# EXPLORING POSSIBLE DOSE-RESPONSE RELATIONSHIPS BETWEEN 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN CONCENTRATION AND HEART RATE VARIABILITY IN HUMANS

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## Introduction

There is evidence that 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) causes toxic polyneuropathy in rats[1,2]. In humans, the existence of a peripheral neuropathy after exposure to polychlorinated dibenzo-*p*-dioxins and -furans (PCDDs/Fs) is still discussed, as studies concerning dioxin effects on the peripheral nervous system are contradictory[3,4,5,6].

The aim of our study is the exploration of possible neurotoxical effects of TCDD on the autonomic nervous system (ANS), which may be subclinically recognized by an impairment of the autonomic innervation of the heart. Heart rate variability (HRV), that is, the amount of heart rate fluctuations around the mean heart beat rate, can be used for noninvasive investigation of the sympathetic and parasympathetic function of the ANS[7,8]. Spectral analysis of HRV is used to define specific incidences of autonomic activities and functions. Aging and obesity affect autonomic activities indicated by HRV, and heavy smoking and alcohol dependency have been suggested as further factors[9,10,11]. Additionally, reduction of HRV has been reported along with autonomic dysregulation as well as with prevalent cardiovascular disease, including hypertension, myocardial infarction, heart failures and sudden cardiac death[12].

We have focussed on the question whether there is a potential ability of TCDD exposure to alter HRV, and if so, whether a dose-response relationship between HRV alterations and the internal TCDD body burden can be established. We used data of a German cohort occupationally exposed to PCDDs/Fs. This study contains data, which will be part of a thesis, that is presently being prepared by one of the authors (A.H.).

## Methods and Materials

The cohort consisted of a subpopulation of former regular employees of chemical plants in Ingelheim and Hamburg, Germany, where exposure to PCDDs/Fs had occurred in the production of lindane and 2,4,5-trichlorophenol, and for whom biomonitoring data had been obtained between 1986 and 1994[13,14]. No remarkable correlations between known conventional risk factors (e.g. age, smoking, systolic blood pressure, triglycerides, cholesterol) for cardiovascular diseases (ICD-9: 390-459) and measured PCDD/F levels have been detected in this cohort[15].

During medical examinations electrocardiogram (ECG) measurements were taken for 5 minutes in a supine resting position after reaching of a steady state and for 10 minutes in a standing resting position. For each position spectral analysis of HRV by the fast Fourier transform was performed. The resulting power spectral density curve was separated into three different frequency segments, high (HF, 0.15-0.50 Hz), low (LF, 0.05-0.15 Hz) and very low (VLF, 0.01-0.05 Hz). The area under the power spectral densitiy curve for each frequency segment was used as HRV-index with $bpm^2$ as measuring unit, where bpm denotes heartbeats per minute. Thus six HRV-indices were available - one per frequency segment and position. To avoid influence of disturbing events, in particular in the standing position, spectral power was repeatedly calculated over time and a trimmed mean was computed. The system Proscicard II (http://www.hchs.de) was used for performing spectral analytical computations.
This analysis was restricted to 125 male workers, for whom both PCDD/F biomonitoring data and HRV data were available. PCDD/F levels, determined on various occasions during 1986-1994, were extrapolated to the day of ECG measurement by using a simple first-order elimination kinetic with half-lifes reported previously[16]. Population characteristics and HRV-indices of interest were described by median, minimum and maximum values. Group differences were tested with Wilcoxon rank sum test. Spearman rank correlation coefficient was used to assess associations among TCDD concentration, basic health variables and confounders on the one hand and the HRV outcome variables on the other. Before multiple linear regression models were fitted, some numerical variables were logarithmically transformed (base 10), whereas smoking (estimated number of cigarettes smoked during lifetime) and alcohol drinking habit (estimated amount of alcohol consumed during lifetime) were categorized into ordinal scales from 0 to 4, using approximate quintiles of the respective frequency distributions. There are diseases (e.g. cardiovascular events, diabetes, affections of the thyroid gland) and certain medications that can alterate the ANS. This fact is represented by the binary covariate *ANS-possibly-altered* categorized by one of the authors (A.M.) based on medical anamneses and clinical examinations. The $\log_{10}$(HRV-index) was linearly regressed on $\log_{10}$(TCDD+1), age, $\log_{10}$(body mass index), $\log_{10}$(systolic blood pressure), smoking-score, alcohol-score and ANS-possibly-altered for each frequency segment (HF, LF and VLF) and position (supine and standing). A significance level of 0.05 was chosen. Due to the exploratory nature of this study all p-values were two-sided and no adjustment for multiple testing was made; instead, the findings were interpreted in light of biological and clinical consistency. The SAS software system was used for statistical computations (SAS Institute Inc., Cary, NC, USA).

**Results and Discussion**

The characteristics of 125 male workers are described in Table 1. Differences between ANS-possibly-altered groups have been tested, see rightmost column of Table 1. Spearman rank correlation coefficients between covariates and outcome variables are presented in Table 2. No statistically significant correlation was observed between TCDD concentration and HRV-indices in all six combinations of frequency segments and positions. Age was significantly negatively correlated with all HRV-indices except *supine/VLF*, and numerically these correlations were amongst the highest observed. If correlation coefficients were calculated separately for both ANS-possibly-altered groups similar patterns had been observed (not shown). In multiple linear regression models for both *standing/LF* and *standing/VLF* only the regression coefficients for age and smoking-score were statistically significant. The regression coefficients and 95 % confidence intervals for $\log_{10}$(TCDD+1) are -0.096 (-0.27, 0.074) and 0.027 (-0.076, 0.13) for *standing/LF* and *standing/VLF*, respectively. Since the regression

models for *standing/HF* and all three supine resting positions showed no significant global F-tests, no further results are shown for them. The exploratory examination of non-linear covariate effects and interactions between covariates did not remarkably change the assessment of TCDD. To assess PCDD/F contamination in a more general fashion, other congeners additionally to TCDD can be considered by defining a toxic equivalency (TEQ) level. Using the TEQ level instead of TCDD has lead to no appreciable differences in the result of our analyses.

**Table 1. Characteristics of study participants.** [#]Median (minimum, maximum), [##]Median, [§]P-value of Wilcoxon rank sum test, *{Number of missing values}

| | | ANS-possibly-altered | | |
|---|---|---|---|---|
| | | No (n=64) | Yes (n=61) | |
| TCDD (ppt) | 36.5 (0, 584)[#] | 26.8[##] | 49.7[##] | 0.0064[§] |
| Age (years) | 54.9 (26.9, 82.9) | 51.4 | 57.8 | 0.0004 |
| Body mass index (kg/m$^2$) | 26.5 (16.4, 49.1) | 26.5 | 26.5 | 0.25 |
| Systolic blood pressure (mm Hg) | 140 (110, 210); {3}* | 130 | 150 | 0.0001 |
| Diastolic blood pressure (mm Hg) | 90 (70, 130); {3} | 85 | 90 | 0.0081 |
| Cigarettes smoked (in thousands) | 124 (0, 789); {8} | 73.1 | 141 | 0.13 |
| Alcohol consumed (kg) | 135 (0, 987); {26} | 125 | 210 | 0.16 |
| HVR-indices (bpm$^2$) | | | | |
| supine/HF | 0.38 (0.013, 8.3) | 0.32 | 0.46 | 0.97 |
| supine/LF | 0.69 (0.052, 8.6) | 0.75 | 0.62 | 0.11 |
| supine/VLF | 1.1 (0.17, 11) | 1.2 | 1.0 | 0.56 |
| standing/HF | 0.27 (0.012, 6.8) | 0.30 | 0.25 | 0.22 |
| standing/LF | 0.81 (0.039, 20) | 1.2 | 0.51 | 0.0004 |
| standing/VLF | 1.6 (0.22, 10) | 2.0 | 1.2 | 0.0015 |

**Table 2. Spearman rank correlation coefficients.** Statistically significant ones (p-value≤0.05) are marked bold.

| | Supine resting position | | | Standing resting position | | |
|---|---|---|---|---|---|---|
| | HF | LF | VLF | HF | LF | VLF |
| TCDD | -0.01 | -0.05 | -0.13 | -0.04 | -0.03 | -0.01 |
| Age | **-0.18** | **-0.27** | -0.13 | **-0.28** | **-0.50** | **-0.40** |
| Body mass index | -0.07 | -0.12 | **-0.19** | -0.11 | -0.06 | -0.07 |
| Systolic blood pressure | -0.03 | -0.09 | 0.01 | -0.10 | **-0.22** | **-0.20** |
| Diastolic blood pressure | -0.01 | 0.00 | 0.08 | -0.05 | -0.07 | -0.03 |
| Cigarettes smoked during lifetime | -0.15 | -0.15 | -0.13 | -0.14 | **-0.32** | **-0.28** |
| Alcohol consumed during lifetime | -0.01 | -0.01 | -0.04 | -0.13 | -0.13 | -0.17 |

The covariates ANS-possibly-altered and systolic blood pressure both show univariate influence on HRV-indices *standing/LF* and *standing/VLF*, see Tables 1 and 2. However, no statistically significant effects of these covariates could be detected in multiple regression. This is most likely due to confounding by age. Workers with a possibly altered ANS are statistically significantly older than workers without, see Table 1. The Spearman rank correlation coefficient between systolic blood pressure and age is 0.34 (p=0.0001). The

observed effects of age and smoking on the HRV-indices are consistent with reported results[9,10]. Both the negligibly small negative correlations coefficients among TCDD and HRV-indices in Table 2 and the results of the multiple regression models do not support the hypothetically assumed potential ability of TCDD to alter HRV. Of course, no dose-response relationships could be established either.

There is still a lot of uncertainty left, whether and how PCDDs/Fs may alter HRV. However, based on our analyses it can be reasonably assumed that such possible effects will be considerably weaker than the observed age effect.

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