ORIGINAL ARTICLE

Changes in lymphocyte subsets in workers exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)

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ABSTRACT

Objectives 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is known to have toxic effects on the haematopoietic system in animals but epidemiological studies in humans have shown inconsistent results. In this cross-sectional study we investigated changes in peripheral blood cell counts and lymphocyte subsets among workers from a Dutch historical cohort occupationally exposed to chlorophenoxy herbicides and contaminants including TCDD.

Methods Forty-seven workers who had been exposed to high levels of TCDD in the past and 38 low-exposed workers were included in the current investigation. Complete blood counts and differential and major lymphocyte subsets were analysed. Current plasma levels of TCDD ($TCDD_{current}$) were determined by high-resolution gas chromatography/isotope-dilution high resolution mass spectrometry. TCDD blood levels at the time of last exposure ($TCDD_{max}$) were estimated using a one-compartment first order kinetic model.

Results Cell counts and lymphocyte subsets were similar between high- and low-exposed workers, except for a non-dose dependent increase in CD4/CD8 ratio among high-exposed workers. Interestingly, most lymphocyte subsets, in particular the B cell compartment, showed a decrease with increasing levels of both $TCDD_{current}$ and $TCDD_{max}$.

Conclusions Overall, our study showed that plasma TCDD levels had no effect on white blood cell counts and major subsets. However, a non-significant decrease in most lymphocyte subsets was noted, with the strongest effect for B cells. The latter finding may suggest that dioxin exposure might have an adverse impact on the haematopoietic system and lends some support to B cell lymphoma induction by dioxin.

What this paper adds

- Human evidence has suggested that 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) may impair cell mediated immunity which likely plays a role in the genesis of lymphomas, in particular non-Hodgkin lymphoma (NHL).
- As NHL has been associated with TCDD exposure in some previous studies, the possible link between NHL and TCDD might be governed by TCDD-related perturbations of immune cells.
- The current study among workers historically exposed to TCDD showed that TCDD levels had no effect on white blood cell counts and major subsets with the possible exception of a decrease in lymphocyte subsets with the strongest effect for B cells.
- The latter finding may suggest that B cell lymphoma induction by dioxin is biologically plausible.

INTRODUCTION

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a persistent environmental contaminant generated as an unwanted by-product of numerous chemical reactions involving chlorine compounds. It produces a broad spectrum of effects on human organs including the skin, liver, reproductive, nervous, haematopoietic and immune systems.[1] This spectrum of toxicities is known to be mediated via its binding to the aryl hydrocarbon receptor (AhR), a specific intracellular protein expressed by major cell types of the immune system.[2]

There is suggestive evidence from human observational studies that TCDD and other dioxin-like compounds with similar structures impair cell mediated immunity. Several cellular targets within the immune-haematopoietic system have been shown to be altered by TCDD, such as antigen specific populations of lymphocytes, including CD4 and CD8 T cells, and B cells.[3–6] However, other studies among TCDD-exposed individuals did not observe such changes.[7–10]

Lymphocyte subsets and their microenvironments, including cytokines and chemokines, likely play a role in the genesis of lymphomas, in particular non-Hodgkin lymphoma (NHL).[11–15] NHL has been associated with exposure to chlorophenoxy herbicides (eg, 2,4,5-trichlorophenoxyacetic acid) or chlorophenols and their contaminants including TCDD and higher chlorinated dioxins in some previous studies.[16–19] Therefore, the possible link between NHL and TCDD might be governed by TCDD-related perturbations (ie, suppression) of immune cells.

Given the inconsistent evidence for the effect of TCDD on the haematopoietic-immune system, we set out to investigate the association between exposure to TCDD and haematological measures, including peripheral blood cell counts and lymphocyte subsets, among subjects historically exposed

to high TCDD levels. Study subjects comprised a subset of a retrospective cohort of Dutch workers, part of the International Agency for Research on Cancer (IARC) multinational study of workers exposed to chlorophenoxy herbicides, chlorophenols and dioxins.[20–22]

MATERIAL AND METHODS

Study population

The cohort study design and exposure assessment have been previously described in detail.[20–21] The cohort consists of workers from two chlorophenoxy herbicide producing factories. Current analyses utilised a subset of workers from one factory (labelled 'A' in previous publications) who were exposed to TCDD as a by-product of production of 2,4,5-trichlorophenoxyacetic acid and 2,4,5-trichlorophenol from 1953 to 1969, and/or during an occupational accident in 1963. Subjects were selected for blood collection based on stratified sampling of (assumed) high-exposed and low-exposed workers. A priori exposure status was based on a detailed occupational history including periods of employment in different departments and positions held. High-exposed workers were selected for blood collection if they were still alive at the end of follow-up (31 December 2006) and (a) they had been exposed due to the industrial accident (both factory workers and contract workers hired to clean-up after the accident) (n=29), or (b) they worked in the main production departments (n=21). High-exposed workers were matched to three presumably low-exposed workers (based on an a priori exposure classification in which several departments were assumed not to be exposed to TCDD) employed in other departments by factory, sex, age (within 5 years) and current residence (first two digits of postal code). If an exposed worker agreed to participate, the first low-exposed worker was invited. If the first low-exposed worker was unable to participate, the second low-exposed worker was invited, and so on. In total, 43 low-exposed workers were selected. All study subjects were male. Written informed consent was obtained from each study subject after the study was explained. Participants were asked to complete a self-administered questionnaire, which included questions on occupational history, personal medical history, medication used in the weeks prior to the blood collection, anthropometric characteristics, smoking status and alcohol intake. Blood plasma samples were collected according to a standard protocol during home visits between May 2007 and September 2008.[21 22]

Haematological measurements

Blood samples were collected in 4 ml EDTA tubes via venipuncture from the participants in the sitting position. Blood samples were generally collected in the afternoon, but occasionally also in early morning or in the evening. Samples were stored on ice at 0°C and were transported to the Department of Medical Immunology, University Medical Centre Utrecht. Haematological parameters including number and proportion of white blood cells and red blood cells, monocytes, granulocytes and platelets as well as haemoglobin concentration and haematocrit were determined by an automated Beckman Coulter AC Tdiff2 counter (Beckman-Coulter, Miami, Florida, USA). Flow cytometric analysis of the B cell and T cell compartments was performed as described previously.[23] The lymphocyte cell subset populations were analysed by flow cytometry on a FACSCalibur flow cytometer (Becton, Dickinson) using Cell Quest Pro data analysis software (Becton Dickinson). Absolute counts of lymphocyte subsets, including T cell and B cell compartments, were calculated by multiplying the percentages of the indicated subset as determined by flow cytometry and absolute lymphocyte counts determined by the Beckman Coulter AC Tdiff2 counter.

Exposure measurements

Heparin plasma samples of all subjects were analysed for TCDD at the Centers for Diseases Control and Prevention (CDC; Atlanta, Georgia, USA) using high-resolution gas chromatography/isotope-dilution high-resolution mass spectrometry. Results were lipid adjusted and reported as parts per trillion (ppt).[24]

Exposure metrics

TCDD is highly persistent with a long half-life in blood and human tissues. As we measured current levels of TCDD ($TCDD_{current}$) approximately 35 years since last exposure (lag), a one-compartment first order kinetic model with a TCDD half-life ($t_{1/2}$) of 7.1 years was used to estimate TCDD blood levels at the time of last exposure ($TCDD_{max}$)[21 22]:

$$TCDD_{max} = background + (measured\ TCDD - background) \times \exp(\ln(2) \times lag/t_{1/2})$$

The lag in this equation is defined as the time since last assumed exposure and was calculated as follows:

- For workers exposed as a result of the accident in 1963, lag was defined as years since the last date they worked in the clean-up.
- For workers working in a 'production' department, lag was defined as years since the last date they worked in this department (the 'production' department closed in January 1971).
- For other workers (not working in any of the above mentioned departments), lag was defined as years since the last date they worked in the factory but no later than 31 December 1976. Exposure to TCDD strongly declined after 1976 when formulation of 2,4,5-trichlorophenoxyacetic acid ceased.

Due to biological persistence, TCDD can be found in the plasma of nearly everybody living in an industrialised country. Therefore, only measured TCDD levels above the estimated background (0.4 ppt) were back-extrapolated, after which the background was added back in. Current TCDD levels and estimated maximum TCDD levels were subsequently used to investigate exposure–response relationships between TCDD levels and haematological measures.

Statistical analysis

Individual TCDD levels (high-exposed, n=15; low-exposed, n=20) which were below the limit of detection were imputed using a maximum likelihood estimation method.[25] TCDD and haematological measures were log-transformed as measured levels appeared to follow a log-normal distribution. Differences in continuous and categorical parameters between high-exposed and low-exposed subjects were tested using a two-sample t test and χ^2 test, respectively.

We explored exposure–response relationships between log-transformed haematological measures as the dependent variable and log-transformed exposure to $TCDD_{current}$ or $TCDD_{max}$ as the independent variable using linear regression analyses. Models were adjusted for potential confounders including body mass index (in kg/m^2; continuous variable), alcohol intake (units/week; continuous variable), smoking status (never, former, current), medication that could affect cell populations (yes/no), and chronic and acute medical conditions (yes/no).

As more than 50% of high- and low-exposed workers had a chronic disease, exposure–response relationships were also investigated using subjects free of chronic disease at the time of blood draw.

Statistical analyses were performed using SAS V.9.2 (SAS Institute, Cary, North Carolina, USA). All p values were two-sided, with $p<0.05$ considered statistically significant.

RESULTS

Characteristics of participants

Peripheral blood cell counts were successfully measured in all workers. We excluded two subjects as their TCDD results were missing and six subjects (two high-exposed and four low-exposed workers) with a previous (non-skin) cancer diagnosis from the analyses to remove the possibility that blood cell counts or plasma TCDD levels may have been changed due to malignant disease or treatment. This resulted in a total of 85 subjects available for analysis: 47 high-exposed workers and 38 low-exposed workers.

Subject characteristics (n=85) are shown in table 1. More than 50% of workers had chronic diseases such as diabetes, cardiovascular diseases and hypertension. High-exposed workers had lower alcohol intake compared to low-exposed workers (p=0.05). Smoking status among high- and low-exposed workers was similar.

Geometric mean (GM) and geometric SD levels of $TCDD_{current}$ and historical maximum exposure ($TCDD_{max}$) were significantly higher in high-exposed workers ($TCDD_{current}$: 3.25±7.43 ppt; $TCDD_{max}$: 79.82±33.28 ppt) compared to low-exposed workers ($TCDD_{current}$: 1.07±6.42 ppt; $TCDD_{max}$: 7.53±32.14 ppt).

Table 1 General characteristics of high and low 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) exposed workers

	High-exposed (n=47)	Low-exposed (n=38)	p Value*
Age (years)†	69.07 (7.45)	68.55 (7.93)	0.75
Body mass index (kg/m^2)†	27.33 (3.10)	26.65 (3.04)	0.31
Alcohol intake (units/week)†	11.48 (13.56)	17.26 (13.16)	0.05
Smoking status, N (%)			0.84
Current smoker	12 (25.5%)	8 (21.1%)	
Former smoker	28 (59.6)	23 (60.5%)	
Never smoker	7 (14.9%)	7 (18.4%)	
Skin cancer, N (%)	4 (8.5%)	3 (7.9%)	0.92
Infectious disease in the past 4 weeks, N (%)	4 (8.7%)	4 (10.5%)	0.78
Chronic disease, N (%)‡	24 (51.1%)	20 (52.6%)	0.89
Chronic inflammatory disease, N (%)§	13 (27.7%)	11 (28.9%)	0.90
Medication, N (%)			0.33
Immunosuppressant	5 (10.6%)	4 (10.5%)	
NSAIDs	14 (29.8%)	6 (15.8%)	
Antibiotics	0	1 (2.6%)	
$TCDD_{current}$ (ppt)**	3.25 (7.43)	1.07 (6.42)	0.002
$TCDD_{max}$ (ppt)††	79.82 (33.28)	7.53 (32.14)	0.001

*p Values from t tests for continuous variables and χ^2 tests for categorical variables.
†Mean (SD).
‡Chronic diseases include diabetes, coronary heart disease and hypertension;
§Chronic inflammatory diseases include chronic obstructive pulmonary disease, psoriasis, sarcoidosis, asthmatic bronchitis, rheumatoid arthritis, liver failure, Crohn's disease, fibromyalgia and allergy.
**Current levels of TCDD, geometric mean (geometric SD (GSD)).
††Estimated maximum levels of TCDD, geometric mean (GSD).
NSAIDs, non-steroidal anti-inflammatory drugs; ppt, parts per trillion.

Haematological measures

Table 2 shows measured blood cell counts, haemoglobin concentration and haematocrit separately for high- and low-exposed subjects. T helper cell numbers increased non-significantly in high-exposed workers compared to low-exposed workers, while cytotoxic T cells were higher among low-exposed workers resulting in a significant difference in the CD4/CD8 ratio between high-exposed and low-exposed workers. However, both ratios were in the normal range (1–4). No consistent differences were observed in other haematological measures between the high- and low-exposed workers.

Linear regression analysis showed that most haematological parameters had an inverse, albeit non-significant, association with $TCDD_{max}$ levels (table 3). We found a significant linear association for B cell and IgG/IgA+ memory B cell counts with $TCDD_{max}$ levels in univariate analyses (table 3). The association between $TCDD_{max}$ and B cells remained significant after adjustment for covariates. Subsequently, we restricted the regression analyses to high- and low-exposed workers separately. These analyses showed that the association, although weak, was present among both high- and low-exposed workers (figure 1). The regression analyses with current TCDD yielded similar results: significant inverse associations between $TCDD_{current}$ and B cell and IgG/IgA+ memory B cell counts in univariate, and B cell counts in multivariate analyses (data not shown).

The sensitivity analysis restricted to chronic disease-free subjects (high-exposed, n=23; low-exposed, n=18) and among non-smoking subjects (n=65) showed similar trends, although results became statistically non-significant because of limited power.

DISCUSSION

In this study we explored the potential haematotoxic effects of exposure to TCDD among workers occupationally exposed to TCDD approximately 35 years prior to blood analysis. Overall numbers of blood cell and lymphocyte subsets were the same among high-exposed and low-exposed workers, except for a significant difference in CD4/CD8 ratio between high- and low-exposed workers. This difference did not seem to be dose-dependent. In most lymphocyte subsets, a dose-dependent decrease with TCDD exposure was observed, although this was statistically significant only in B cells.

Consistent with our finding, Oh *et al*[6] reported a non-significant higher ratio of T helper cells (CD4) to T cytotoxic cells (CD8) among South Korean waste incineration workers, exposed to, among other compounds, dioxins. In contrast, Webb *et al*[3] reported a significant increase in CD8 percentages and a significant decrease in CD4/CD8 ratio in exposed subjects among residents of an environmentally contaminated site in Missouri, including dioxin contamination, as compared to non-exposed subjects. Another study among industrial workers showed a significant increase in CD8 counts among TCDD-exposed workers compared to the control group of non-exposed workers, although the CD4/CD8 ratio did not change significantly.[10] However, other studies have found no difference in CD4 and CD8 counts and CD4/CD8 ratio among dioxin-exposed subjects as compared to non-exposed subjects.[4 5 8 26–33] Although the evidence for a possible association between TCDD and the CD4/CD8 ratio is inconsistent, it is noteworthy that elevated CD4/CD8 ratios have been reported in the literature to be associated with lymphoma risk.[34]

Our results showed a non-significant inverse association between TCDD exposure levels and most lymphocyte subsets,

Table 2 Haematological measurements of high-exposed and low-exposed workers (n=85)

	High-exposed (n=47)		Low-exposed (n=38)		
	n	GM (GSD)	n	GM (GSD)	p Value*
RBC (10^9/l)		4740.00 (1.08)		4710.87 (1.07)	0.71
Haemoglobin (g/dl)		9.01 (1.08)		9.09 (1.08)	0.63
Haematocrit		0.44 (1.08)		0.44 (1.08)	0.89
Platelet count (10^9/l)		243.08 (1.35)		242.70 (1.27)	0.98
WBC (10^6/l)		7360.3 (1.26)		7295.7 (1.29)	0.87
Monocytes (10^6/l)		333.15 (1.86)		371.77 (1.42)	0.34
Granulocytes (10^6/l)		4765.00 (1.26)		4785.92 (1.38)	0.94
Lymphocytes (10^6/l)		2074.31 (1.52)		2007.08 (1.37)	0.69
B cells (CD19) (10^6/l)		192.34 (2.46)		202.24 (2.19)	0.79
Naive B cells (10^6/l)	44	108.25 (2.14)	37	109.34 (1.91)	0.94
IgM+ memory B cells (10^6/l)	45	11.18 (5.24)	37	13.52 (2.91)	0.65
IgG/IgA+ memory B cells (10^6/l)	45	16.19 (2.72)	37	19.00 (2.28)	0.48
T cells (10^6/l)		1400.20 (1.48)		1359.60 (1.40)	0.72
T helper cells (CD4) (10^6/l)	46	867.99 (1.51)	38	787.17 (1.54)	0.29
CD38/CD4 (10^6/l)	46	6.43 (2.55)	38	6.59 (2.45)	0.90
Naive CD4 (10^6/l)	42	289.95 (2.64)	37	231.45 (2.57)	0.30
Memory CD4 (10^6/l)	42	495.68 (1.65)	37	492.16 (1.53)	0.95
Cytotoxic T cells (CD8) (10^6/l)	46	375.26 (1.88)	38	441.46 (1.61)	0.19
CD38/CD8 (10^6/l)	46	5.14 (2.92)	38	4.67 (2.62)	0.67
Naive CD8 (10^6/l)	42	87.56 (2.35)	38	85.24 (2.70)	0.90
Memory CD8 (10^6/l)	42	258.75 (2.25)	38	310.40 (1.78)	0.25
LGL cells (10^6/l)	45	149.95 (2.44)	38	117.65 (2.32)	0.21
Natural killer cells (10^6/l)		338.13 (1.75)		312.45 (1.64)	0.50
CD4/CD8 ratio	46	2.75 (1.71)	38	2.09 (1.16)	0.05

GM, geometric mean; GSD, geometric SD; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; LGL, large granular lymphocytes; RBC, red blood cells; WBC, white blood cells.
*p Values are from t tests of log-transformed values.

in particular the B cell compartment. A reduction in lymphocyte counts among TCDD-exposed subjects as compared to non-exposed subjects has been shown in some studies.[4 5 6 31] However, other studies have shown no changes[7 9 28 35] or an increase in some lymphocyte subsets[3 30] in TCDD-exposed subjects.

A study among Operation Ranch Hand veterans exposed to Agent Orange, which was usually contaminated with TCDD, showed that B cell counts significantly increased in the lowest exposed category of TCDD exposure, but not in the higher exposed categories, compared to a non-exposed control group[5 36]; other studies did not observe significant changes in B cell counts.[3 4 6 9 30 31 35] However, some of these studies suffer from certain inadequacies such as small numbers,[3 9 30 35] no report of quantitative blood TCDD exposure level,[30 35] TCDD measured in tissues other than blood[3] or estimated from work history/or environment,[4 6 31] and potential residual confounding.[4 31] Despite the inconsistent findings in epidemiological studies, there is a significant body of animal and in vitro research demonstrating a direct effect of TCDD on B cell maturation, function and differentiation as well as various lines of evidence supporting a possible role of the AhR in these effects.[37] Moreover, a subset of genes has been identified in B cells that are transcriptionally altered by TCDD-activated AhR.[37] However, whether the molecular mechanisms for B cell suppression are the same in humans are as yet unclear and need further investigation.

Although linear regression analysis showed weak but statistically significant declines in B cell counts, the results of t tests between subjects classified as high- and low-exposed based on job titles did not reveal a difference. Analyses restricted to the high- and low-exposed workers separately showed that log-transformed continuous TCDD levels significantly correlated with B cell counts among both groups (figure 1). Exposure levels between high- and low-exposed workers largely overlap, and although average levels of TCDD between high- and low-exposed workers are different, the contrast was not great enough to provide a significant difference in TCDD levels between the two groups. The fact that the association is similar among the high- and low-exposed groups strengthens the observation that TCDD plasma levels were related to a decrease in many lymphocyte subsets, in particular B cells. Whether these effects are related to past or peak exposures, or due to current or cumulative TCDD levels is difficult to disentangle as in our study $TCDD_{max}$ and $TCDD_{current}$ are highly correlated (Pearson correlation coefficient 0.97, $p<0.01$). Although the back extrapolation method used in this study is standard, there are some limitations as several factors have been related to elimination of dioxins (eg, amount of body fat, smoking, age, genetics, etc) and as such would influence the back-extrapolation. Unfortunately, we do not know most of these factors, precluding the use of more complex models. However, inclusion of such factors would likely result in a weaker correlation between $TCDD_{max}$ and $TCDD_{current}$ enabling possibly the differentiation in effect between the two measures. This would be informative as an effect with $TCDD_{current}$ is likely more relevant for effects on current cell counts, whereas an association with $TCDD_{max}$ would be reflective of a long-lasting effect on the blood forming system.

There are some limitations to our study. Small sample size prohibited more extensive sensitivity analyses. Moreover, analyses of a large number of blood outcomes may produce

Table 3 Dose–effect relationships between haematological measurements and $TCDD_{max}$

	Univariate (n=85)		Multivariate* (n=85)	
	Estimate	95% CI	Estimate	95% CI
RBC (10^9/l)	−0.001	−0.005 to 0.004	−0.001	−0.006 to 0.004
Haemoglobin (g/dl)	−0.0001	−0.005 to 0.006	0.0001	−0.005 to 0.005
Haematocrit	−0.001	−0.006 to 0.003	−0.001	−0.006 to 0.004
Platelet count (10^9/l)	−0.011	−0.027 to 0.005	−0.006	−0.024 to 0.013
WBC (10^6/l)	−0.009	−0.024 to 0.005	−0.009	−0.024 to 0.006
Monocytes (10^6/l)	−0.026	−0.057 to 0.005	−0.029	−0.063 to 0.005
Granulocytes (10^6/l)	−0.007	−0.023 to 0.009	−0.007	−0.023 to 0.010
Lymphocytes (10^6/l)	−0.013	−0.034 to 0.010	−0.010	−0.035 to 0.014
B cells (10^6/l)	−0.058	−0.106 to −0.009	−0.056	−0.108 to −0.004
Naive B cells (10^6/l)	−0.032	−0.077 to 0.012	−0.024	−0.066 to 0.018
IgM+ memory B cells (10^6/l)	−0.033	−0.123 to 0.056	−0.017	−0.115 to 0.080
IgG/IgA+ memory B cells (10^6/l)	−0.068	−0.125 to −0.012	−0.024	−0.082 to 0.033
T cells (10^6/l)	−0.014	−0.036 to 0.008	−0.003	−0.026 to 0.019
T helper cells (CD4) (10^6/l)	−0.015	−0.040 to 0.010	−0.005	−0.030 to 0.020
CD38/CD4 (10^6/l)	0.006	−0.049 to 0.060	0.001	−0.062 to 0.065
Naive CD4 (10^6/l)	−0.036	−0.094 to 0.023	−0.021	−0.087 to 0.044
Memory CD4 (10^6/l)	−0.017	−0.045 to 0.011	−0.010	−0.041 to 0.022
Cytotoxic T cells (CD8) (10^6/l)	−0.021	−0.055 to 0.013	−0.007	−0.046 to 0.031
CD38/CD8 (10^6/l)	0.011	−0.050 to 0.072	0.006	−0.066 to 0.078
Naive CD8 (10^6/l)	−0.044	−0.100 to 0.011	−0.037	−0.099 to 0.025
Memory CD8 (10^6/l)	−0.017	−0.060 to 0.027	−0.010	−0.060 to 0.040
LGL cells (10^6/l)†	0.017	−0.035 to 0.069	0.029	−0.032 to 0.091
Natural killer cells (10^6/l)	0.021	−0.010 to 0.052	0.011	−0.025 to 0.046
CD4/CD8 ratio	0.021	−0.070 to 0.112	0.014	−0.092 to 0.120

The parameter estimate reflects a change per unit of $TCDD_{max}$ exposure (parts per trillion) on the log scale; all analyses are based on log-transformed values of haematological measures and $TCDD_{max}$, estimated maximum levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)

*Covariates included in the multivariate models were age, body mass index, alcohol intake, smoking, chronic disease, chronic inflammatory disease, infectious disease within 4 weeks before blood sampling, and medication.

IgM, immunoglobulin M; IgG, immunoglobulin G; IgA, immunoglobulin A; LGL, large granular lymphocytes; RBC, red blood cells; WBC, white blood cells.

statistically significant associations simply by chance. Finally, workers with relatively high exposures may have died or been unable to participate, which might have led to selective survival bias in our results.

Although the study did not provide strong support for possible haematotoxicity of TCDD, our finding of B cell declines may be noteworthy in light of previous studies which have implicated B cell activation and the B and T cell interactions as

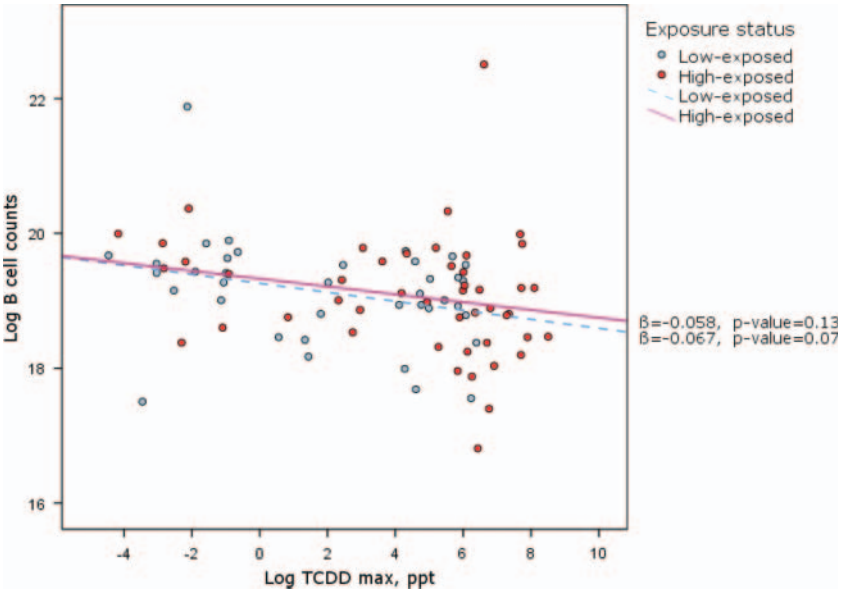

Figure 1 Correlation between B cell counts and $TCDD_{max}$ levels among high- and low-exposed workers; both values are log-transformed; β=Linear regression slope estimate with p-value of the slope estimate. Results did not materially change after removing the outliers. $TCDD_{max}$ indicates the 2,3,7,8-tetrachlorodibenzo-p-dioxin blood levels at the time of last exposure. This figure is only reproduced in colour in the online version.

relevant for the development of lymphomas.[14 38] The fact that we see a specific effect on B lymphocytes is interesting as most lymphomas originate from B cells. In our previous study of the long-term effects of TCDD on humoral immunity, lower complement component 4 (C4) levels were found with increasing TCDD exposure.[22] Previous studies showed that decreased C4 levels might have a role in the survival of auto-reactive B cells.[12] Given that altered immunity including immunosuppression is an established risk factor for NHL,[39] these results support the biological plausibility that TCDD could be involved in the development of lymphomas and provide some support to the observed suggestion of an increased risk of NHL in this cohort of workers exposed to chlorophenoxy herbicides, chlorophenols and contaminants.[40]

CONCLUSION

In conclusion, our study suggests that plasma TCDD levels might be associated with a decrease in most lymphocyte subsets with the strongest effect for B cells. The latter finding may suggest that dioxin exposure can have an adverse impact on the haematopoietic system and that B cell lymphoma induction by dioxin is biologically plausible.

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