Long-term effects on humoral immunity among workers exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)

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ABSTRACT

Objectives Epidemiological studies have shown inconsistent effects on immunological parameters in subjects exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). In this study we investigated changes in humoral immunity and prevalence of atopic diseases among workers from a Dutch historical cohort occupationally exposed to chlorophenoxy herbicides and contaminants including TCDD.

Methods 45 workers who had been exposed to high levels of TCDD in the past and 108 non-exposed workers (39 from the same factory as the exposed subjects (internal control group) and 69 from a comparable factory but without TCDD exposure (external control group)) were included in the study. Blood immunoglobulin (Ig) and complement factor (C) concentrations and specific IgE antibodies to a panel of common allergens were measured using quantitative nephelometry or ELISA. TCDD plasma levels were measured and back-extrapolated to the time of last exposure (TCDDmax) using a one-compartment first order kinetic model.

Results A borderline significant negative association between both current and predicted TCDD levels and C4 was found in multivariate analyses ($\beta=-0.020$; 95% CI=−0.040—0.010 and $\beta=-0.020$; 95% CI=−0.030—0.00, respectively). History of eczema was significantly associated with current TCDD levels in both crude (OR=1.5; 95% CI=1.03—2.2) and adjusted models (OR=1.7; 95% CI=1.08—2.7).

Conclusions Our results do not support an association between TCDD exposure and markers of humoral immunity except possibly C4. Interestingly, decreased levels of C4 have been linked to lymphoma risk, which provides some support to the putative link between TCDD and non-Hodgkin lymphoma.

What this paper adds

- 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) has been linked to non-Hodgkin's lymphoma (NHL) in several studies and as NHL has a strong immunological component, TCDD may be associated with NHL through modulation of the immune system.
- Several animal studies have found evidence of TCDD suppression of humoral and cell-mediated immune responses, but there is limited evidence of such an association in humans.
- The current study among workers historically exposed to high levels of TCDD did not provide strong support for possible long-term immunological effects of TCDD exposure with the possible exception of a borderline significant decrease in C4 levels.
- It is noteworthy that decreased levels of C4 have been linked to lymphoma risk.

INTRODUCTION

Previous epidemiological studies have shown a possible relationship between occupational exposure to chlorophenoxy herbicides, chlorophenols and their contaminants (eg, dioxins and furans) and risk of several cancers including soft tissues sarcomas, non-Hodgkin's lymphoma (NHL) and lung cancer.[1–6] Manufacture of some chlorophenoxy herbicides (eg, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T)) has been prohibited in many countries because of possible contamination with polychlorinated dibenzo-p-dioxins (PCDDs), including the highly toxic 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD).[7] TCDD is an unwanted by-product of numerous chemical reactions involving chlorine compounds and is highly persistent in the environment and biological organisms.[8]

Immunotoxicity related to TCDD has been described in several animal studies.[9 10] Results of human epidemiological studies on this topic, however, have been largely inconsistent. For example, several epidemiological studies, but not all, have shown perturbations in immunoglobulin levels in TCDD exposed subjects.[11–15] Immune suppression increases susceptibility to various infectious diseases and lympho-proliferative diseases such as lymphoma. It is well known that severe immune deficiency in humans increases the risk for NHL.[16 17] Moreover, NHL has been associated with exposure to chlorophenoxy herbicides or chlorophenols in several case—control[18 19] and some recent cohort studies,[7 20 21] particularly with TCDD exposure. Therefore, one could hypothesis that the possible link between NHL and TCDD might be governed by TCDD related perturbations (ie, suppression) of the immune system.

Besides possible effects on immunological parameters, several animal and in vitro studies have suggested that TCDD may exacerbate atopic conditions, in particular atopic dermatitis.[22 23] However, to date only a few investigations have studied TCDD exposure in relation to prevalence of atopic diseases in humans.

Given the limited evidence for an association between TCDD and immunological parameters and given the putative link between immunological factors and NHL, we set out to explore the possible long-term immunological effects among subjects historically exposed to TCDD. Moreover, occurrence of different atopic diseases in exposed workers compared to non-exposed workers was investigated, with the inference that excess prevalence may indicate immunological related health impacts of TCDD. Workers were selected from a retrospective cohort of workers exposed to chlorophenoxy herbicides, chlorophenols and dioxins. The cohort study was part of the IARC multinational study of workers exposed to chlorophenoxy herbicides, chlorophenols and dioxins.[3 6 7 24]

MATERIAL AND METHODS

Cohort population

The Dutch herbicide cohort has been described in detail elsewhere.[3 6 24] Briefly, the cohort consists of workers from two chemical factories: factory A (workers employed between 1955 and 1985, n=1167) and factory B (workers employed between 1965 and 1986, n=1143) involved in the production and formulation of chlorophenoxy herbicides. In factory A, one of the main products was 2,4,5-T. Other pesticides manufactured in factory A were 2,4,5-trichlorophenol (2,4,5-TCP), lindane, dichlobenil and tetradifon. Contamination with TCDD and other dioxins is possible during production of 2,4,5-T and 2,4,5-TCP. In March 1963, an uncontrolled reaction occurred in an autoclave in factory A where 2,4,5-TCP was synthesised at the time. After the explosion, the contents of the autoclave were released into the factory hall, including dioxins such as TCDD.[3 24] In factory B, the main products were 4-chloro-2-methylphenoxyacetic acid (MCPA), 4-chloro-2-methylphenoxy propanoic acid (MCPP) and 2,4-dichlorophenoxyacetic acid (2,4-D), which are unlikely to be contaminated with TCDD.[6 25]

Study population

Subjects were selected for blood collection based on stratified sampling of (assumed) exposed and unexposed workers. For each worker, exposure status was based on a detailed occupational history including periods of employment in different departments and positions held. Exposed workers were selected for blood collection if (1) they had been exposed due to the accident in factory A (both factory workers and hired contract workers who had also been involved in clean-up after the accident) (n=28) or if (2) they had worked in main production departments (n=20). Exposed workers were individually matched to three presumably non-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) as an internal control group. If an exposed worker agreed to participate, the first non-exposed worker was invited. If the first non-exposed worker was unable to participate, the second non-exposed worker was invited, and so on. Some non-exposed subjects (n=4) were matched as control subjects to two exposed workers. A random sample of workers from factory B (n=78) was used as an external control group in the analyses, since they were unlikely to be exposed to TCDD. These subjects were not individually matched on age. All study subjects were male. Written informed consent from each study subject was obtained after the study was explained. Participants were asked to fill in a self-administered questionnaire, which included information on their occupational history, personal medical history, medication used in the weeks before the study, anthropometric characteristics, smoking status and alcohol intake. Peripheral non-fasting blood samples were collected during home visits between May 2007 and September 2008. Heparinised blood samples were centrifuged within the hour and plasma was kept cold at 4°C until stored within 6 h at −80°C. The study was approved by Medical Ethics Committee of the University Medical Center Utrecht, the Netherlands.

Serum immunoglobulin concentrations

Blood immunoglobulin (Ig) and complement factor (C) concentrations were measured by quantitative nephelometry (IgA, IgG, IgM, IgD, C3 and C4) and ELISA (total IgE). Specific immunoglobulin E (IgE) antibodies to common allergens (house dust mite, cat skin scrape, dog skin scrape, birch pollen and two grass pollens (timothy and English rye); Allergon AB, Angelholm, Sweden) were measured by ELISA.[26] A sample was considered positive if corrected optical density (OD) was higher than 0.05. Atopy was defined as a positive reaction to one or more common allergens.

Exposure measurements

Heparin plasma samples of all subjects were analysed for the presence of polychlorinated dibenzo-p-dioxins (PCDDs, including TCDD), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) at the Centers for Disease Control and Prevention (CDC), Atlanta, USA using high-resolution gas chromatography/isotope-dilution high resolution mass spectrometry with results reported as parts per trillion (ppt), lipid adjusted (Boers D, Portengen L, Turner WE, *et al*. Modelling of historical TCDD exposure in a cohort of workers exposed to chlorophenoxy herbicides, chlorophenols and contaminants. *J Expo Sci Environ Epidemiol*, submitted).

Atopic diseases

Lifetime history of atopic diseases including asthma, hay fever, eczema and allergy was ascertained by self-administered questionnaire (Boers D, Portengen L, Turner WE, *et al*. Modelling of historical TCDD exposure in a cohort of workers exposed to chlorophenoxy herbicides, chlorophenols and contaminants. *J Expo Sci Environ Epidemiol*, submitted).

Exposure metrics

As TCDD is highly persistent with a long half-life in blood and human tissue, exposures to TCDD can be measured in blood or fatty tissues years after the initial exposure has ended. In this study we measured current levels of TCDD ($TCDD_{current}$) approximately 35 years after last exposure. To predict TCDD blood levels at the time of last exposure ($TCDD_{max}$), we extrapolated current TCDD levels to time of assumed last exposure, which differs between subjects, using the following one-compartment first order kinetic model with 7.1 years as the half-life ($t_{1/2}$) (Boers D, Portengen L, Turner WE, *et al*. Modelling of historical TCDD exposure in a cohort of workers exposed to chlorophenoxy herbicides, chlorophenols and contaminants. *J Expo Sci Environ Epidemiol*, submitted):

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

Current TCDD levels and predicted maximum TCDD levels were subsequently used to investigate exposure–response relationships between TCDD levels and blood immune markers.

Statistical analysis

Immune markers measured in concentrations below their respective detection limits were imputed based on the maximum likelihood estimation (MLE) procedure.[27] TCDD and immune marker concentrations were log-transformed as measured levels appeared to follow a log-normal distribution. Differences in continuous and categorical parameters between exposed and non-exposed subjects were tested using the t test (paired t test in matched and two sample t test in unmatched analyses) and χ^2 test, respectively.

We calculated exposure–response relationships between immune markers as dependent variables and exposure to $TCDD_{current}$ and $TCDD_{max}$ using linear regression analyses for continuous outcome variables or logistic regression for binary outcome variables (ie, specific IgE antibodies and presence of atopic disease). Additional adjustments for potential confounders including body mass index (BMI, in kg/m^2; continuous variable), alcohol intake (units/week; continuous variable), smoking (categorical variable), medication used (categorical variable) and chronic and acute medical conditions (categorical variable) were considered.

Statistical analyses were performed using SPSS v 16 and SAS v 9.1. All p values were two-sided, with $p<0.05$ considered as statistically significant.

RESULTS

Characteristics of participants

Blood immunoglobulin and complement factor concentrations were measured successfully in 169 out of 170 workers. TCDD was measured successfully in 164 workers. We excluded 16 subjects (seven workers from factory A and nine from factory B) with a previous cancer diagnosis (except skin cancer) from the analyses to remove the possibility that immune marker levels may have been changed due to malignant disease or medications used. This resulted in 153 subjects available for analysis: 84 workers from factory A (45 exposed workers and 39 non-exposed workers) and 69 external non-exposed workers from factory B.

Subject characteristics (n=153) are shown in table 1. Mean age differed between exposed workers (69.7±7.03 years) from factory A and non-exposed subjects from factory B (59.2±9.1 years). Around 50% of exposed and non-exposed workers from both factories had chronic diseases such as diabetes, cardiovascular diseases and hypertension. The proportions of current smokers among exposed workers and both groups (internal and external) of non-exposed workers were similar, whereas exposed workers had lower alcohol intake compared to both groups of non-exposed workers, which was significant when compared with the internal non-exposed group (p=0.02). The geometric mean (GM) and geometric SD (GSD) of $TCDD_{current}$ were 3.3±7.7 ppt (10th–90th percentiles (P_{10}–P_{90}) 0.1–30.9) in exposed workers and 1.2±5.4 ppt (P_{10}–P_{90} 0.08–7.2) and 0.4±5.1 ppt (P_{10}–P_{90} 0.07–3.8) in internal and external non-exposed groups, respectively. Historical maximum exposure ($TCDD_{max}$) was significantly higher in exposed workers (GM±GSD 81.9±35.6; P_{10}–P_{90} 0.1–2269.7) compared to both internal (8.9±26.6; 0.08–433.22) and external non-exposed workers (0.4±5.1; 0.07–3.8).

Serum immunoglobulin and complement factor concentrations

Table 2 shows serum levels of all immune markers and percentages of subjects with positive specific antibodies against common allergens for exposed and non-exposed subjects. By excluding cancer subjects, a total of 41 matched pairs (four non-exposed workers were matched to eight exposed workers) were included in the paired t test, while 45 exposed workers from factory A and 69 non-exposed workers from factory B were included in the independent two-sample t test. No consistent differences were observed in immunological markers between the exposed workers and either of the control groups.

Table 1 General characteristics of exposed and non-exposed workers

	Factory A			Factory B	
	Exposed (n=45)	Non-exposed (n=39)	p Value	Non-exposed (n=69)	p Value
Age*	69.7 (7.03)	68.8 (7.9)	0.6	59.2 (9.1)	<0.001
Body mass index*	27.2 (3.0)	26.4 (3.1)	0.2	27.2 (3.6)	0.9
Alcohol intake (units/week)*	10.8 (13.5)	17.6 (13.1)	0.02	15.1 (16.5)	0.2
Smoking status, n (%)					
Non-smoker	8 (17.8%)	7 (17.9%)	0.9	14 (20.3%)	0.9
Former smoker	27 (60.0%)	23 (59.0%)		38 (55.1%)	
Smoker	10 (22.2%)	9 (23.1%)		17 (24.6%)	
Medication, n (%)					
Immunosuppressants	4 (8.9%)	4 (10.3%)	0.3	2 (2.9%)	0.1
NSAIDs	15 (33.3%)	7 (17.9%)		14 (20.3%)	
Antibiotics	0	0		1 (1.4%)	
Skin cancer, n (%)	4 (8.9%)	3 (7.7%)	0.8	3 (4.3%)	0.3
Infectious diseases in the past 4 weeks, n (%)	3 (6.8%)	4 (10.3%)	0.6	6 (8.7%)	0.7
Chronic diseases, n (%)†	24 (53.3%)	21 (53.8%)	0.9	32 (46.4%)	0.5
Chronic inflammatory diseases, n (%)‡	12 (26.3%)	9 (23.1%)	0.7	18 (26.1%)	0.9
$TCDD_{current}$, ppt§	3.3 (7.7)	1.2 (5.4)	0.001	0.4 (5.1)	<0.001
$TCDD_{max}$, ppt¶	81.9 (35.6)	8.9 (26.6)	0.001	0.4 (5.1)	<0.001

*Mean (SD).
†Chronic diseases included diabetes, coronary heart disease and hypertension.
‡Chronic inflammatory diseases included chronic obstructive pulmonary disease, psoriasis, sarcoidosis, asthmatic bronchitis, rheumatoid arthritis, liver failure, Crohn's disease, fibromyalgia and allergy.
§Current levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) ($TCDD_{current}$) parts per trillion, geometric mean (geometric SD).
¶Back-extrapolated levels of TCDD ($TCDD_{max}$) parts per trillion, geometric mean (geometric SD).
NSAIDs, non-steroidal anti inflammatory drugs.

Dose–response analyses

Current measured levels of TCDD ($TCDD_{current}$) in workers of factory A were back-extrapolated to time of assumed last exposure and regression analyses were carried out for both current and maximum TCDD exposure levels ($TCDD_{max}$). We found a significant linear association for IgA and IgM with current TCDD levels (see table 3). As around 50% of subjects had chronic medical conditions which might affect immune markers, we restricted the analyses to subjects without chronic disease (n=75). In these analyses, the association of IgA with current TCDD exposure remained significant. However when including other covariates in the model, the association with IgA became statistically non-significant. A consistent borderline statistically significant inverse association for C4 was found with current TCDD.

The regression analyses with $TCDD_{max}$ gave essentially similar results in that a significant increase in IgA level and a significant decrease in C4 with increasing $TCDD_{max}$ level were observed in univariate and fully adjusted regression models, respectively.

Specific IgE for at least one of the five common allergens (atopy) was detected in 19 (12.8%) workers and was not associated with current TCDD levels in univariate (OR 1.13; 95% CI 0.88 to 1.46) or adjusted models (OR 1.11; 95% CI 0.84 to 1.48) (see table 4). Most sensitised workers had IgE to house dust mite (6.8%) or

Table 2 Serum immunoglobulins and complement factors (geometric mean (geometric SD)) of exposed and non-exposed subjects

	Exposed (n=41)	Non-exposed, factory A (n=41)	p Value*	Exposed (n=45)	Non-exposed, factory B (n=69)	p Value
IgG (g/l)	11.0 (1.2)	11.1 (1.2)	0.8	10.9 (1.2)	10.4 (1.3)	0.3
IgM (g/l)	0.8 (1.7)	0.9 (1.7)	0.7	0.8 (1.7)	0.8 (1.7)	0.7
IgA (g/l)	2.5 (1.6)	2.6 (1.4)	0.8	2.5 (1.5)	2.4 (1.6)	0.6
IgD (mg/l)†	22.2 (4.5)	23.0 (4.0)	0.7	21.2 (4.6)	13.3 (6.8)	0.2
IgE (g/l)	27.2 (7.3)	37.5 (9.6)	0.5	22.9 (8.6)	18.8 (8.6)	0.6
C3 (g/l)	1.1 (1.2)	1.1 (1.1)	0.6	1.1 (1.2)	1.2 (1.2)	0.4
C4 (g/l)	0.2 (1.3)	0.2 (1.3)	0.2	0.2 (1.3)	0.3 (1.4)	0.2
Birch pollen, n (%)	2 (4.9%)	1 (2.4%)	0.6	2 (4.4%)	1 (1.4%)	0.3
Grass-mix pollen, n (%)‡	4 (9.8%)	3 (7.3%)	0.7	4 (8.9%)	4 (5.8%)	0.5
Dog skin scrape, n (%)	0	0	–	0	1 (1.4%)	0.4
Cat skin scrape, n (%)	0	1 (2.4%)	0.3	0	1 (1.4%)	0.4
House dust mite, n (%)	2 (4.9%)	3 (7.3%)	0.6	3 (6.7%)	5 (7.2%)	0.9
Atopy, n (%)§	6 (14.6%)	5 (12.2%)	0.7	7 (15.6%)	8 (11.6%)	0.5

*Paired t tests were used for continuous variables.
†More than 50% of IgD samples were imputed.
‡Timothy and English rye grass pollen.
§Atopy was defined as a positive reaction to one or more common allergens.

grass pollen (7.4%); however, none of the specific IgE antibodies were associated with current TCDD levels (data not shown). Lifetime history of eczema was significantly associated with current TCDD levels in both crude (OR 1.51; 95% CI 1.03 to 2.20) and adjusted models (OR 1.71; 95% CI 1.08 to 2.71). We found no significant associations between back-extrapolated TCDD levels and specific IgE or any other reported atopic diseases.

DISCUSSION

Immunotoxicity related to TCDD in humans has been investigated in several studies showing largely inconsistent results. In the current investigation, we explored the effect of exposure to TCDD on humoral immune markers among workers occupationally exposed to TCDD approximately 35 years after last exposure. Overall levels of all immunoglobulins were slightly lower in exposed workers compared with internal non-exposed workers, while we found that exposed workers had higher levels of immunoglobulin compared with external non-exposed workers. However, these differences were not statistically significant. In addition, regression models adjusted for chronic inflammatory and infectious diseases within 4 weeks before blood collection, medication used, alcohol consumption, smoking, age and BMI did not show significant changes in immunoglobulins levels in relation to TCDD exposure. In

Table 3 Dose–response relationships between immunological parameters and $TCDD_{current}$ and $TCDD_{max}$

	General linear model‡					
	Univariate					
	All subjects (n=148)		Subjects without chronic disease (n=75)		Multivariate§ (n=148)	
	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI
$LnTCDD_{current}$*						
IgG	0.009	−0.008 to 0.026	0.013	−0.011 to 0.036	0.004	−0.020 to 0.020
IgA	0.039	0.004 to 0.074	0.049	0.004 to 0.094	0.030	−0.010 to 0.070
IgM	−0.044	−0.087 to 0.00	−0.037	−0.096 to 0.022	−0.050	−0.100 to 0.004
IgD¶	−0.073	−0.212 to 0.065	−0.109	−0.290 to 0.072	−0.050	−0.200 to 0.080
IgE	−0.016	−0.197 to 0.166	−0.154	−0.404 to 0.096	−0.040	−0.480 to 0.096
C3	−0.006	−0.021 to 0.010	−0.013	−0.034 to 0.008	−0.010	−0.020 to 0.010
C4	−0.016	−0.040 to 0.008	−0.028	−0.060 to 0.003	−0.020	−0.040 to 0.010
$LnTCDD_{max}$†						
IgG	0.006	−0.003 to 0.016	0.010	−0.005 to 0.022	0.010	−0.010 to 0.020
IgA	0.022	0.003 to 0.041	0.034	0.009 to 0.060	0.020	−0.002 to 0.040
IgM	−0.016	−0.040 to 0.008	−0.010	−0.043 to 0.024	−0.020	−0.050 to 0.010
IgD¶	−0.010	−0.087 to 0.067	−0.002	−0.106 to 0.103	−0.004	−0.090 to 0.080
IgE	0.016	−0.084 to 0.116	−0.045	−0.189 to 0.099	−0.010	−0.120 to 0.110
C3	−0.006	−0.014 to 0.003	−0.010	−0.021 to 0.003	−0.010	−0.020 to 0.001
C4	−0.012	−0.026 to 0.001	−0.015	−0.033 to 0.002	−0.020	−0.030 to 0.00

The parameter estimate reflects a change per unit of exposure (parts per trillion) on the log scale.
*Current levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) ($TCDD_{current}$).
†Back-extrapolated levels of TCDD ($TCDD_{max}$).
‡All analyses are based on log-transformed values of immunoglobulins and dioxin.
§Covariates included in the multivariate models were chronic inflammatory disease, infectious disease within 4 weeks before blood sampling, medication, alcohol intake, smoking, age and body mass index.
¶More than 50% of IgD samples were imputed.

Table 4 Dose–response relationships between atopy, history of eczema, asthma, hay fever and allergic disease and $TCDD_{current}$ and $TCDD_{max}$ (n=148)

	Univariate OR (95% CI)	Multivariate‡ OR (95% CI)
$LnTCDD_{current}$*		
History of allergic disease	1.13 (0.80 to 1.58)	1.14 (0.79 to 1.64)
History of eczema	1.51 (1.03 to 2.20)	1.71 (1.08 to 2.71)
History of hay fever	1.08 (0.80 to 1.47)	1.08 (0.77 to 1.52)
History of asthma	1.28 (0.84 to 1.96)	1.35 (0.75 to 2.43)
Atopy	1.13 (0.88 to 1.46)	1.11 (0.84 to 1.48)
$LnTCDD_{max}$†		
History of allergic disease	1.07 (0.89 to 1.27)	1.09 (0.89 to 1.34)
History of eczema	1.13 (0.95 to 1.35)	1.20 (0.97 to 1.48)
History of hay fever	1.08 (0.92 to 1.28)	1.10 (0.91 to 1.36)
History of asthma	1.17 (0.94 to 1.45)	1.36 (0.94 to 1.96)
Atopy	1.08 (0.95 to 1.24)	1.10 (0.93 to 1.30)

The OR reflects a change per unit of exposure (parts per trillion) on the log scale.
*Current levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) ($TCDD_{current}$).
†Back-extrapolated levels of TCDD ($TCDD_{max}$); all analyses are based on log-transformed values of both TCDD values.
‡Covariates included in the models: age, body mass index, smoking and alcohol intake.

a study on waste incineration workers exposed to TCDD in South Korea, slight decreases in several immunoglobulin (IgG, IgA, IgM and IgE) levels were detected compared to a control group, but these differences were statistically non-significant.[11] However, in this study workers were also exposed to other toxicants such as heavy metals, polycyclic aromatic hydrocarbons (PAHs) and other organic materials. Other recent studies have shown significant changes in one or more immunoglobulins including decreased IgG levels,[12–14 28] increased IgG levels[29] or increased IgE levels.[14] It is important to note that TCDD levels in the present study were measured approximately 35 years after last exposure, which is relatively long compared to other studies (10–20 years after exposure).[12 13]

There is limited evidence in the literature on blood levels of complement factors in relation to TCDD exposure. A study of TCDD exposed subjects conducted 20 years after the Seveso accident showed a non-significant positive association between blood TCDD and C4.[13] Similarly, in another study a significant positive association for C4 with current TCDD levels was reported.[29] These results are in contrast to our results which seemed to indicate decreasing C4 levels with increasing measured TCDD and $TCDD_{max}$ levels. In the study conducted by Ott *et al*, regression models were only corrected for age, BMI and smoking.[29] However, other potential confounders such as acute and chronic disease and use of medication were not taken into account. Compared with the Seveso study which included both males and females within a wide age range, our study subjects were middle-aged or elderly men. Moreover, the magnitude of TCDD exposure and long time interval between past maximum exposure and current measurement which vary between studies might explain some of the differences observed between studies.

We did not find any significant relationship between the presence of IgE specific antibodies and TCDD levels. To our knowledge there are no previous investigations that relate IgE specific antibodies to TCDD levels except for a study on Flemish adolescents (Belgium) that showed an effect of exposure to dioxin-like compounds and IgE specific antibodies. In this study a negative association between the odds of having a positive response to house dust mites, cat skin scrape or grass pollen was related to the serum concentrations of dioxin-like compounds. In addition, a negative correlation between serum IgE and dioxin-like compounds was observed.[15] However, given the small sample size of our study, the elderly study population and the low prevalence of sensitisation to common allergens, it is possible that we missed a possible weak association between positive IgE specific antibodies and TCDD levels.

Alterations in allergic immune responses after TCDD exposure have been investigated in several studies. Recent animal studies documented that dioxin exposure exacerbates atopic dermatitis with no increase in IgE antibody production.[22 30] A study on Korean Vietnam War veterans exposed to Agent Orange (a mixture of 2,4,5-T and 2,4-D used as a defoliant in the Vietnam War, which could be contaminated with TCDD) indicated that not only did IgE levels increase, but also the immune system response skewed towards type 2 immune responses, which could indicate an enhanced susceptibility to various allergic diseases.[14] In our study, a non-significant inverse association between total IgE and $TCDD_{current}$ and $TCDD_{max}$ levels was found. Moreover, we found a significant association between history of eczema and measured TCDD levels. Possible confounding of history of eczema by other skin manifestations of TCDD exposure such as chloracne was explored. None of the subjects with self-reported eczema (n=11) reported a history of chloracne, while three of the subjects without eczema (n=137) reported a history of chloracne. Therefore, there is no indication that the reported association between TCDD and eczema was confounded by a history of chloracne. Consistent with our findings, Kim *et al* reported that Korean Vietnam War veterans exposed to Agent Orange had an increased frequency of eczema compared to non-Vietnam veterans (adjusted for potential confounders).[31] These findings raise the possibility that TCDD affects the pathogenesis of eczema independent of IgE signalling.[22] However, our study did not show a significant association between eczema and $TCDD_{max}$, which might indicate that the association found with $TCDD_{current}$ could be a chance finding. Given the strong correlation between $TCDD_{current}$ and $TCDD_{max}$ (R_{sp}=0.97), it would be expected that both TCDD measures would provide similar results.

There were several limitations to our study. First, we selected an internal non-exposed group of workers assumed to be non-exposed to TCDD based on detailed job information (exposure classified as exposure to chlorophenoxy herbicides) from factory A. However, recent analyses have shown that exposure to TCDD was more widespread than previously thought within this factory (Boers D, Portengen L, Turner WE, *et al*. Modelling of historical TCDD exposure in a cohort of workers exposed to chlorophenoxy herbicides, chlorophenols and contaminants. *J Expo Sci Environ Epidemiol*, submitted). Moreover, our results showed that the average levels of TCDD in non-exposed workers from factory A are higher than the levels of non-exposed workers from factory B. Therefore, it seems that using an internal group of non-exposed workers might have biased the results towards the null through misclassification of non-exposed workers. The external non-exposed workers, however, were not closely matched to the exposed workers with regard to age. Although outcomes were adjusted for age, some residual confounding might have remained due to regional differences and differences in work setting. Furthermore, examination of long-term immunological effects of TCDD exposure was complex as it was difficult to differentiate between effects that might be related to high levels of exposure in the past ($TCDD_{max}$) or current measured levels of TCDD. Differences in associations between $TCDD_{max}$ and $TCDD_{current}$ with eczema may reflect this complexity. Finally, bias due to selective survival may have influenced our results.

Although the study did not provide strong support for possible long-term effects on humoral immunity of TCDD, it is noteworthy that decreased levels of C4 have been linked to lymphoma risk.[32 33] Low C4 levels might potentially have a role in the survival of autoreactive B cells. Prolonged survival of B cells could increase the risk that unfavourable mutations might occur, resulting in malignancy.[34] Therefore, our findings for C4 might provide some support for the putative link between TCDD and NHL.

CONCLUSIONS

Our study showed that plasma TCDD levels were not associated with markers of humoral immunity with the possible exception of a borderline significant decrease in C4 levels. Given the observed heterogeneity in results from different studies, it can be hypothesised that perturbation of the humoral immune response due to TCDD exposure, if it occurs at all, may be subtle. However, the immune system is complex with both humoral and cellular components playing an important role. Therefore, more in-depth characterisation of both the humoral (eg, cytokine expression profiles) and cellular components might provide additional insights into the possible immunological effects of TCDD in humans.

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REFERENCES

1. **Becher H,** Flesch-Janys D, Kauppinen T, *et al*. Cancer mortality in German male workers exposed to phenoxy herbicides and dioxins. *Cancer Causes Control* 1996;**7**:312—21.
2. **IARC (International Agency for Research on Cancer).** Polychlorinated Dibenzo-para-Dioxins and Polychlorinated Dibenzofurans. *IARC Monogr Eval Carcinog Risk Hum* 1997;**69**.
3. **Hooiveld M,** Heederik DJ, Kogevinas M, *et al*. Second follow-up of a Dutch cohort occupationally exposed to phenoxy herbicides, chlorophenols, and contaminants. *Am J Epidemiol* 1998;**147**:891—901.
4. **Dich J,** Zahm SH, Hanberg A, *et al*. Pesticides and cancer. *Cancer Causes Control* 1997;**8**:420—43.
5. **De Roos AJ,** Hartge P, Lubin JH, *et al*. Persistent organochlorine chemicals in plasma and risk of non-Hodgkin's Lymphoma. *Cancer Res* 2005;**65**: 11214—26.
6. **Boers D,** Portengen L, Bueno-De-Mesquita HB, *et al*. Cause-specific mortality of Dutch chlorophenoxy herbicide manufacturing workers. *Occup Environ Med* 2010;**67**:24—31.
7. **Kogevinas M,** Becher H, Benn T, *et al*. Cancer mortality in workers exposed to phenoxy herbicides, chlorophenols, and dioxins an expanded and updated international cohort study. *Am J Epidemiol* 1997;**145**:1061—75.
8. **Holsapple MP,** Snyder NK, Wood SC, *et al*. A review of 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced changes in immunocompetence: 1991 update. *Toxicology* 1991;**69**:219—55.
9. **Inouye K,** Ito T, Fujimaki H, *et al*. Suppressive effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on the high-affinity antibody response in C57BL/6 mice. *Toxicol Sci* 2003;**74**:315—24.
10. **Vorderstrasse BA,** Bohn AA, Lawrence BP. Examining the relationship between impaired host resistance and altered immune function in mice treated with TCDD. *Toxicology* 2003;**188**:15—28.
11. **Oh E,** Lee E, Im H, *et al*. Evaluation of immuno- and reproductive toxicities and association between immunotoxicological and genotoxicological parameters in waste incineration workers. *Toxicology* 2005;**210**:65—80.
12. **Neubert R,** Maskow L, Triebig G, *et al*. Chlorinated dibenzo-p-dioxins and dibenzofurans and the human immune system: 3. Plasma immunoglobulins and cytokines of workers with quantified moderately-increased body burdens. *Life Sci* 2000;**66**:2123—42.
13. **Baccarelli A,** Mocarelli P, Patterson DG Jr, *et al*. Immunologic effects of dioxin: new results from Seveso and comparison with other studies. *Environ Health Perspect* 2002;**110**:1169—73.
14. **Kim H-A,** Kim E-M, Park Y-C, *et al*. Immunotoxicological effects of Agent Orange exposure to the Vietnam War Korean veterans. *Ind Health* 2003;**41**:158—66.
15. **Van Den Heuvel RL,** Koppen G, Staessen JA, *et al*. Immunologic biomarkers in relation to exposure markers of PCBs and dioxins in flemish adolescents (Belgium). *Environ Health Perspect* 2002;**110**:595—600.
16. **Engels EA,** Cerhan JR, Linet MS, *et al*. Immune-related conditions and immune-modulating medications as risk factors for non-Hodgkin's Lymphoma: a case-control study. *Am J Epidemiol* 2005;**162**:1153—61.
17. **Grulich AE,** Vajdic CM, Cozen W. Altered immunity as a risk factor for non-Hodgkin lymphoma. *Cancer Epidemiol Biomarkers Prev* 2007;**16**:405—8.
18. **Hardell L,** Eriksson M, Degerman A. Exposure to phenoxyacetic acids, chlorophenols, or organic solvents in relation to histopathology, stage, and anatomical localization of non-Hodgkin's lymphoma. *Cancer Res* 1994;**54**:2386—9.
19. **Hardell L,** Lindstrom G, Van Bavel B, *et al*. Some aspects of the etiology of non-Hodgkin's lymphoma. *Environ Health Perspect* 1998;**106**:679—81.
20. **Bertazzi PA,** Consonni D, Bachetti S, *et al*. Health effects of dioxin exposure: a 20-year mortality study. *Am J Epidemiol* 2001;**153**:1031—44.
21. **Bodner KM,** Collins JJ, Bloemen LJ, *et al*. Cancer risk for chemical workers exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Occup Environ Med* 2003;**60**:672—5.
22. **Ito T,** Inouye K, Nohara K, *et al*. TCDD exposure exacerbates atopic dermatitis-related inflammation in NC/Nga mice. *Toxicol Lett* 2008;**177**:31—7.
23. **Kimata H.** 2,3,7,8-tetrachlorodibenzo-p-dioxin selectively enhances spontaneous IgE production in B cells from atopic patients. *Int J Hyg Environ Health* 2003;**206**:601—4.
24. **Bueno-De-Mesquita HB,** Doornbos G, Van Der Kuip DAM, *et al*. Occupational exposure to phenoxy herbicides and chlorophenols and cancer mortality in the Netherlands. *Am J Ind Med* 1993;**23**:289—300.
25. **Heederik D,** Hooiveld M, Bueno-De-Mesquita HB. Modelling of 2,3,7,8-tetrachlorodibenzo-p-dioxin levels in a cohort of workers with exposure to phenoxy herbicides and chlorophenols. *Chemosphere* 1998;**37**:1743—54.
26. **Doekes G,** Douwes J, Wouters I, *et al*. Enzyme immunoassays for total and allergen specific IgE in population studies. *Occup Environ Med* 1996;**53**:63—70.
27. **Helsel DR.** More than obvious: better methods for interpreting nondetect data. *Environ Sci Technol* 2005;**39**:419A—23A.
28. **Halperin W,** Vogt R, Sweeney MH, *et al*. Immunological markers among workers exposed to 2,3,7,8- tetrachlorodibenzo-p-dioxin. *Occup Environ Med* 1998;**55**:742—9.
29. **Ott MG,** Zober A, Germann C. Laboratory results for selected target organs in 138 individuals occupationally exposed to TCDD. *Chemosphere* 1994;**29**:2423—37.
30. **Fujimaki H,** Nohara K, Kobayashi T, *et al*. Effect of a single oral dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin on immune function in male NC/Nga Mice. *Toxicol Sci* 2002;**66**:117—24.
31. **Kim JS,** Lim HS, Cho SI, *et al*. Impact of Agent Orange exposure among Korean Vietnam veterans. *Ind Health* 2003;**41**:149—57.
32. **Ioannidis JPA,** Vassiliou VA, Moutsopoulos HM. Long-term risk of mortality and lymphoproliferative disease and predictive classification of primary Sjögren's syndrome. *Arthritis Rheum* 2002;**46**:741—7.
33. **Theander E,** Henriksson G, Ljungberg O, *et al*. Lymphoma and other malignancies in primary Sjögren's syndrome: a cohort study on cancer incidence and lymphoma predictors. *Ann Rheum Dis* 2006;**65**:796—803.
34. **Pillemer SR.** Lymphoma and other malignancies in primary Sjögren's syndrome. *Ann Rheum Dis* 2006;**65**:704—6.