CONFOUNDING FACTORS INFLUENCING SERUM DIOXIN CONCENTRATIONS IN THE FRENCH DIOXIN AND INCINERATORS STUDY

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Abstract

When comparing a potentially exposed group to dioxins to a referent group, it is crucial to identify all the confounding factors. In the French Dioxin and Incinerators Study, serum analyses of PCDDs, PCDFs and PCBs were performed in 1030 adults randomly selected to identify the determinants of the body-burden of these compounds in the population living close to waste incinerators. The impact of age, body mass index (BMI) and other potential confounders on the serum levels of all these compounds was assessed.

Many factors were relevant for determining current serum levels of dioxins: age, BMI, gender, recent change in weight, smoking status, occupation, location of residence, background food intake, burning activities, and recreational activity. For example, we observed an average increase of approximately 0.3 pg TEQ PCDD/Fs/g lipids per year of age. We recommend that future studies consider all these factors when comparing a group potentially exposed to dioxins to a referent group to avoid misinterpretation.

Introduction

Several studies provide evidence that serum dioxin levels can vary with many factors, such as age and body mass index (BMI). Age has been found to be an important determinant, with older persons generally having higher levels. The amount of body fat has also been correlated with dioxin levels. However, some studies find no association or a negative association with serum dioxin levels and BMI. [1]

When comparing a potentially exposed group to a referent group, it is crucial to identify all these factors and to control them to determine whether the difference of dioxin levels between the two groups is due to the environmental exposure independently of these confounding factors. [1,2,3,4]

The objective of the French Dioxin and Incinerators Study was to determine whether the emissions of municipal solid waste incinerators contribute to the body-burden of PCDDs, PCDFs and PCBs in the surrounding population in comparison to referent people and to know if some factors, such as local food consumption, could influence their serum levels. The first step before providing an answer to this question was to study the different factors that could explain the variation in serum dioxin levels among the population independently of the incinerators. So, in the present work, we investigate the different potential confounders of serum dioxin concentrations.

Materials and Methods

The French Dioxin and Incinerators Study has been carried out by the National Institute of Public Health Surveillance (InVS) in collaboration with the French National Food Safety Agency (Afssa). 1030 adults (30-65 years old) have been included between March and July 2005 in 8 different areas in France around municipal solid waste incinerators. They were selected through a stratified two stage random sampling. In each of the 8 areas, about 130 people falling within the following criteria were studied: living for at least 10 years around the incinerator, without occupational exposure, and no breastfeeding in the past 15 years for women. Exposed people were living in the impact area of the incinerator's plume and non-exposed people (referents) were living beyond 20 km of the incinerator and not exposed to known dioxins sources. In each of the exposed or not exposed study groups, the population studied was divided in two groups:

1) people eating home-grown or food produced locally (poultry, meat, eggs, milk, fruit and vegetables…),

2) people not eating home-grown or locally-produced food.

After having given their informed consent, the participants provided about 200 ml of blood under fasting conditions in the morning with the assistance of staff from the French National Agency for Blood.
Exposure was assessed by serum concentrations of the 17 classical dioxins, furans (PCDDs and PCFs), 12 DL-PCBs and 4 marker PCBs (IUPAC 118, 138, 153, 180). A fast automated extraction and clean-up procedure was used for low-level analysis. Samples were analyzed by GC–HRMS on the lipid fraction of serum. The total lipids content was measured by enzymatic summation method. Blind controls were used to assess the quality of the analyses. Concentrations of dioxins and PCBs are expressed in pg WHO_{1998}-TEQ per gram of lipids. Total TEQ corresponds to the TEQ sum for PCDDs, PCDFs and the 12DL-PCBs.

The questionnaire consisted of a one hour face-to-face interview with different sections: questions to determine the eligibility of each participant, socio-demographic, environmental and food (general and local) questionnaires. Basic demographic (age, gender, education, marital status) and health questions (height, weight, recent weight change, smoking status, childbearing and breastfeeding) were included. The participants were asked to recall possible dioxin exposure pathways in the past. Questionnaires included a full residential history (since the beginning of use of incinerators), occupations, recreational activities and environmental exposure: urbanism (rural, suburb, city), type and date of construction of the household, barbecue, type of heating, burning, exposure to a fire, number of minutes per week in a vehicle.
Food intake was quantified by a validated food frequency and portions questionnaire detailed for the food vectors of animal lipids, which are also the food vectors of dioxins. There were 3 questionnaires : 1) on the general diet involving 109 frequencies of consumption of food products combined with several items on portions (food groups : meat (beef, pork, poultry,…), fish, sea shell and shellfish, eggs, milk and dairy products, oils and fats, vegetables (leafy, roots), fruit, starchy food, 2) on locally-produced food diet (132 items; frequency, portion, duration per year, etc) and 3) on production of cattle, poultry, eggs, milk in the area of the plume. Food consumption was expressed in grams of food products or in grams of lipids of the food products for food from animal origin.
To study the potential confounders, univariate and multivariate analyses were used. All analyses were adjusted for survey sampling weights. We used the log-transformation for blood levels of dioxins furans and PCBs, since the preliminary investigation indicated that serum dioxins, furans and PCBs levels were consistent with a log-normal distribution. SAS, R, and Stata softwares were used for statistical analysis.

Results and discussion
Table 1 and table 2 show factors that influence the serum dioxin levels independently to the exposure to the incinerator. Individual characteristics of the participants were important determinants: age, gender, BMI, recent change of body weight, smoking status and the current social and occupational category. We also adjusted for the 8 studied areas. Other factors, related to individual's activities and environment were also associated with serum dioxin levels: background food, living in a rural or urban area, leisure activities associated to dioxin exposure, heating with wood by chimney or stove. Some of these individual factors were already highlighted in international publications; that reinforces the coherence of the data collected here.

Age is the factor which most strongly influences serum dioxin and PCBs concentrations. Serum levels increase by 15% every 5 years for the total TEQ and by 10 % for PCDDs/PCDFs, which correspond to an average increase of approximately 0.3 pg PCDDs/PCDFs/g lipids per year of age. It is known that these substances accumulate in the body with time, particularly in adipose tissue and that serum concentrations increase with age. The relationship of age and current serum dioxin levels is thought to be mediated by a birth cohort effect[1]. Slightly higher increases were documented in the past in various industrialized countries, from 0.4 to 0.8 pg TEQ/g of lipids per year of age, but now lower increases are found (around 0.3 pg/g lipids, like our result).
The serum dioxin levels in **women** were on average a little higher than in men (30.8 versus 26.6 pg total TEQ/ g lipids), which was also observed in studies carried out in Spain[5], Germany[6] and in Taiwan[4]. The reason is not clearly elucidated: difference in diet, body fat or hormone? The three answers are possible.
In the present study, the serum dioxin levels increase with **BMI**, as already reported in other studies.[1,4] Dioxin levels were (in average) 1 to 2 pg of total TEQ/g lipids lower in people with normal BMI than in those who are overweight. BMI is generally associated with a more important food intake, and so with a more important dioxin intake. In addition, people with more body fat eliminate dioxins more slowly than people with less body fat[7].

Moreover, **recent changes in weight** also influence the serum dioxin levels; serum levels are higher with recent body weight loss and lower with recent body weight gain. Body weight loss is accompanied by a mobilization of dioxins stored in adipose tissue which are then found in blood; gained body weight has the opposite effect.
The concentrations were different depending on the socio-professional category with higher levels in **farmers** (in pg total TEQ/g lipids: 37.0, 27.5, 28.8, 24.6, respectively in farmers, craftsmen, employees and workers). This observation was not described previously; it can result from food practices or activities with particular exposure to dioxins such as the use of certain pesticides or burning activities.
The well-known, but surprising relationship with **smoking status** was found in our study; dioxin concentrations in smokers are on average lower by approximately 4 pg TEQ total/g lipids than those observed in non-smokers and ex-smokers. The current explanation is based on the hypothesis that smoking would lead to a particular metabolism of dioxins which accelerates its elimination.

Since **domestic combustion of wood** has been identified as a source of dioxins, it is not surprising to observe slightly higher serum dioxin concentrations in residents having a chimney or a wood stove (difference a little higher than 1 pg total TEQ /g lipids, significant only for PCDD/Fs). Materials used for wood combustion can modify the air quality of the dwellings when a part of combustion gases and fine particles return inside; these emissions inside the house are more or less important. The European inventory of dioxin release carried out in 2000 estimated between 20 and 25 % the contribution of the domestic combustion of wood to the total rejections; these non-industrial wastes remain nevertheless difficult to quantify.
Individual behaviours, such as practice of **recreational activities** are also likely to lead to dioxins exposure. In this study, such activities were found associated with serum dioxin levels (increase of approximately 3 pg total TEQ /g lipids). Works on treated wood (beams, old pieces of furniture) can generate dioxins, PCBs exposure. By the past, wood could be painted with old paintings or be treated with preservatives for wood containing PCBs. Another protective and fungicide agent of wood is pentachlorophenol, largely used in the past, which contains dioxin residues. Some herbicides (such as 2,4,5 T) used till recently also contained dioxin residues, by-products released at the time of the manufacture of these chemicals.
The type of **urbanization** seems to play a role. Serum dioxin levels are slightly higher in rural zone than in suburban zone, which is coherent with a previous observation in the InVS study on dioxins in the mother's milk conducted in 1998 (www.invs.sante.fr).The concentrations of PCDDs in the mother's milk increased with the duration of residence in a rural zone. This result raised the question of a possible impact of agrochemical products or burning activities which can lead to dioxin exposure.

Food is known as the major source of dioxins, except in case of exposure to particular sources. Consequently, it is not surprising to find significant correlations between serum dioxin concentrations of the studied population and its "background” food consumption (i.e. bought at the market, not locally produced). These associations are particularly found in animal products which mainly contribute to dioxins exposure. In the study, we observed a significant positive association between the offal consumption and serum dioxin concentrations (PCDD/Fs), as well as a negative association with the consumption of pork products (respectively $p = 0.03$ and $p = 0.02$). These associations seem to be related to the relative contamination of these products, higher for offals, lower for pork products. However, the specific contribution of this type of food to the total dioxin intake remains low[8]. Food intake of the major groups of food vectors for dioxins (fish and sea products, dairy products), were only marginally associated with serum concentrations. The consumption of sea products was correlated with serum dioxin only for the residents from littoral areas and the association between consumption of dairy products and serum dioxin concentrations was positive but marginally significant ($p = 0,095$).

In conclusion, many confounding factors were relevant for determining current serum levels of dioxins. We recommend adjusting for age, BMI, gender, recent change in body weight, smoking status, occupation, location of residence (in multicentric studies), background food intake and burning activities when comparing a group potentially exposed to dioxins to a referent group to avoid misinterpretation.

Table 1 – Percentage increase in serum dioxin levels associated with an inter-quartile increase in the confounding factor levels

Factors		PCDD/Fs				PCDD/Fs + DL-PCBs			
	p75-p25	% of variation	CI95%		Pr > |t|	% of variation	CI95%		Pr > |t|
Age	12.5 years	**27.1%**	21.0%	33.6%	<0.0001	**41.8%**	34.6%	49.3%	<0.0001
BMI	5.5	**5.5%**	2.9%	8.1%	<0.0001	**4.6%**	1.6%	7.7%	0,0025
Background food intake :									
Pork	8.5 g	**-2.9%**	-5.4%	-0.4%	0.0227				
Offal	1.4 g	**3.1%**	0.3%	6.1%	0.031	**3.1%**	0.0%	6.2%	0.0487
Eggs	2.3 g	**1.4%**	-0.5%	3.4%	0.1535				
Dairy products	25.3 g	**2.8%**	-0.5%	6.2%	0.095				

Table 2 – Geometric means adjusted for the confounding factors for PCDD/Fs and PCDD/Fs + DL-PCBs in pg TEQ/g lipids

Factors	Classes	PCDD/Fs pg TEQ/g lipids	95% Conf. Intervalle		p	PCDD/F+ DL-PCBs pg TEQ/g lip	95% Conf. Intervalle		p
Gender	Males	13.1	12.3	13.9	<0.0001	26.6	25,1	28,2	<0.0001
	Females	15.5	14.5	16.4		30.8	28.9	32.8	
Fluctuation of weight (last 6 months)	Gain weight	13.2	12.3	14.1	0.0003	26.2	24.3	28.1	0.0002
	Loss weight	15.5	14.5	16.7		31.8	29.4	34.3	
Smoking status	Smokers	12.9	11.8	14.2	0.003	26.0	24.0	28.3	0.0006
	Ex-smokers	14.6	13.8	15.5		29.5	27.7	31.4	
	Non-smokers	15.2	14.4	16.0		30.5	28.9	32.3	
Chimney use	No	13.9	13.2	14.6	0.06	28.1	26.6	29.7	0.2355
	Yes	14.6	13.7	15.5		29.2	27.3	31.1	
Leisure activities linked to dioxins	No	13.4	12.7	14.1	0.0003	27.3	25.8	28.8	0.0023
	Yes	15.1	14.1	16.1		30.1	28.1	32.1	
Location of residence	Urban area	13.4	12.1	14.8	0.11	27.5	25.0	30.2	0.2632
	Suburban	14.1	13.1	15.2		28.4	26.2	30.8	
	Rural area	15.2	14.3	16.2		30.1	28.3	32.0	

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