*-DDT tertiles were more than five times more likely and more than eight times more likely to have a mutation, respectively, than tumours of cases in the lower tertile (p for trend=0·005). For *p,p′*-DDE, the corresponding odds ratios were 2·7 and 5·3 (p for trend=0·031).

The Spearman's correlation coefficient between *p,p′*-DDT and *p,p′*-DDE was 0·65 (p<0·01), and between *p,p′*-DDE and total PCBs the coefficient was 0·30 (p=0·03). When the potential confounding effects of lipids, other covariates, and total PCBs were accounted for, the association between K-*ras* mutation and both *p,p′*-DDT and *p,p′*-DDE remained significant (table 2). No significant interactions were noted.

Serum concentrations (ng/mL)	Controls (n=26)	p values for comparison of controls *vs* cases of exocrine pancreatic cancer		
		All 51 cases	34 mutated	17 wild-type
***p,p′*-DDT**				
Mean (SD)	0·53 (0·67)	0·047*	0·003*	0·643*
Median	0·20	<0·001†	<0·001†	0·130†
***p,p′*-DDE**				
Mean (SD)	9·41 (7·25)	0·030*	0·006*	0·646*
Median	7·59	0·009†	0·002†	0·288†
PCB 138				
Mean (SD)	1·13 (0·77)	0·661*	0·113*	0·096*
Median	1·07	0·185†	0·058†	0·718†
PCB 153				
Mean (SD)	1·26 (0·64)	0·455*	0·099*	0·289*
Median	1·26	0·122†	0·030†	0·369†
PCB 180				
Mean (SD)	1·30 (0·58)	0·040*	0·004*	0·935*
Median	0·85	0·009†	0·003†	0·504†

*Mann-Whitney's *U* test. †Covariance analysis, adjusted for total lipids, age, sex, and tobacco, alcohol, and coffee consumption.

Table 3: **Serum concentrations of compounds among the 26 hospital controls, and significance of differences between them and all 51 cases of pancreatic cancer, 34 cases with a K-*ras* mutation, and 17 cases without a K-*ras* mutation**

The mutation spectrum was available for 15 of the 34 mutated cases. A specific association was observed between *p,p′*-DDE and a glycine to valine substitution (GGT→GTT) at K-*ras* codon 12: all six cases with this transversion had *p,p′*-DDE serum concentrations above the median of the 51 cases combined, compared with only three of nine cases with other mutations (odds ratio 24·1, p=0·028). For *p,p′*-DDT the odds ratio was 15·9 (p=0·044). Compared with wild-type cases, cases with the valine mutation were substantially more likely to have *p,p′*-DDE concentrations above the median (odds ratio 18·2, p=0·019). For *p,p′*-DDT the odds ratio was 53·9 (p<0·001). There was no association between serum concentrations of these two compounds and the other mutations.

Serum concentrations of *p,p′*-DDT and *p,p′*-DDE were significantly higher among the 51 cases of pancreatic cancer than among the 26 controls (table 3). Concentrations among cases with wild-type K-*ras* were similar to controls. These results were unchanged in multivariate analyses by tertiles. For instance, the 51 cases were more than five times more likely to be in the upper tertile of *p,p′*-DDE concentration than the 26 controls (multivariate-adjusted odds ratio 5·6 [95% CI 1·3–24·6]; p for trend=0·025). The odds ratio for the 34 mutated cases was 10·5 (1·9–59·3; p for trend=0·007). None of the odds ratios that compared wild-type cases with controls was significantly increased.

Serum concentrations of ten PCB congeners were detected, but seven were non-detectable or non-quantifiable in over half of the cases. All 51 cases of pancreatic cancer had detectable concentrations of congeners 153 and 180, and PCB 138 was detected in 43 cases. Values for these three congeners are shown in table 1. Patients with pancreatic cancer whose tumours had a K-*ras* mutation had significantly higher concentrations of the 3 congeners than wild-type cases (p<0·05). The chance of having a mutated tumour was

more than four to eight times higher for cases in the upper tertile than for cases in the lower tertile (p for trend <0·05 in all instances, table 1).

Correlation coefficients between each of the three predominant PCBs and total lipids were between 0·3 and 0·4 (p<0·05 in all cases). The coefficient between *p,p′*-DDE and PCB 153 was 0·43 (p<0·01). Estimates for the three PCBs were essentially unchanged in multivariate analysis (table 2). No association was observed between mutation spectrum and PCB congeners.

Concentrations of the three PCBs were higher in the 51 cases than in the 26 controls, but differences were significant only for PCB 180 (table 3). The 51 cases were more than four times more likely to be in the upper tertile of PCB 180 than the 26 controls (multivariate-adjusted odds ratio 4·6 [95% CI 1·1–19·0]; p for trend=0·037). The odds ratio for the 34 mutated cases was 7·4 (1·6–34·4; p for trend=0·012). Again, none of the odds ratios to compare wild-type cases with controls was significant.

Hexachlorobenzene was detected and quantified in all 51 cases and 26 controls; the respective means were 10·51 ng/mL (SD 7·86; median 8·64) and 9·17 (5·62; 8·75; p=0·663). The difference between mutated and wild-type cases was also very small (p=0·448). Similarly, concentrations of β-hexachlorocyclohexane were not significantly different between cases and controls (p=0·139). The respective means were 10·67 ng/mL (8·14; 8·42) and 15·03 (10·24; 12·38). The concentration did not differ between the two types of cases (p=0·215). α- and γ- hexachlorocyclohexane, octachlorostyrene, and pentachlorobenzene were found in smaller concentrations. None of those compounds showed significant differences between K-*ras* mutated cases and non-mutated cases.

Discussion

We have shown that patients with exocrine pancreatic cancer whose tumours had a K-*ras* mutation had significantly higher concentrations of *p,p′*-DDT, *p,p′*-DDE, and PCB congeners 138, 153, and 180 than patients without a mutation. The results do not necessarily imply that organochlorines play a direct part in activation of K-*ras*. Rather, the compounds might enhance the effects of K-*ras* mutagens or might provide a growth advantage to the mutated cells.

Several observations suggest that our findings are not due to chance. The likelihood that the serum concentrations of the five organochlorine compounds were in the upper tertile was five to eight times higher in cases with K-*ras* mutation than in cases with wild-type K-*ras*, and adjusted estimates were even higher. For each of these markedly raised point estimates, the 95% CI excluded the null value of unity. The association of *p,p′*-DDE and *p,p′*-DDT with the GGT→GTT transversion (Gly→Val) was substantial. The findings were already apparent in simple analyses (not just after multiple tests), and a dose-response curve was commonly observed, although precision was limited by our small dataset. In addition, the association depended on the types of chlorinated organics detected: hexachlorobenzene and hexachlorocyclohexane were detected in all patients, yet were not associated with K-*ras* mutations.

Pancreatic neoplasms containing K-*ras* mutations are induced in laboratory animals by several chemical carcinogens.[15–18] The association that we observed between *p,p′*-DDE, *p,p′*-DDT, and the GGT→GTT transversion could point to a more specific effect of the DDT family, or it could reflect the action of some carcinogen promoted by DDT analogues, such as benzo[*a*]pyrene, which in experimental models has been linked with G→T transversions in the K-*ras* gene.[15–17] DDE and PCBs such as congeners 138, 153, and 180 induce specific cytochrome P450 enzymes,[1,5,9,23,24] some of which affect the activation of environmental carcinogens.[25] The three congeners predominant in our study were not associated with any specific K-*ras* mutation, which may reflect a wider promoting effect on a number of K-*ras* mutagens.

Organochlorine compounds might also promote pancreatic carcinogenesis because of their endocrine-disrupting effects.[1,2,11,12] Androgen and oestrogen receptors have been shown in normal and neoplastic pancreas, and steroids modulate pancreatic carcinogenesis in rodents.[26] Some PCBs can bind to oestrogen receptors and stimulate the expression of genes involved in cell proliferation, including *ras* genes.[9] Oestrogens can be complete carcinogens capable of tumour initiation by gene mutations, and their hormonal effects may complete the development of tumours.[27] DDT and the more oestrogenic PCBs are established animal carcinogens,[3–9] but whether they are directly mutagenic in human beings is less clear than is their effect as tumour promoters.

The association between exposure to organochlorine compounds and K-*ras* activation has a sound time rationale. Present serum concentrations reflect exposures that occurred in the past decade.[1,3–7] Background exposures more than 10 years ago were likely to have been higher than our data show, because less time had elapsed since the use of DDT and PCBs was restricted. K-*ras* activation is an early event in the genesis of pancreatic tumours.[15–18] Therefore, organochlorines were present in the patients' bodies during the time (10 or more years before diagnosis) when K-*ras* mutations most probably occurred and played a part in the progression of precursor lesions to invasive cancer. In Spain, organochlorine residues in food may have decreased since use of organochlorine pesticides was progressively restricted in the mid-1980s. However, recent surveys showed that 78–100% of meat samples contained DDE, all sampled foods contained hexachlorobenzene and hexachloro-cyclohexane, and 50% of fish samples contained PCB residues; the congeners detected were 138, 153, and 180.[28,29]

Cancer-induced lipid mobilisation is unlikely to have biased the comparison between K-*ras* and wild-type cases, since there were no significant differences between the two types of cases in terms of prediagnostic symptoms and signs (eg, weight loss, cachexia), or in tumour stage at diagnosis. Organochlorine concentrations were unrelated to these factors. Differences in blood lipids between the two types of cases and between cases and controls were small.

The comparison of the 51 cases with the 26 controls suggests that organochlorines might increase the risk of pancreatic cancer, but the primary message of the comparison is that cases with mutated K-*ras* had higher serum concentrations of organochlorines than the general population (study base), whereas cases with wild-type K-*ras* and controls had similar concentrations. Having a control group from a single hospital limits the case-control component of the study, but is unlikely to bias the conclusion that the association was specific for cases with mutated K-*ras*. Patients from the different hospitals did not differ in their clinical and sociodemographic characteristics; they also had very similar mutation rates and concentrations of the organochlorine compounds.

Some clear increases in risk of pancreatic cancer have been associated with exposure to chlorinated organics (mostly occupational) but, overall, epidemiological evidence is limited.[1,8,13,14] Epidemiological studies are susceptible to misclassification of personal cumulative exposure to organochlorine compounds, because these stem from many sources and chemical mixtures. Measurement of serum concentrations gives more accurate and specific estimates of individual internal dose.[24] Observed serum concentrations were similar to those observed in other groups from the general population;[1,3,4] hence, background environmental exposure (eg, through contaminated foods)[5,23,29] seems a more likely source than occupational exposure.

Our findings suggest that organochlorine compounds such as *p,p′*-DDT, *p,p′*-DDE, and some PCBs could play a part in the pathogenesis of exocrine pancreatic cancer through modulation of K-*ras* activation. If extended by other studies, the results could improve our understanding of the causes of pancreatic cancer, and might help to clarify the relation between organochlorine compounds and risks of several other neoplasms.

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Contributors

Miquel Porta, Francisco Real (principal investigators), and Núria Malats (study coordinator) conceived the study and wrote the paper. Juli Rifà, Luisa Guarner, Alfredo Carrato, Antonio Salas, and Montserat Andreu contributed to identification of patients, recruitment, care, and follow-up. Josep Corominas and Antonio Salas reviewed all pathological material. Francisco Real and Núria Malats were responsible for K-*ras* analyses. Joan Grimalt and Mary Santiago-Silva analysed organochlorine compounds. Miquel Porta and Manuel Jariod analysed the data. All authors contributed to the design and conduct of the study, to interpretation of results, and to revisions of the paper.

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