

# DETECTION OF PERSISTENT ORGANIC POLLUTANTS IN BLOOD SERUM OF ELECTRICIANS AND WELDERS IN LATVIA

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*Persistent organic pollutants (POPs) are chemical substances that persist in the environment, bioaccumulate through the food web, and pose a risk of causing adverse effects to human health and the environment. The aim of the study was to assess the POP (polychlorinated biphenyls (PCBs), polybrominated diphenylethers (PBDEs), DDT and their derivatives) levels in blood serum to identify possible risk group workers. Blood serum samples (116 in total) were collected from two groups of employees — electricians, who can come in contact with PCB-containing transformer and capacitor oil, and welders, who were used as a control group. Sample purification was done by double solid phase extraction. The concentrations of POPs in blood serum were determined by gas chromatography with electron capture detector GC/ECD and recovery controlled by internal standard CB-174. None of the 116 samples contained the full range of tested POPs. However, all samples contained at least one of pesticides, and a marker PCB and mono-ortho PCB. Blood serum samples of 52% of electricians and 97.8% of welders contained non-ortho PCB compared to 84% and 74.7%, respectively, for PBDE's. The concentrations of 18 detected PCBs, 4 detected PBDEs and 6 chlorinated pesticides and their metabolites varied in wide ranges and the differences in mean values between groups were not statistically significant ( $p > 0.05$ ). The estimated concentrations of POPs correspond to the lowest levels detected in other countries. Mean concentrations of low-chlorinated marker PCBs were higher in the electrician group, suggesting that the employment sites are contaminated with PCBs, or that employees have contact with PCB-containing items.*

**Key words:** polichlorinated biphehyls (PCB), polibrominated diphenylethers (PBDE), chlorinated pesticides, blood serum, toxic equivalents (TEQ).

## INTRODUCTION

Persistent organic pollutants (POPs) are resistant to environmental degradation by chemical, biological, and photolytic processes. This means that stopping the production of these compounds cannot exclude the possibility of ongoing release of final products of the substances and their accumulation in the environment. Classic POPs include insecticides like DDT and its derivatives, and other compounds such as polychlorinated biphenyls (PCBs) and polybrominated diphenylethers (PBDEs).

The use of the pesticide DDT has been banned in Latvia since 1965 but DDT and its metabolites can be still detected in the environment. Commercial DDT is a mixture of several closely related compounds, such as DDE and DDD, which are the major metabolites and breakdown products of

DDT in the environment. For this reason it is important to determine not only DDT but also its derivatives.

Polychlorinated biphenyls (PCBs) are mixtures containing chlorinated compounds. Theoretically, 209 different congeners of PCB are possible, but only about 130 of these have been identified in commercial products (Anonymous, 2000a).

Long-term exposure to PCBs may have serious effects on the liver, immune system, endocrine system, reproductive system, and thyroid hormone levels, which in turn may affect normal growth and development (Ross, 2004).

Polybrominated diphenylethers (PBDEs) are members of a broader class of brominated chemicals used as flame retardants. PBDEs are endocrine disruptors, they have immunotoxicity, cytotoxicity and developmental neurotoxicity

(Anonymous, 2004). Information about the health effects of PBDEs is insufficient. Information on levels of PCBs and PBDEs in blood serum of humans in Latvia is generally lacking.

Electricians working with capacitor and transformer oil represent a risk group for possible exposure of PCB in the work environment.

The aim of study was to conduct blood serum analysis for persistent organic pollutants and to compare the results between the two groups of workers (electricians and welders).

## MATERIALS AND METHODS

Subjects of the study were 25 electricians (age  $57 \pm 12$  years) working with high voltage electrical equipment and transformers containing oil (work experience  $29 \pm 13$  years). The control group consisted of 91 welders (age  $42 \pm 14$ ) from different metalworking enterprises (work experience  $14 \pm 13$  years). In total, 116 blood samples from persons working in different Latvian regions and enterprises were analysed. Data was collected on workers' age and occupation, and also on eating and smoking habits.

Blood serum analysis included testing for the presence of twenty eight substances — eighteen PCBs including six marker PCBs (CB-28, CB-52, CB-101, CB-138, CB-153, and CB-180), eight mono-ortho PCBs (CB-105, CB-114, CB-118, CB-123, CB-156, CB-157, CB-167, and CB-189) and four Non-ortho PCBs (CB-77, CB-81, CB-126, and CB-169); six pesticides (o,p'-DDE, p,p'-DDE, o,p'-DDD, p,p'-DDD, o,p'-DDT, and p,p'-DDT) and four PBDEs (BDE-47, BDE-99, BDE-100, BDE-153).

The concentrations of POP's were determined using recognised WHO standard methods (Anonymous, 2000b). Extraction was conducted using a modified method described by Kočan *et al.* (1994). The collected blood serum samples were stored in a freezer at  $-18^{\circ}\text{C}$ . The samples were thawed and internal standard (CB 174) was added one day before purification. The samples were purified by double solid-phase extraction. Before extraction, 10 ml of 15% 1-propanol-water mixture was added to samples that were then homogenised in an ultrasonic bath for 10 minutes. Extraction was carried out in solid phase extraction tubes "Supelclean" LC-18 SPE Tubes 6 ml (1 gm). The columns were inserted in an industrially produced solid phase extractor that could simultaneously extract 12 columns under vacuum. The columns were activated with 10 ml methanol and 20 ml  $\text{H}_2\text{O}$ . The samples were immediately quantitatively transferred into columns, 5 ml of 15% 1-propanol water mixture was added, and kept for one hour in vacuum to dry. Then, POPs were eluted with 10 ml hexane-dichloroethane mixture (9:1). To break down fats present in the extract, 0.5 ml concentrated sulfuric acid was added and shaken for two minutes.

In the next step of sample purification, samples were passed through columns filled with 1.0 g purified and activated

fluorosil, and 1 g of 44% sulfuric acid silica gel mixture (prepared by mixing 40 grams of activated silica gel with 19 ml of 96% sulfuric acid) and 1 g of anhydrous sodium sulphate placed on the top of each column. The columns were activated with 8 ml of hexane and samples were then immediately introduced by quantitative transfer, followed by elution using 10 ml of hexane. To the obtained extracts, 10  $\mu\text{l}$  of iso-octane was added and samples were then evaporated almost to dryness. The residue of each sample was dissolved in 100  $\mu\text{l}$  iso-octane and transferred to a 2 ml gas chromatography vial with 250  $\mu\text{l}$  insert.

Analyses were carried out using a gas chromatograph Varian 3800 with electron capture detector (ECD). Chromatographic parameters were: injector temperature  $280^{\circ}\text{C}$ , detector temperature  $320^{\circ}\text{C}$ , carrier gas — the flow 1 ml/min, column — DB-5 60 m X 0.25 mm. The column temperature regimen was: start at  $110^{\circ}\text{C}$ , hold 1.5 min then with rate  $30^{\circ}\text{C}/\text{min}$  heat to  $200^{\circ}\text{C}$ , and then with rate  $2.5^{\circ}\text{C}/\text{min}$  heat until  $300^{\circ}\text{C}$  and hold for 5 min. Total analyses time was 49.7 min.

Quality of quantitative and qualitative measurements was tested using various certified standards: 18 PCB, 6 pesticide and 4 PBDE individual and complex solutions (obtained from Acustandard). Recovery was using PCB-174, which was added to samples before purification.

Concentrations of dioxine-like mono-ortho and non-ortho PCBs were expressed in toxicity equivalents (WHO-TEQ) to obtain comparable results with data from other studies (Anonymous, 2003).

Statistical Results Processing was performed by using SPSS 16.0, Microsoft Excel and MedCalc 12.2.0.0 softwares were used in separate cases. Generally recognized statistical methods (Teibe and Berķis, 2001; Paula and Arhipova, 2002; Teibe, 2007) were used in data statistical analysis.

## RESULTS

The concentrations of all POP types in blood serum samples of both groups were very variable. None of the 116 samples contained the full range of tested POP's, but all samples contained at least one pesticide, and a marker and mono-ortho PCB. Blood serum samples of 52% of electricians and 97.8% of welders contained non-ortho PCB, compared to 84% and 74.7%, respectively, for PBDE's. The least common POP in blood serum was BDE-153, which was not detected in any samples from electricians, and was found only in ten samples from the welder group. In the calculation of mean values, half of the minimum of detection level in place of zero values were used, to avoid biased values. The average recovery by CB-174 was 84.5% for the electrician group and 84.6% for the welder group.

The summed concentration of all marker-PCBs in blood serum of the electrician group was 2.087 ng/g, compared to 2.282 ng/g in the welders group (Table 1). The obtained re-

Table 1

THE CONCENTRATION (NG/G) OF PCB-MARKER COMPOUNDS IN BLOOD SERUM SAMPLES (N = 116) OF ELECTRICIANS (EG) AND WELDERS (WG)

WG mean	0.297	0.592	0.298	0.391	0.447	0.256	2.282
SD	0.184	0.396	0.212	0.308	0.408	0.199	1.075
Ranges, min	< 0.005 <sup>a</sup>	< 0.004 <sup>a</sup>	< 0.003 <sup>a</sup>	< 0.005 <sup>a</sup>	< 0.004 <sup>a</sup>	< 0.005 <sup>a</sup>	0.553
Ranges, max	0.925	1.683	0.858	1.575	2.413	1.092	5.706
t-test	0.003	0.071	0.306	0.086	0.026	0.119	0.091
p-value	0.998	0.944	0.762	0.932	0.980	0.906	0.928

<sup>a</sup> Below the detection limit

Table 2

THE CONCENTRATION (NG/G) OF MONO-ORTHO PCB COMPOUNDS AND TOXIC EQUIVALENTS (WHO-TEQ PG/G) IN BLOOD SERUM SAMPLES (N = 116) OF ELECTRICIANS (EG) AND WELDERS (WG) GROUPS

SD	0.283	0.045	0.093	0.126	0.124	0.225	0.131	0.085	0.514
Ranges, min	< 0.004 <sup>a</sup>	< 0.004 <sup>a</sup>	< 0.006 <sup>a</sup>	< 0.006 <sup>a</sup>	< 0.010 <sup>a</sup>	< 0.005 <sup>a</sup>	< 0.004 <sup>a</sup>	< 0.005 <sup>a</sup>	0.026
Ranges, max	2.008	0.309	0.447	0.663	0.584	0.905	0.616	0.387	3.639
WG WHO-TEQ pg/g serum	0.013	0.023	0.010	0.009	0.080	0.087	0.001	0.008	0.232
t-test	0.007	0.960	0.083	0.118	0.401	0.393	0.796	0.553	0.791
p-value	0.995	0.345	0.934	0.907	0.692	0.697	0.433	0.585	0.435

<sup>a</sup> Below the detection limit

<sup>b</sup> TEF, toxicity equivalence factor for the mono-ortho-PCB group compounds, taking into consideration dioxin-type toxic features (Anonymous, 2003).

Table 3

THE CONCENTRATION (NG/G) OF NON-ORTHO PCB COMPOUNDS AND TOXIC EQUIVALENTS (WHO-TEQ PG/G) IN BLOOD SERUM SAMPLES (N = 116) OF ELECTRICIANS (EG) AND WELDERS (WG) GROUPS

EG mean	0.069	0.348	0.250	0.107	0.775
SD	0.068	0.195	0.118	0.093	0.295
Ranges, min	< 0.005 <sup>a</sup>	< 0.010 <sup>a</sup>	< 0.010 <sup>a</sup>	< 0.008 <sup>a</sup>	0.151
Ranges, max	0.267	0.663	0.472	0.310	1.134
EG WHO-TEQ pg/g serum	0.007	0.035	25.031	1.072	26.144
WG mean	0.112	0.405	0.157	0.199	0.872
SD	0.127	0.595	0.161	0.197	0.486
Ranges, min	< 0.005 <sup>a</sup>	< 0.010 <sup>a</sup>	< 0.010 <sup>a</sup>	< 0.008 <sup>a</sup>	0.013
Ranges, max	0.697	2.845	0.809	1.007	2.845
WG WHO-TEQ pg/g serum	0.011	0.040	15.669	1.991	17.711
t-test	0.259	0.762	0.084	0.202	0.126
p-value	0.797	0.453	0.934	0.841	0.901

<sup>a</sup> Below the detection limit

<sup>b</sup> TEF, toxicity equivalence factor for the mono-ortho-PCB group compounds, taking into consideration dioxin-type toxic features (Anonymous, 2003).

sults showed no statistically significant differences between groups ( $p > 0.05$ ).

The total mean concentration of dioxin-like mono-ortho PCBs expressed in toxicity equivalents TEQ in the electrician group was 0.249 pg/g serum (Table 2), which was almost at the same level as in the welder group (0.232 pg/g). The total TEQ of non-ortho PCB group compounds (26.144 pg/g) in blood serum in the electrician group was 1.5 times higher than in the welder group (17.711 pg/ml) (Table 3). These differences were not statistically significant ( $p > 0.05$ , see Tables 2 and 3).

The higher TEQ of non-ortho PCBs in blood serum of the electrician group was due to a higher mean concentration of CB-126 — 0.250 ng/g compared to 0.157 ng/g in the welder group. However, the occurrence of detectable levels of CB-126 was higher in the welder group (62.6%) than in the electrician group (40%).

Similarly to the results for PCBs, the differences the two groups in pesticide concentrations were not statistically significant. The summed mean concentrations, of all DDT-related compounds (2,4-DDT, 4,4-DDT, DDE's, and DDD's) was higher in blood serum of the welder group

Table 4

THE CONCENTRATION (ng/g) OF PESTICIDES IN BLOOD SERUM SAMPLES (n = 116) OF ELECTRICIANS (EG) AND WELDERS (WG) GROUPS

SD	0.115	0.066	0.020	0.089	0.051	0.073	0.227
Ranges, min	< 0.005 <sup>a</sup>	< 0.007 <sup>a</sup>	< 0.009 <sup>a</sup>	< 0.008 <sup>a</sup>	< 0.009 <sup>a</sup>	< 0.006 <sup>a</sup>	0.026
Ranges, max	0.441	0.221	0.078	0.303	0.227	0.258	0.884
WG mean	0.171	0.263	0.055	0.072	0.066	0.069	0.705
SD	0.459	0.606	0.074	0.062	0.069	0.054	0.713
Ranges, min	< 0.005 <sup>a</sup>	< 0.007 <sup>a</sup>	< 0.009 <sup>a</sup>	< 0.008 <sup>a</sup>	< 0.009 <sup>a</sup>	< 0.006 <sup>a</sup>	0.033
Range, max	2.829	3.223	0.374	0.315	0.339	0.225	3.652
t-test	0.745	0.404	0.224	0.140	0.361	0.910	0.180
p-value	0.462	0.689	0.824	0.889	0.721	0.371	0.859

<sup>a</sup> Below the detection limit

Table 5

THE CONCENTRATION (NG/G) OF POLIBROMINATED DIPHENYL ETHERS IN BLOOD SERUM SAMPLES (N = 116) OF ELECTRICIANS (EG) AND WELDERS (WG) GROUPS

WG mean	0.551	0.284	0.242	0.217	0.636
SD	0.594	0.395	0.276	0.156	0.807
Ranges, min	< 0.005 <sup>a</sup>	< 0.008 <sup>a</sup>	< 0.006 <sup>a</sup>	< 0.009 <sup>a</sup>	< 0.005 <sup>a</sup>
Ranges, max	2.316	2.656	0.876	0.595	3.532
t-test	0.178	0.645	0.679		0.354
p-value	0.860	0.524	0.502		0.726

<sup>a</sup> Below the detection limit

<sup>b</sup> Was not found in any sample

(0.705 ng/g) than in the electrician group (0.570 ng/g), but the differences were not statistically significant (Table 4).

Occurrence of detectable levels of PBDEs in blood serum samples was less common than for other POPs. In particular, BDE-153 was not found in blood serum of any of the electricians only samples from 10 welders (11%). Also for this POPs group, the differences between two groups were not statistically significant ( $p > 0.05$ ), and total mean PBDE concentrations were close: 0.736 ng/g in blood serum in the electrician group and 0.636 ng/g in the welder group (Table 5).

## DISCUSSION

Previous studies have shown that the concentrations of POPs in blood serum are very variable, which corresponds to findings of this study. High levels of POPs in an organism are typically associated with fatty food consumption. In our study, the consumption of types of fatty products differed among subjects. There have been more representative studies that covered a larger number of respondents and were performed repeatedly. For example, in the USA, a review of 45 studies on PCBs concentrations in blood serum covering 16 914 samples analysed during a period of 41 years from 1963 to 2003 (Hopf *et al.*, 2009) showed that total concentrations of PCBs in blood serum decreased with

time. The mean total PCB concentration reported from eighteen of these studies that were published after 1999 was  $3.76 \pm 3.19$  ppb (ng/g), compared to  $1.59 \pm 1.22$  ng/g in the studied electrician group and  $1.70 \pm 1.10$  ng/g in the welder group. The higher total PCB concentration in the US studies might be explained by the home location of the studied population near large industrial transformers and capacitors, by the consumption of more fish and other seafood, or incidence of cancer. In a study conducted by Jackson *et al.* (2010), the concentration of total PCBs in maternal blood serum (n = 44) was 4.18 ng/g serum, and in child serum at the age of 24 months (n = 17) — 0.88 ng/g serum.

All marker PCBs were detected in only one sample from the electrician group, but in 41 samples (45%) from the welder group. The least common PCB in electrician blood serum was CB-180, which was detected in only two samples. While the total marker PCB concentrations were similar among the two groups, higher concentrations of individual CBs occurred in the electricians group, particularly for low chlorinated PCBs. Summed and mean concentrations of CB-28, CB-52 and CB-101 in blood serum were higher in the electrician group (1.604 ng/g) than in the welder group (1.187 ng/g). Higher concentrations of a PCBs in blood serum may indicate that the site of employment is contaminated with PCBs, or that employees have contact with PCB-containing items (Liebl *et al.*, 2004; Broding *et al.*, 2007; 2008). The lack of significant differences between groups in concentrations might be explained by lack of a statistical difference in fat products consumption between groups.

The total toxic equivalent (TEQ) of mono-orto and non-orto polychlorinated biphenyls in the electrician group was 26.39 pg/g, compared to 17.94 pg/g in the welder group. Although the dioxin-like PCB concentration in blood serum of the electrician group was 1.5 times higher, the difference between groups was not statistically significant ( $p > 0.05$ ). In the electrician group the TEQ was higher than in the welder group due to high concentration of CB-126, which is the most toxic PCB. In a study in Sweden on a population of pregnant women (n = 325), TEQ of organochlorine compounds was 3.21 pg/g (Glynn *et al.*, 2007). A similar TEQ



(3.70 ng/g) for organohalogen chemicals was obtained in the United Kingdom (Thomas *et al.*, 2006) in analysis of blood serum from 154 volunteers. A higher TEQ was obtained in Taiwan (Lung *et al.*, 2005) for blood serum from people (n = 414) exposed to PCBs in 1979 via an oil contamination event oil and from children (n = 21) born after the accident. The mean TEQ value in the children group was  $32.0 \pm 29$  pg/g, compared to  $84 \pm 10$  pg/g for the adult group. Higher TEQ in the welder and electrician groups than in studies conducted in Sweden and the United Kingdom occurred since only mono-ortho PCBs were determined. In our study the concentrations of mono-ortho PCB were 0.249 pg/g in the electrician group and 0.232 pg/g in the welder group, which are less than in the above mentioned studies.

Previous studies have shown that the largest proportion of DDX concentration is represented by p,p'-DDE (Walizewski *et al.*, 1999; Thomas *et al.*, 2006; Draper *et al.*, 2007; Glynn *et al.*, 2007). In blood serum of the electrician group, p,p'-DDE concentration was 0.095 ng/g, compared to 0.263 ng/g in the welder group serum. In other countries, reported concentrations of p,p'-DDE and total DDT are higher. For example, in human blood serum of inhabitants of Veracruz, Mexico, p,p'-DDE concentration was  $14.5 \pm 28.0$  ng/g serum and total DDT concentration was  $16.4 \pm 30.8$  ng/ml (Walizewski *et al.*, 1999). In a study conducted in the USA, concentration of p,p'-DDE in blood serum varied in the range from 0.17 to 8.9 ng/ml (Draper *et al.*, 2007). Lower concentrations of DDT-like pesticides in occurred in Latvia, which can be explained by less use of the given substances in our economy.

Polybrominated diphenyl ethers are quite new POP problem for Latvia. Studies have shown that concentrations of PBDEs in blood and other biological material increase with time (Toms *et al.*, 2006). The reason for this is wide use of products containing these compounds. The concentrations of this POPs in our study varied in a broad ranges from concentration in serum below detection levels up to 1.216 ng/g in electricians and 3.532 ng/g in welders. Similarly, in Australia, POP concentrations varied from 6.4 ng/g lipid weight to 80.0 ng/g lipid weight and in Netherlands from 0.010 ng/g serum to 1.012 ng/g serum (Ruud and Peters, 2004; Toms *et al.*, 2006). The study in Australia included 85 groups and 8132. The results were given in ng/g lipid, which can be recalculated to ng/g serum because there was information on mean fat content. Mean total PBDE concentration in blood serum was 0.736 ng/g in the electrician group serum and 0.636 ng/g in the welders group. Higher levels occurred in Australia in blood of serum of adults 16 year (0.083 ng/g serum in 2002/2003 and 0.100 ng/g in 2004/2005) but lower levels in a population of Californian pregnant women (1.4 and 1.9 ng/ml serum) (Toms *et al.*, 2006; Draper *et al.*, 2007). As reported in other studies, BDE-47 was the most frequently found PBDE and with higher concentrations (Sjodin *et al.*, 1999; Thomas *et al.*, 2006; Draper *et al.*, 2007). In our study mean concentration of BDE-47 in serum was 0.344 ng/g serum in the electrician

group and 0.551 ng/g in the welders group, compared to 0.027 ng/g in the Australian study. As observed for POPs, also PBDE levels did not significantly differ between groups. In comparison with other studies, concentrations of total PBDEs in our study were generally higher. This can be explained by specific conditions in work places of the subjects.

## CONCLUSIONS

1. The study groups (electricians and welders) are comparable; the general characteristic indicators (age, work experience, smoking and feeding habits) did not significantly differ and the data meet the research criteria.
2. High mean concentrations of low chlorinated marker PCB (CB-28, CB-52 and CB-101) may indicate that the employment sites are contaminated with PCBs, or that employees have contact with PCB-containing items.
3. The mean toxic equivalent (WHO-TEQ pg/g) in the electrician group was higher than in the welders group samples, but due to the large variability the difference was not statistically significant ( $p < 0.05$ ).
4. Statistically significant difference between groups was not observed due to the fact that electricians and welders have no differences in fatty food consumption.
5. The concentrations of PCBs and DDT-like pesticides in blood serum were similar or lower than observed in USA, United Kingdom, Sweden, Mexico, and Taiwan. The detected levels of PBDEs in general were higher than in other studies but distribution of concentrations and occurrence of detectable levels were similar.

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## NOTURĪGO ORGANISKO PIESĀRŅOTĀJU NOTEIKŠANA LATVIJAS ELEKTRIĶU UN METINĀTĀJU ASINS SERUMĀ

Pētījuma mērķis bija novērtēt noturīgo organisko piesārņotāju (NOP) klātbūtni nodarbināto asins serumā (116 paraugi), lai varētu spriest par polihlorēto bifenilu (PCB), polibromēto difenilēteru (PBDE), DDT un tā derivātu iespējamo risku veselībai. Novērtētas divas nodarbināto grupas: elektrīķi, kas darba vidē kontaktē ar NOP saturošām transformatoru un kondensatoru eļļām, un metinātāji kā kontroles grupa. NOP koncentrācija tika noteikta ar gāzu hromatogrāfijas metodi ar elektronu satveres detektoru, atkārtotam bija kontrolēta ar polihlorēto bifenilu CB-174. Nevienam no analizētajiem serumu paraugiem nesaturēja visu analizēto NOP savienojumu spektru, bet katrā paraugā bija vismaz viens katras analizētās grupas savienojums. Noteikto 18 PCB, 4 PBDE un 6 hlolu saturošo pesticīdu un to metabolītu koncentrācijas svārstījās plašā amplitūdā, bet vidējo lielumu atšķirības starp grupām nebija statistiski ticamas ( $p > 0,05$ ). Vidējās marķieru PCB koncentrācijas bija augstākas elektrīķu grupas paraugiem, kas norāda, ka darba vidē viņiem ir kontakts ar PCB saturošām eļļām un vide ir piesārņota ar šiem savienojumiem.