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SHORT COMMUNICATION
Dioxin/polychlorinated biphenyl body burden, diabetes and endometriosis: findings in a population-based study in Belgium

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Dioxins and polychlorinated biphenyls (PCBs) are persistent organic pollutants widely distributed in the food chain, which is the main source of human exposure. Their effects on human health at background exposure levels are still poorly understood. Recent epidemiological evidence suggests a possible association between these pollutants and diabetes. We report here the results of a population-based study in Belgium on 257 (142 women and 115 men) environmentally exposed subjects, including 10 cases of endometriosis and nine cases of diabetes. Seventeen 2,3,7,8-polychlorinated dibenzodioxins/dibenzofurans (PCDD/Fs or dioxins), four coplanar PCBs (International Union of Pure and Applied Chemistry [IUPAC] nos 77, 81, 126 and 169) and 12 PCB markers (IUPAC nos 3, 8, 28, 52, 101, 118, 138, 153, 180, 194, 206 and 209) were quantified in serum fat from fasting blood samples in order to estimate the body burden of these pollutants. Whilst no difference was found between women with endometriosis and their controls, diabetic patients had significantly increased serum levels of dioxins, coplanar PCBs and the 12 PCB markers. After adjustment for age and other covariates, serum total toxic equivalent activity (sum of PCDD/Fs and coplanar PCBs) and 12 PCB marker concentrations in diabetics were 62% ($p = 0.0005$) and 39% ($p = 0.0067$) higher, respectively, than in controls. The risk of diabetes was significantly increased in subjects in the top decile for adjusted concentrations of dioxins (odds ratio 5.1, 95% confidence interval [CI] 1.18–21.7), coplanar PCBs (odds ratio 13.3, 95% CI 3.31–53.2) or 12 PCB markers (odds ratio 7.6, 95% CI 1.58–36.3). These findings warrant further studies to assess the significance of the associations between diabetes and environmental exposure to polychlorinated pollutants.

Keywords: dioxin, polychlorinated biphenyls, diabetes, endometriosis, body burden.

Introduction

Polychlorinated dibenzodioxins/dibenzofurans (PCDD/Fs or dioxins) and polychlorinated biphenyls (PCBs) are persistent lipophilic pollutants that accumulate in the food chain and the human body (Bernard *et al.* 2002). Although the average dioxin and PCB body burden has declined over recent decades in most industrialized countries, some groups in the general population still show an elevated body burden of these pollutants as a result of their dietary habits and current or past exposures. This is especially true for people who live near pollution

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sources such as old incinerators. For these groups, dioxins and PCBs might remain a matter of concern in view of the recent epidemiological evidence of a possible link between halogenated aromatic compounds and some chronic diseases such as endometriosis (Rier and Foster 2002) and diabetes. For instance, Longnecker *et al*. (2001) reported that PCB serum levels were on average 30% higher in pregnant women with diabetes (primarily type 1) than in control subjects. Other studies have also found weak associations between serum lipid 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and diabetes, not only in cases of accidental or occupational exposure but also at background exposure levels (Remillard and Bunce 2002).

The findings reported in this study provide further indication of a link between dioxins/PCBs and diabetes, but do not support the implication of these compounds in the aetiology of endometriosis in environmentally exposed populations.

Methods

After approval by the local ethical committee, a total of 257 subjects (142 women and 115 men) were recruited into this study during 2000–2001 from five areas of Belgium. They included (i) 58 subjects aged 25–67 years living within the vicinity of an iron and steel plant (Cockerill); (ii) 52 subjects aged 26–71 years living around a waste dumping site (Mont-Saint-Guibert); (iii) 33 subjects aged 33–65 years recruited from the vicinity of a municipal solid waste incinerator (MSWI) in an industrial area (Pont-de-Loup); (iv) 51 subjects aged 21–80 years living in the vicinity of a MSWI in a rural area (Thumaide); and (v) 63 subjects aged 33–66 years recruited as referents living in rural areas in Southern Belgium (Daverdisse, Nassogne and Bertrix) with no known source of pollution by dioxins and PCBs. All subjects were recruited on a volunteer basis via a mail targeting adult subjects with a long residence time in the same area and regularly consuming locally produced foods. Subjects with a possible occupational exposure to dioxins or PCBs (e.g. workers at the investigated plants) were excluded. Information about dietary habits was obtained by a self-administered questionnaire. The two incinerators at Pont-de-Loup and Thumaide had been in operation since 1978 and 1980, respectively, and their dioxin emissions had exceeded 50 ng toxic equivalents (TEQ)/Nm^3 until 1998–1999, leading to very high levels of dioxin contamination in cows' milk from nearby farms (up to 38.9 pg TEQ/g fat). After giving informed consent and filling out a detailed questionnaire, volunteers provided approximately 200–250 ml of blood under fasting conditions in order to evaluate the body burden of dioxins and PCBs. Seventeen PCDD/Fs, four dioxin-like non-*ortho*-PCBs (coplanar PCBs; International Union of Pure and Applied Chemistry [IUPAC] nos 77, 81, 126 and 169) and 12 PCB markers (IUPAC nos 3, 8, 28, 52, 101, 118, 138, 153, 180, 194, 206 and 209) were quantified by gas chromatography–high resolution mass spectrometry (GC–HRMS) on the lipid fraction of the serum. All results were reported per gram of fat. Concentrations of dioxins and coplanar PCBs were expressed in TEQs using the 1998 international toxic equivalent factors of the World Health Organization.

Information about the health status of the subjects was obtained from the questionnaire. A total of nine subjects were reported to suffer from type 2 diabetes (five women and four men), which was controlled by hypoglycaemic drugs such as sulphamides ($n = 6$), by insulin ($n = 1$) or by diet alone ($n = 2$). These diabetic subjects were residents of the vicinity of the MSWI in the rural area ($n = 3$), the iron and steel plant ($n = 2$), the waste-dumping site ($n = 2$) and the referent area ($n = 2$). Out of a total of 142 women, 10 were reported to have suffered from endometriosis. These women were living in the vicinity of the MSWI in the industrial area ($n = 4$), of the MSWI in the rural area ($n = 1$), the iron and steel plant ($n = 4$) and the waste-dumping site ($n = 1$).

Dioxin and PCB concentrations in the diabetics were compared with those in the rest of the population ($n = 248$) as controls. Cases of endometriosis were compared with women in the rest of the population ($n = 132$) as controls. The differences between cases of endometriosis or diabetes and their respective controls were assessed using the Student's t-test applied to log-transformed data (using inverse transformation for the body mass index [BMI]). A stepwise multiple linear regression model was used to identify determinants of dioxin, coplanar PCB and 12 PCB markers concentrations by testing the following independent variables: diabetes, endometriosis, age, gender, BMI, area of residence, smoking habits, weight loss, fat intake and fish consumption. After adjustment for the covariates, all case and control dioxin and PCB values were again compared using the Student's t-test. Odds ratios were calculated on the total population divided into two groups using the 90th percentile of the adjusted dioxin, coplanar PCB and 12 PCB markers concentrations as the cut-off (SAS Procedures, Enterprise Guide 2.0).

Table 1. Subject characteristics and mean dioxin and PCB concentrations.

	Endometriosis		Diabetes	
	Cases (n = 10)	Controls (n = 132)	Cases (n = 9)	Controls (n = 248)
Age (years)	49.0 (40.3–57.7)	51.2 (49.3–52.8)	56.0 (52.6–59.4)	51.5 (50.3–52.8)
BMI (kg/m^2)	26.9 (24.3–30.2)	25.1 (24.3–26.0)	26.2 (23.0–30.4)	25.6 (25.1–26.2)
Serum lipids (g/l)	7.69 (6.85–8.64)	7.36 (7.07–7.63)	7.85 (6.67–9.25)	7.38 (7.18–7.58)
Weight loss	2 (22%)	19 (16%)	3 (33%)	30 (12%)
Fat intake (g/week)[a]	165 (114–238)	167 (150–183)	252 (153–416)	181 (169–194)
PCDD/Fs (pg TEQ/g fat)	26.2 (18.2–37.7)	25.6 (24.3–28.9)	46.6 (34.7–62.5)	25.2 (23.6–26.8)*
Coplanar PCBs (pg TEQ/g fat)	7.97 (5.05–12.6)	7.45 (6.69–8.30)	16.2 (9.47–27.7)	7.2 (6.65–7.73)*
Total TEQ (pg TEQ/g fat)[b]	34.6 (23.7–50.4)	34.5 (31.7–37.6)	64.2 (46.7–88.3)	32.8 (30.8–35.0)*
12 PCB markers (ng/g fat)	294 (215–401)	372 (351–403)	652 (512–831)	402 (383–423)*

Values are the geometric mean with the 95% CI in parentheses, except for age (arithmetic mean and 95% CI), BMI (inverse transformed) and weight loss (number and percentage of subjects who lost weight within the year before examination). The endometriosis controls were exclusively women.

[a] Fat intake was calculated from the questionnaire (consumption of poultry, bovine and swine products).

[b] PCDD/Fs plus coplanar PCBs.

* Statistically significant difference between cases and controls ($p < 0.05$) using the Student's t-test.

Results

As shown in table 1, subjects with diabetes and endometriosis were well matched with their respective controls for age, BMI and serum lipids. There were no statistically significant differences in weight loss and fat intake between controls and cases of endometriosis or diabetes, even though the mean fat intake appeared to be higher in diabetics. Concentrations of PCDD/Fs and coplanar PCBs in the serum of women with endometriosis were not different from those of controls. The mean total TEQ activity (sum of PCDD/Fs and coplanar PCBs) in the serum of these women was almost identical to that in the controls, whereas the mean concentration of the 12 PCB markers was slightly lower. In addition, no differences were found when the comparison was restricted to the five cases of endometriosis living in the vicinity of incinerators: for these cases the mean concentrations of dioxins (22.5 pg TEQ/g fat), coplanar PCBs (5.9 pg TEQ/g fat) and 12 PCB markers (217 ng/g fat) was slightly lower than in the controls. By contrast, the mean serum levels of PCDD/Fs, coplanar PCBs and 12 PCB markers were significantly increased in diabetics compared with the rest of the population (table 1).

These findings were confirmed by multiple regression analyses testing the influence of diabetes, endometriosis, age, gender, BMI, area of residence, smoking habits, weight loss, fat intake and fish consumption. The analysis performed on women did not reveal any significant association between endometriosis and dioxins or PCBs. In contrast, when considering the whole population, diabetes emerged as a significant determinant (partial $r^2 = 0.03$) of PCDD/Fs levels, which also correlated positively with age (partial $r^2 = 0.16$), residence around the MSWI

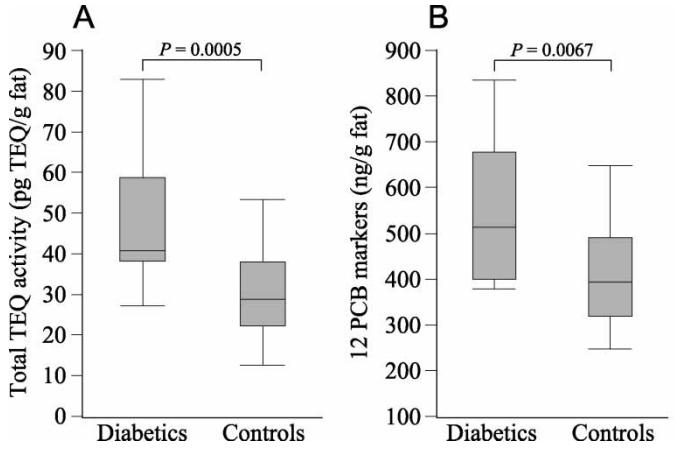

Figure 1. Distribution of serum dioxin and PCB concentrations in diabetic and non-diabetic subjects. Data are box plots showing the 5th, 25th, 50th, 75th and 95th percentiles. (A) Total TEQ activity (PCDD/Fs plus coplanar PCBs); (B) 12 PCB markers. Diabetics and controls were compared using the Student's *t*-test.

in the rural area (partial $r^2 = 0.11$), BMI (partial $r^2 = 0.02$) and fat intake (partial $r^2 = 0.015$). The serum levels of coplanar PCBs were also positively correlated with diabetes (partial $r^2 = 0.04$), together with age (partial $r^2 = 0.11$), BMI (partial $r^2 = 0.09$), residence around the rural MSWI (partial $r^2 = 0.05$) and fish consumption (partial $r^2 = 0.03$). Diabetes also correlated positively with the 12 PCB markers (partial $r^2 = 0.02$) together with age (partial $r^2 = 0.24$) and fat intake (partial $r^2 = 0.06$). Figure 1 illustrates the increase in total TEQ and 12 PCB markers in the serum of diabetic subjects after adjustment for other covariates; the body burden of these pollutants in diabetics was on average 62% ($p = 0.0005$) and 39% ($p = 0.0067$) higher than that of controls, respectively. However, this increase in the body burden of dioxins and PCBs in diabetics was not congener-specific, as all the congeners were found to rise in diabetics in the same proportions (results not shown). Of note, congener patterns found in diabetics corresponded to those of the background environmental contamination and did not show any increase in the congeners (the low-chlorinated furans) characterizing the Belgian 1999 PCB/dioxin incident (Bernard *et al.* 1999). This suggests there is no detectable influence of this punctual source of pollution on dioxin and PCB body burdens.

In order to further explore the link between diabetes and dioxins or PCBs, we estimated the probability of being diabetic by dividing the total population into two groups using the 90th percentile of the dioxin, coplanar PCB and 12 PCB markers concentrations adjusted for other covariates as the cut-off. The odds ratios were statistically significant in the top deciles, reaching values of 5.07 (95% confidence interval [CI] 1.18–21.7) for dioxins, 13.3 (95% CI 3.31–53.2) for coplanar PCBs and 7.58 (95% CI 1.58–36.3) for the 12 PCB markers.

Discussion

Although based on a relatively small number of cases, our results do not provide evidence supporting the hypothesis that dioxins or PCBs are involved in the

aetiology of endometriosis, at least at current exposure levels in the environment. The cases of endometriosis recruited in this study, even those living in the vicinity of incinerators, had almost identical serum concentrations of dioxins and coplanar PCBs to the controls, while the levels of the 12 PCB markers were slightly lower than those of the controls. These findings are in accordance with those of the recent survey conducted in Seveso residents (Eskenazi *et al.* 2002). Despite dioxin (TCDD) levels largely above those in the present study and also a larger population (601 female residents including 19 cases of endometriosis), this study detected only a small non-significant increase in the risk of endometriosis and failed to show a clear dose–response relationship. Our findings in diabetics are, however, much more surprising, since this group showed a highly significant elevation of serum levels of dioxins, coplanar PCBs and the 12 PCB markers. In addition, for these three categories of pollutants, a significant increase in the risk of diabetes was found in the most exposed subjects (top decile). All these observations remained statistically significant after adjustment for possible confounders (age, BMI, fat intake, fish consumption or place of residence) identified by multivariate analysis.

These associations between diabetes and dioxins or PCBs, however, have to be confirmed by other studies involving larger numbers of cases and should be interpreted with caution, since the direction of the potential causality still remains to be established. There are presently no data allowing us to determine whether the higher levels of dioxins and PCBs in diabetics truly reflect a higher exposure to these pollutants, which in turn may contribute to diabetogenesis, or whether they are merely the consequence of diabetes-induced metabolic perturbations facilitating the accumulation of these pollutants. Indeed, diabetes is associated with a variety of metabolic changes, which quite conceivably could alter the metabolism of dioxins and PCBs. For instance, it is known that diabetes can alter the pharmacokinetics of some drugs (Gwilt *et al.* 1991) and also affect the activity of cytochrome P450 (Pass *et al.* 2002). Diabetes, especially type 2 diabetes, is also known to cause a dysregulation of fat metabolism, which in turn might influence the distribution and elimination of lipophilic compounds such as dioxins and PCBs. The possibility of a slower elimination of dioxins in diabetes is, however, not supported by a recent study on Vietnam veterans, in whom no difference in TCDD half-life was found between diabetic and non-diabetic patients (Michalek *et al.* 2003). In our study, concentrations of serum lipids in diabetics were similar to those in the controls, and there were no alterations in the dioxin and PCB congener patterns seen in the serum fat from diabetics, which also tends to argue against the hypothesis of a slower biotransformation of these compounds in diabetes. Finally, diabetes could be hypothesized to increase the serum fat to adipose tissue ratio of dioxins and PCBs, but whereas a difference in the tissue distribution of these compounds is usually associated with a shift in the congener patterns, this was not found to be the case in our study.

The second hypothesis is that dioxins or PCBs might play a role in the aetiology of diabetes. Such a possibility is suggested by several epidemiological studies linking a higher dioxin or PCB body burden to an increased risk of diabetes or modified glucose metabolism. These studies have been conducted on industrial workers and other populations experiencing high levels of exposure, but also in

populations exposed to background levels, suggesting that these pollutants may act at relatively low levels of exposure. Interestingly, it has recently been hypothesized (Remillard and Bunce 2002) that dioxins and PCBs could promote diabetes by interaction with peroxisome proliferator-activated receptor-γ, a ligand-activated transcription factor controlling lipid metabolism and homeostasis that is linked with diabetes (Smith 2001). If such a mechanism could be demonstrated, this would provide a plausible biological explanation for the associations seen in epidemiological studies.

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References

Bernard, A., Hermans, C., Broeckaert, F., De Poorter, G., De Cock, A. and Houins, G. 1999, Food contamination by PCBs and dioxins. *Nature*, **401**, 231–232.

Bernard, A., Broeckaert, F., De Poorter, G., De Cock, A., Hermans, C., Saegerman, C. and Houins, G. 2002, The Belgian PCB/dioxin incident: analysis of the food chain contamination and health risk evaluation. *Environmental Research*, **88**, 1–18.

Eskenazi, B., Mocarelli, P., Warner, M., Samuels, S., Vercellini, P., Olive, D., Needham, L. L., Patterson, D. G. Jr., Brambilla, P., Gavoni, N., Casalini, S., Panazza, S., Turner, W. and Gerthoux, P. M. 2002, Serum dioxin concentrations and endometriosis: a cohort study in Seveso, Italy. *Environmental Health Perspectives*, **110**, 629–634.

Gwilt, P. R., Nahhas, R. R. and Tracewell, W. G. 1991, The effects of diabetes mellitus on pharmacokinetics and pharmacodynamics in humans. *Clinical Pharmacokinetics*, **20**, 477–490.

Longnecker, M. P., Klebanoff, M. A., Brock, J. W. and Zhou, H. 2001, Polychlorinated biphenyl serum levels in pregnant subjects with diabetes. *Diabetes Care*, **24**, 1099–1101.

Michalek, J., Ketchum, N. and Tripathi, R. 2003, Diabetes mellitus and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin elimination in veterans of Operation Ranch Hand. *Journal of Toxicology and Environmental Health A*, **66**, 211–221.

Pass, G. J., Becker, W., Kluge, R., Linnartz, K., Plum, L., Giesen, K. and Joost, H. G. 2002, Effect of hyperinsulinemia and type 2 diabetes-like hyperglycemia on expression of hepatic cytochrome p450 and glutathione S-transferase isoforms in a New Zealand obese-derived mouse backcross population. *Journal of Pharmacology and Experimental Therapeutics*, **302**, 442–450.

Remillard, R. B. and Bunce, N. J. 2002, Linking dioxins to diabetes: epidemiology and biologic plausibility. *Environmental Health Perspectives*, **110**, 853–858.

Rier, S. and Foster, W. G. 2002, Environmental dioxins and endometriosis. *Toxicological Sciences*, **70**, 161–170.

Smith, S. A. 2001, Peroxisome proliferator-activated receptors and the regulation of mammalian lipid metabolism. *Biochemical Society Transactions*, **30**, 1086–1090.