LEVELS OF DIOXIN IN SERUM OF PREGNANT WOMEN LIVING NEARBY A PAPER MILL INDUSTRY, IN THE PROVINCE OF VALDIVIA, CHILI.

Scippo M.-L.[1], Brose F.[1], Focant J.-F.[2], Bertran E.[3], Gallardo W. [3], Rybertt S.[4], Pilleux L.[4], Gòmez J.[4] and Lòpez Sierra M.[4]

1 CART, Laboratory of Food Analysis, University of LIEGE, Bd de Colonster, 20, BatB43b, B-4000, LIEGE, BELGIUM
2 CART, Laboratory of Mass Spectrometry, University of LIEGE, Allée de la Chimie, BatB6, B-4000, LIEGE, BELGIUM
3 Health Regional Dept, XIV Región de Los Ríos, Chilean health, Chacabuco 700, Valdivia, Chile
4 Hematology Unit, Dept. of Medecine, Faculty of Medicine, Bueras 1003, University Austral of Valdivia, Chile

Mauricio López Sierra, Hematology Unit, Dept. of Medicine, Faculty of Medicine, Bueras 1003, University Austral of Chile, Valdivia, Chile, . Telephone: 56-63-221150. Fax: 56-63-214157. E-mail: mlopezs@uach.cl.

Abstract
We present here a limited study of dioxin measurement in serum of 26 pregnant women living in the surroundings of a paper mill industry, in the province of Valdivia, in Chile.
By the way of a questionnaire, several relevant data were recorded, such as the height and weight of the women, their dwelling time, their stage of pregnancy, their parity, their occupation, their education level, their possible pathology (endometriosis, cancer, diabetes, etc), if they are smoker or not smoker, or if they leave with smokers and how many. The dioxin content of serum of these pregnant women ranges between 10 and 55 pg BEQ / g fat, with a mean and a median value of 35 pg BEQ / g fat. These concentrations are in the concentration range found in Europe for non-exposed populations

Introduction
Paper mill industry is well known to be a source a dioxin contamination of people living around. We present here a limited study of dioxin measurement in serum of 26 pregnant women living in the surroundings of a paper mill industry (active since 2000), in the province of Valdivia, in Chile.
A large proportion of the 18,000 residents of the studied area relies on fishing and farming activity for subsistence and consequently consumes large amounts of seafood, vegetables and meat. This population possibly is highly exposed to PCBs and to dioxin-like compounds such as 2,3,7,8-substituted PCDDs and PCDFs as well as dioxin-like PCBs. Most of these food items that could contribute the most to this exposure are produced in the region close to the paper mill industry. An other source of possible pollution is the water, because the industrial waste of the paper mill industry are conduced to the Cruces river, and this water is utilized for human consumption and for the farming (irrigation and water for animals).

Materials and methods
Blood samples of 26 pregnant women aged 15-37 years were collected at San José de la Mariquina's clinical office, in the province of Valdivia, Chile. All individuals leave 7 km away of the paper mill industry located at the North side of the river Cruces, receiving its effluents. None of them had been occupationally exposed to dioxins. Several other data were recorded, by the way of a questionnaire, such as the height and weight of the women, their dwelling time, their stage of pregnancy, their parity, their occupation, their education level, their possible pathology (endometriosis, cancer, diabetes, etc), if they are smoker or not smoker, or if they leave with smokers and how many . None of the women displayed any pathology. After collection, serum samples were immediately stored at -20ºC until analysis. Dioxin were analysed using the DR-CALUX cell line (Biodetection system), in which cells were stably transfected with the upstream region of mouse *cyp1a1* gene (from -1301 to -819) containing four Dioxin Responsive Elements (DREs), the MMTV viral promoter and the luciferase gene as described by Garrison and co-workers [1].

For the extraction, samples (10 ml of serum) were acidified with 1 ml of HCl 1 M, before liquid - liquid extraction with 3 times 10 ml of hexane, and purification on acidic silica (33% H_2SO_4). Extracts were then dissolved in DMSO prior to analysis on DR-CALUX cells, as already described [2]. Results were expressed as pg of TCCD equivalents (TCDD eq) per L of serum or per g of lipids. As the sample amount was not enough in this limited study to measure lipid content in all samples, results were expressed per g fat, using an average fat content of 6 g fat per L of serum.
The limit of quantification (LOQ) of method for human serum analyses (determined using unfortified foetal calf serum) was 80 pg BEQ/L or 13 pg BEQ per g fat.
The validity of the cell based assay to measure TCDD eq of dioxins in human serum was ensured by analyzing matrix-specific control samples (human serum samples previously analyzed by GC-IDHRMS for their PCDD/Fs and DL-PCBs content). High Resolution Gas Chromatography/Mass Spectrometry (HRGC/MS) analysis to measure PCDD, PCDF and DL-PCBs in serum were performed as already describe [3].

Results and Discussion

Performance of the DR-CALUX to measure dioxins in human serum

The performance of the DR-CALUX to measure dioxins in human serum was evaluated using a "home-made" quality control sample, well characterized by repeated GC-IDHRMS analyses.
The intra-laboratory reproducibility measured with a limited number of samples (n=4) was 23%, while the mean trueness (mean ratio between the GC-IDHRMS assigned value and the four repeated DR-CALUX results) was 70 %.
Additionally, 5 serum samples previously analyzed with GC-IDHRMS were analyzed using the DR-CALUX assay. The mean trueness for these 5 samples was 60%.
This very limited number of samples is certainly not sufficient to make a statistical analysis, but it shows that the DR-CALUX method works in our laboratory to detect dioxin levels in human serum.
These results indicate a slight underestimation of the evaluation of the dioxin content in human serum, as already described earlier[4].
Interestingly, when we calculated the total TEQ content of the 5 serum samples used as control by applying the 2005 WHO-TEF instead of he 1998 WHO-TEF, the mean trueness get better (80%), because of the lower contribution of the DL-PCBs, which are known to be less active in the DR-CALUX assay.
We observe the same situation if we use the REP determined in our laboratory [2] to calculate the TEQ content of the 6 control serum samples (Table 1).

Table 1 : Results of the analysis of quality control serum samples.

Control sample n°	1	2	3	4	5	Mean % of the TCDD eq content
pg 1998 WHO PCDD/Fs + DL-PCBs TEQ / L	***280***	***290***	***230***	***160***	***140***	***60 %***
pg 2005 WHO PCDD/Fs + DL-PCBs TEQ / L	***270***	***209***	***139***	***113***	***90***	***80 %***
pg REP calculated PCDD/Fs + DL-PCBs TEQ / L	***186***	***135***	***92***	***74***	***60***	***110 %***
pg TCDD eq / L (DR-CALUX measurement)	***201***	***140***	***140***	***80***	***90***	

Compared to other cell based assays, we can note that the CALUX determination of dioxin in serum using the XDS (Xeno Detection System) CALUX (which uses the mouse cells) lead to an overestimation [5], probably because of the difference between rat and mouse REPs.

Dioxin content in serum from pregnant women of the province of Valdivia (Chili)

The results obtained for the serum of interest (corrected for the mean recovery of 60%) are presented in Table 2, as well as some other data of interest.
As the sample amount was not enough in this limited study to measure lipid content in all samples, results were expressed per g fat, using an average fat content of 6 g fat per L of serum. These results are thus presented as indicative of the dioxin content in the serum of the 26 pregnant women of this study.
The dioxin content of serum of 25 pregnant women out of 26 ranges between 10 and 55 pg TCDD eq / g fat, with a mean and a median value of 35 pg TCDD eq / g fat. One woman displays a higher concentration of 93 pg BEQ / g fat, but this outlier could be due to a unusual fat content in the serum of this woman.
These concentrations are in the concentration range found in Europe for non-exposed populations.
Costopoulo and co-workers [6],cited some references from the literature showing concentration ranging from 10 (in Greece, Athens) to 40 pg PCDD/Fs/DL-PCBs TEQ / g fat (in Finland). In this study, concentrations reported for Australia, New Zealand and USA were respectively 15, 19 and 26 pg PCDD/Fs/DL-PCBs TEQ / g fat. In Czech Republic [7], concentrations of around 40 to 50 pg total WHO-TEQ/g fat in a population living near a pesticide plant, while the concentration is the control group was 23 pg total WHO-TEQ / g fat.
In Belgium, levels around 75 pg total TEQ /g fat were reported [4]. In Asiatic countries, background levels seem to be lower (less than 20 pg / g fat) [8, 9].

In this limited study, we observed no clear correlation between the different factors recorded and the indicative dioxin serum content of the women, but the low number of samples, together with the lack of lipid serum content data do not allow performing a deep statistical analysis.
Most of the women of the study (17 out of 26) leave in the area of interest since their birth, while 5 women are living there since less than 10 years, and 3 out of these 5 women display dioxin levels below 25 pg BEQ / g fat.
We can also note that for 14 of these women, this is their first pregnancy, and from these 14 women, 10 display dioxin levels higher than 30 (ranging from 33 to 93) pg TCDD eq / g fat. This can be explained by the fact that on the contrary to the other women of the study, these women never lactated children and keep a higher body burden of dioxins [10].

In conclusion, dioxin levels of pregnant women living in the vicinity of a paper mill industry, in the province of Valdivia, in Chili, are in the range of the European situation, except for one woman displaying a higher level. This case, if it is confirmed, should be analyzed deeply to identify the cause of that contamination.

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References

1. Garrison, P.M., Tullis K., Aarts J.M., Brouwer A., Giesy J.P. and Denison M.S. Fundam *Appl Toxicol*, 1996; 30: 194.
2. Scippo, M.-L., EPPE G., De Pauw E., Maghuin-Rogister G. *Talant*a, 2004 ; 63**:** 1193.
3. Focant J.-F., Eppe G., Massart A.-C., Scholl G., Pirard C., De Pauw E. *Journal of Chromatography A* 2006 ; 1130, 97.
4. Koppen G., Covaci A., Van Cleuvenbergen R., Schepens P., Winneke G., .Nelen V., Schoeters G. *Toxicology Letters*, 2001; 123: 59.
5. Van Wouwe N., Windal I. , Vanderperren H., Eppe G., Xhrouet C., Massart A-C , Debacker N., Sasse A., Baeyens W., De Pauw E., Sartor F., Van Oyen H., Goeyens L. *Talanta*, 2004; 63: 1157.
6. Costopoulou D., Vassiliadou I., Papadopoulos A., Makropoulos V., Leondiadis L. *Chemosphere*, 2006; 65: 1462.

7. Cerna M., Krate J., Zejglicova K., Brabec M., Maly M., Smıd J., Crhova S., Grabic R., Volf J. *Chemosphere*, 2007; 67: S238.
8. Chen J.W., Wang S.-H., Yu H.-Y., Liao P.-C., . Lee C.-C. *Chemosphere*, 2006; 65: 1667.
9. Park H., Ikonomou M.G., Kim H.-S., Choi J.-W., Chang Y.-S. *Environment International*, 2009 ; 35 : 580.
10. Focant J. -F., Pirard C., Thielen C., De Pauw E. *Chemosphere*, 2002; Volume 48: 763.

Table 2 : Dioxin content (DR-CALUX measured, expressed as TCDD eq) in serum of pregnant women leaving at the vicinity of a paper mill industry in the province of Valdivia, Chile, and other relevant information. * smoker woman. ** BMI = Body mass index [weight (Kg) / height (m) *height (m)].

Sample N°	pg TCDD eq / g fat (corrected for 60% recovery)	Age (year)	BMI**	Weeks of pregnancy	Parity	Dwelling time	Occupation
1	10	21	21.1	12	2	21	House wife
2	12	17	22.6	12	0	11	Student
3	14	26	33.6	39	2	26	Restaurant employee
4	17	23	33.3	30	3	5	House wife
5	19	20	23.9	7	0	2	Nurse
6	19	19	20.5	11	0	19	Nurse
7	19	36	27.0	10	7	16	House wife
8*	24	19	36.6	24	0	6	House wife
9	24	27	20.6	13	4	27	House wife
10	29	18	25.5	27	2	18	House wife
11	31	27	34.4	30	3	2	House wife
12	33	21	32.9	23	2	21	Fruit exportation plant
13	33	18	21.0	9	0	18	Student
14	36	15	21.8	29	0	15	Student
15	38	20	23.1	9	2	18	Nurse
16	38	17	27.6	11	0	17	House wife
17	40	22	22.3	8	2	5	House wife
18*	45	17	30.8	29	0	17	House wife
19	45	16	25.4	5	0	16	Student
20	50	26	26.8	8	0	26	House wife
21	50	18	17.6	13	0	18	Student
22	52	17	26.9	25	0	17	Student
23	52	27	29.0	34	3	27	House wife
24	55	23	27.4	5	4	12	House wife
25	55	16	23.3	26	0	16	Student
26	93	23	23.9	29	0	23	Student