ORIGINAL ARTICLE

Plasma dioxin levels and cause-specific mortality in an occupational cohort of workers exposed to chlorophenoxy herbicides, chlorophenols and contaminants

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The Central Bureau of Statistics (CBS) Netherlands provided data on causes of death used in this investigation.

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ABSTRACT

Background We recently reported increased risks for all cancers and urinary cancers in workers exposed to chlorophenoxy herbicides using data from the Dutch herbicide cohort study. These risks could not be linked to the qualitative exposure proxies available. Here, we re-investigate exposure–response relationships using a (semi)quantitative measure of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) exposure.

Methods Plasma TCDD levels of 187 workers were used to develop a predictive model for TCDD exposure. Cox proportional hazards model was used to investigate associations between time-varying TCDD exposure and cause-specific mortality. Sensitivity analyses were performed to assess the impact of key assumptions in exposure assessment.

Results Predicted TCDD levels were associated with mortality from all causes (HR 1.08; 95% CI 1.03 to 1.13), ischaemic heart disease (IHD; HR 1.19; 95% CI 1.08 to 1.32) and non-Hodgkin's lymphoma (NHL; HR 1.36; 95% CI 1.06 to 1.74). No relationships were found between TCDD exposure and mortality from all cancers, respiratory or urinary cancers, which were previously linked to qualitative proxies of TCDD exposure in this cohort. Sensitivity analyses showed that results were relatively robust to slight changes in exposure estimation.

Conclusions Modelled TCDD exposure does not explain the previously reported increased risks for cancer mortality in this cohort except for a possible association with NHL. A small increase in ischaemic heart disease was observed, however we cannot exclude that this finding was due to residual confounding. Although risk estimates for some of the rarer outcomes were still rather imprecise, we do not expect more precise estimates from longer follow-up of this cohort due to the long time-span since last exposure to TCDD.

What this paper adds

- Occupational exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) has been associated with increased risk of all cancers, respiratory cancers, soft tissue sarcoma and non-Hodgkin's lymphoma (NHL).
- In a recent follow-up, excess risk of mortality from urinary and genital cancers was reported, although this excess risk could not be linked to a specific exposure.
- As excess mortality from respiratory cancers and NHL could not be confirmed, analyses were repeated with a more quantitative exposure of estimates TCDD.
- However, the previously reported excess risk of mortality in this cohort could not be explained by estimated TCDD levels except for a possible association with NHL.

INTRODUCTION

Chlorophenoxy herbicides are widely used in agriculture, and chlorophenols are used as a raw material for the manufacture of chlorophenoxy herbicides and for wood preservation.[1 2] Chlorophenoxy herbicides and chlorophenols may be contaminated during production by polychlorinated dibenzo-*p*-dioxins (PCDDs), including 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and polychlorinated dibenzofurans (PCDFs). Since the 1980s, a considerable amount of epidemiological research has been conducted due to concerns about the potential health effects of exposure to these compounds.[3–6] Serious health effects have been reported, including cancer (eg, non-Hodgkin's lymphoma (NHL), soft tissue sarcoma), lung cancer[3 5] and immune deficiencies.[7 8]

The first studies used detailed job information, including duration of employment and positions held, to investigate the effects of exposure to these compounds.[9–11] Later studies also analysed blood levels of dioxins, furans and polychlorinated biphenyls (PCBs) to investigate exposure–response relationships. Although biological sampling was often done years after exposure had ended, this was an improvement and justified by the long half life of several years of dioxins, and most furans and PCBs in blood and human tissues.[12–14] However, epidemiological results from these studies remained heterogeneous.

In this study, we investigated the effect of TCDD exposure on cause-specific mortality among workers from two Dutch chlorophenoxy herbicide producing facilities (referred to as factory A and factory B). In factory A, the main products were

2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 2,4,5-trichlorophenol (2,4,5-TCP), which can be contaminated with TCDD. Moreover, in 1963, an industrial accident in a reactor resulted in release of the contents, including TCDD, into the production hall of factory A.[5 10] In factory B, the main products were 2,4-dichlorophenoxyacetic acid (2,4-D), 4-chloro-2-methylphenoxy propanoic acid (MCPA) and 4-chloro-2-methylphenoxyacetic acid (MCPP).[10] During the production of 2,4-D, MCPA and MCPP, lower chlorinated dioxins can be formed,[9 11] but significant exposure to TCDD is unlikely.

In a recently published paper based on the third follow-up of cause-specific mortality in this cohort,[15] we found increased risk of all cancers and urinary cancers. Although these results were consistent with those obtained during the second follow-up of this cohort,[5] no clear exposure–response relationships with qualitative proxies of exposure could be found. In the present study, we set out to investigate the association between TCDD exposure and cause-specific mortality in more detail using (semi) quantitative estimates of TCDD exposure.

MATERIALS AND METHODS

Dutch herbicide cohort

The Dutch herbicide cohort consists of workers from two factories (A and B) involved in the manufacturing of chlorophenoxy herbicides. In factory A, 2,4,5-T, the main product, was produced from the 1950s until the early 1970s. Other pesticides produced in this factory included 2,5-dichlorophenol (2,5-DCP), 2,4,5-TCP, dichlobenil, tetradifon, and in later years lindane, MCPA and MCPP. In March 1963, an uncontrolled reaction occurred in an autoclave where 2,4,5-TCP was synthesised. The contents of this autoclave, including dioxins like TCDD, were released into the production hall after an explosion.

In factory B, 2,4-D was produced from the mid 1960s until the early 1970s. In later years the factory mostly produced MCPA and MCPP. More detailed information on the companies and their products can be found in an earlier publication.[10]

Study population

For factory A, all workers ever employed between 1955 and 1985 (n=1167) were enrolled in the study, including 85 contract workers who were hired to clean up after the accident in 1963. Sixteen subjects were excluded from further analyses because of either unknown date of birth (n=10) or unknown end of follow-up (n=6). One (deceased) individual who was included as a male worker in earlier analyses appeared to be a female upon linkage to the death registry.[10] In factory B, all workers ever employed between 1965 and 1986 (n=1143) were enrolled. One worker was excluded from further analyses because of unknown end of follow-up. Also in this factory, one (deceased) individual who was included as a male in earlier analyses appeared to be a female upon linking to the death registry. All female workers were excluded from further analyses since there were relatively few of them in the cohort (n=192).

Information on vital status was obtained from municipal records (the 'GBA'). The vital status of cohort members was updated until 31 December 2006. Cause-specific mortality for deceased workers was obtained by linkage to death certificates at the Central Bureau of Statistics (CBS). Linkage was unsuccessful for 12 deceased workers and these were therefore excluded from further analyses. The Medical Ethics Committee of the University Medical Center Utrecht, the Netherlands, approved the study.

Exposure assessment

Exposure assessment was based on a predictive model for TCDD plasma levels at the time of assumed last exposure that was derived by two-stage regression modelling. A comprehensive description of the sampling, blood collection and exposure modelling is provided in a supplementary online appendix. 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. The selected subsample of the cohort included subjects from factory A (n=101) and B (n=86) and covered most departments.

Potential predictors for current log-transformed TCDD levels were identified by linear regression of measured TCDD on subject characteristics, and indicators of ever-working and/or time-spent working in any of 12 distinct departments. The derived model was then fitted to TCDD levels at the assumed time of last exposure by back-extrapolation of current levels using a first-order one-compartment kinetic model with a half life estimate ($t_{1/2}$) of 7.1 years.[14 16]

Workers in factory B (n=86) were assumed not to be occupationally exposed, and were used to estimate background plasma TCDD levels. No relationship was found between current plasma TCDD levels and time spent working in any of the departments. An alternative (a priori) model was fitted to the data using the a priori assumed exposure status as used in earlier epidemiological analyses[10] as predictor. Predictions from this model were different from those of the empirical model both in absolute exposure levels and in relative ranking for some subjects, but the overall (rank) correlation between predictions from the two models was high (Spearman's r=0.79).

Statistical analyses

We used a Cox proportional hazards model with exposure as time-varying covariate to investigate exposure–response relationships between TCDD levels and cause-specific mortality. TCDD levels at event times were calculated from predicted TCDD levels at time of assumed last exposure by using the same first-order one-compartment kinetic model that was used for back-extrapolation. TCDD levels were approximately log-normally distributed, and all analyses were based on log-transformed (ln) TCDD values. To allow for a latency period, TCDD levels were lagged 1 year by excluding exposures that occurred in the year before for all non-cancerous causes of death and 10 years for all cancers. Deaths that occurred during the latency period were considered to be unrelated to the exposure of interest.

All workers from factory B were considered to be non-exposed (as confirmed by plasma TCDD measurements (geometric mean 0.4 ppt) for a subset of n=86 workers), and this group therefore made up the larger portion of the reference population in the statistical analyses. To investigate the extent to which results were dependent on the assumption that workers in factory A and B were comparable for factors other than exposure, all main analyses were also carried out excluding workers from factory B.

HRs and corresponding 95% CIs adjusted for age were calculated for the total cohort and for factory A using a continuous TCDD exposure variable. To study exposure–response relationships for selected causes of death that were considered to be of special interest (ie, all causes of death, all cancers, lung cancer, urinary cancers, NHL) or that showed an association with TCDD in the linear model (ie, stomach cancer, ischaemic heart disease (IHD) and accidents, poisoning and violence) in

more detail, TCDD plasma levels were also categorised in three exposure strata (low, medium and high TCDD exposure) with non-exposed workers as reference. Cut points were based on tertiles of the exposure distribution for exposed cancer cases at their time of death, and were therefore different for outcomes using different lagged exposures.

As a sensitivity analysis, exposure–response relationships were also investigated using the alternative (a priori) model for estimating TCDD exposure levels. All analyses were performed using the STATA software package v 9.0.[17]

RESULTS

The final study population consisted of 2056 male workers, of whom 1410 were still alive at the end of follow-up, 541 had died, 16 were lost to follow-up and 89 had emigrated (table 1). Workers from factory A were on average older than workers from factory B. For instance, 7.6% of the workers in factory A were first employed before 1955, compared to only 0.1% for factory B. Workers from factory A also worked relatively shorter time periods than workers in factory B. In factory A, 80 contract workers and 58 factory workers were involved in cleaning up after the industrial accident in 1963.

Table 1 Cohort characteristics for male workers from factory A and factory B, the Netherlands, 1955–2006

	Factory A (n=1020)		Factory B (n=1036)	
Cohort characteristics	**Total**	**Mean**	**Total**	**Mean**
Person-years of observation	33 162	32.5	29 961	28.9
	N	%	N	%
Vital status				
Alive	626	61.4	784	75.7
Deceased	339	33.1	202	19.5
Lost to follow-up	8	0.8	8	0.8
Emigrated	47	4.7	42	4.1
Year of first employment				
Before 1955	77	7.6	1	0.1
1955–1964	403	39.5	114	11.0
1965–1974	316	31.0	516	49.8
1975 and later	224	22.0	405	39.1
Duration of employment				
0–5 years	612	60.0	399	38.5
5+ years	408	40.0	637	61.5
Time since last employment*				
<10 years	106	10.4	80	7.7
10–20 years	96	9.4	109	10.5
20–30 years	439	43.0	692	66.8
>30 years	379	37.2	155	15.0
Ever working in department†				
Synthesis	4	0.4	12	1.2
Formulation	15	1.5	1	0.1
Packing	13	1.3	14	1.4
Maintenance/repair	58	5.7	173	16.7
Laboratory	78	7.7	40	3.9
Chemical effluent/waste	6	0.6	32	3.1
Cleaning (regular)	2	0.2	4	0.4
Transportation/shipping/stores/ warehouse	81	7.9	19	1.8
Plant supervision	22	2.2	31	3.0
Other exposure	100	9.8	27	2.6
Unclassified exposure	1	0.1	1	0.1
Production	86	8.4	95	9.2
Manual workers	369	36.2	485	46.8
Office workers	80	7.8	280	27.0
Mixed manual/office workers	48	4.7	124	12.0
Clean-up (contract workers)	80	7.8	NA	–
Clean-up (factory workers)	58	5.7	NA	–

*Calculated as time since last employment at end of follow-up.
†Workers may have worked in multiple departments.

HRs for TCDD levels and cause-specific mortality are presented in table 2 for the total cohort and for factory A only. In the total cohort, a significantly increased risk for mortality from all non-cancer causes (HR 1.09; 95% CI 1.03 to 1.16 for each unit increase in exposure on the log-scale), NHL (HR 1.36; 95% CI 1.06 to 1.74), IHD (HR 1.19; 95% CI 1.08 to 1.32) and accidents, poisoning and violence (HR 1.26; 95% CI 1.06 to 1.49) was found. Exclusion of workers from factory B resulted in a lower HR for NHL, but higher HRs for stomach cancer and benign and unspecified cancers. Previously reported excess mortality from urinary cancers in factory A appeared to be unrelated to TCDD levels in the current analysis (bladder cancer HR 0.92; 95% CI 0.66 to 1.27, kidney cancer HR 0.83; 95% CI 0.46 to 1.49). Similar results were obtained when analyses were repeated for alternative latency times of 1 and 5 years.

Exposure–response relationships were explored in more detail using a categorised exposure variable to investigate possible non-linear relationships between TCDD and mortality for selected causes of death. TCDD exposure levels were categorised into four strata, using tertiles of the exposure distribution for exposed cancer cases. Due to the small number of cases for some outcomes, these relationships were studied in the total cohort only, that is, including workers from factory B (table 3). Results show limited evidence of consistent exposure–response relationships for all non-cancer causes of death (low TCDD levels HR 1.02; medium TCDD levels HR 1.34; high TCDD levels HR 1.52; compared to background), urinary cancers (low HR 0.76; medium HR 1.32; high HR 2.04), NHL (low HR 2.99; medium HR 5.28; high HR 10.28), IHD (low HR 1.17; medium HR 1.00; high HR 2.60) and accidents, poisoning and violence (low HR 0.70; medium HR 1.64; high HR 2.80).

To allow more direct comparison with earlier analyses in this cohort[5 14] and to investigate the extent to which these results depended on our department-based predictive exposure model, we re-analysed the data using exposure estimates from our alternative (a priori) prediction model. For ease of interpretation we used the same cut-off points for exposure categorisation, although this resulted in a more uneven distribution of cases. Estimates from this analysis were mostly similar to those based on predicted exposures from the 'main' prediction model for most outcomes, although HRs tended to be a little higher (table 3). HRs for urinary cancers were all above 2.0 in this alternative analysis, although the estimates were rather imprecise and failed to reach statistical significance.

DISCUSSION AND CONCLUSIONS

The Dutch herbicide cohort was created to investigate the possible health effects of occupational exposure to chlorophenoxy herbicides, chlorophenols and contaminants (ie, dioxins). Recently, we reported on the relationship between exposure to chlorophenoxy herbicides, chlorophenols and contaminants and cause-specific mortality using a qualitative measure of exposure.[15] A slightly increased mortality from all cancers was observed in both factory A (HR 1.31; 95% CI 0.86 to 2.01) and factory B (HR 1.54; 95% CI 1.00 to 2.37), while increased mortality from urinary cancers (HR 4.2; 95% CI 0.99 to 17.89) and genital cancers (HR 2.93; 95% CI 0.61 to 14.15) was observed in factory A only.

Table 2 Hazard ratios for lagged (ln-transformed) TCDD plasma levels and selected causes of death for male workers in the total cohort, and for factory A only

	Total cohort		Factory A	
Causes of death (ICD-10)	**n**	**HR (95% CI)**	**n**	**HR (95% CI)**
Exposure lagged 10 years				
All cancers (C00–D48)	192	1.01 (0.94 to 1.10)	112	1.03 (0.92 to 1.16)
Digestive cancers (C15–C26)	49	0.96 (0.80 to 1.15)	25	1.00 (0.77 to 1.28)
Stomach (C16)	14	1.06 (0.77 to 1.47)	6	1.52 (1.05 to 2.20)
Pancreas (C25)	7	1.17 (0.82 to 1.65)	6	0.89 (0.50 to 1.57)
Respiratory cancers (C33–C39)	54	1.00 (0.86 to 1.17)	30	1.08 (0.87 to 1.33)
Trachea, bronchus and lung (C33–C34)	52	0.98 (0.84 to 1.15)	28	1.07 (0.86 to 1.33)
Malignant melanoma of skin (C43–C44)	7	1.29 (0.90 to 1.84)	5	1.27 (0.76 to 2.23)
Genital and urinary cancers (C51–C68)	37	1.09 (0.92 to 1.30)	27	1.01 (0.79 to 1.32)
Genital cancers (C51–C63)	15	1.04 (0.75 to 1.43)	8	1.29 (0.85 to 1.94)
Prostate (C61)	14	1.08 (0.79 to 1.49)	8	1.29 (0.85 to 1.94)
Urinary cancers (C64–C68)	23	1.10 (0.90 to 1.35)	19	0.92 (0.66 to 1.27)
Bladder (C67)	15	1.07 (0.83 to 1.38)	11	0.98 (0.66 to 1.45)
Kidney (C68)	8	1.16 (0.82 to 1.63)	8	0.83 (0.46 to 1.49)
Lymphatic and haematopoietic cancers (C81–C96)	23	1.12 (0.94 to 1.35)	17	0.96 (0.71 to 1.30)
Non-Hodgkin's lymphoma (C82–C83, C85)	7	1.36 (1.06 to 1.74)	6	1.27 (0.95 to 1.71)
Leukaemia (C91–C95)	9	0.90 (0.59 to 1.37)	5	0.74 (0.38 to 1.42)
Benign and unspecified cancers (D00–D48)	7	1.02 (0.70 to 1.48)	3	1.39 (1.18 to 1.64)
Exposure lagged 1 year				
All non-cancer causes (A00–B99, D50–Y89)	349	1.09 (1.03 to 1.16)	227	1.06 (0.98 to 1.15)
Infectious and bacterial diseases (A00–B99)	5	0.46 (0.14 to 1.51)	2	0.40 (0.05 to 3.15)
Disease of endocrine system and blood (D50–E89)	11	1.19 (0.94 to 1.51)	10	0.91 (0.62 to 1.34)
Mental disorders (F00–F99)	6	1.10 (0.72 to 1.69)	4	1.10 (0.69 to 1.76)
Disease of nervous system and sense organs (G00–H95)	13	1.19 (0.96 to 1.47)	10	1.10 (0.87 to 1.40)
Diseases of circulatory system (I00–I99)	182	1.07 (0.98 to 1.16)	114	1.04 (0.94 to 1.16)
Ischaemic heart disease (I20–I25)	93	1.19 (1.08 to 1.32)	60	1.24 (1.09 to 1.43)
Other heart diseases (I30–I51)	27	0.77 (0.56 to 1.05)	14	0.67 (0.42 to 1.04)
Cerebrovascular diseases (I60–I67)	39	0.98 (0.83 to 1.16)	24	0.90 (0.73 to 1.11)
Diseases of respiratory system (J00–J98)	52	0.97 (0.81 to 1.15)	31	1.04 (0.84 to 1.28)
Digestive system (K00–K92)	16	0.96 (0.74 to 1.24)	12	0.74 (0.48 to 1.15)
Genital, urinary system (N00–N99)	9	1.23 (0.89 to 1.72)	7	1.18 (0.77 to 1.81)
Ill-defined and unspecified causes (R00–R99)	21	1.25 (0.99 to 1.56)	16	1.10 (0.79 to 1.52)
Accidents, poisoning and violence (S00–Y09)	30	1.26 (1.06 to 1.49)	19	1.42 (1.13 to 1.78)

HRs are adjusted for age.
ICD-10, International Classification of Diseases, Tenth revision; n, number of deaths; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

Previously reported excess mortality from respiratory cancers, NHL or IHD in the second follow-up[5] of this cohort (using both qualitative and semiquantitative exposure estimates) could not be confirmed in this recent third follow-up.[15] Semiquantitative exposure assessment for the second follow-up was based on a limited number (n=47) of blood samples from workers in factory A only. Relatively high TCDD levels for workers involved in the production of chlorophenoxy herbicides and in cleaning up after the industrial accident in 1963 were found. Although these measurements were valid, they did not cover all departments. As a result, it was difficult to assess the extent of exposure in factory A. To overcome these limitations, we collected blood from 187 male workers in both factories, covering most departments.

No relationship was found between predicted TCDD plasma levels and cause-specific mortality from all cancers (HR 1.01; 95% CI 0.94 to 1.10). However, we did find increased risks of NHL (HR 1.36), all non-cancer mortality (HR 1.09), IHD (HR 1.19) and accidents, poisoning and violence (HR 1.26). When workers from factory B were excluded to rule out the possibility that results were driven by non-exposure related differences between factories A and B, essentially similar results were observed.

We subsequently explored exposure–response relationships for a few selected causes of death in more detail. These analyses were limited to some selected causes of death in order to reduce the potential for chance findings. Results from these analyses were generally consistent with those from the linear models, and

Table 3 HRs for selected causes of death for male workers from the total cohort by tertiles of (lagged) TCDD plasma levels; low, medium and high compared with workers with background TCDD levels

		Empirical model*		A priori model†	
Causes of death (ICD-10)	**TCDD level (ppt)**	**n**	**HR (95% CI)**	**n**	**HR (95% CI)**
All non-cancer causes (A00–B99, D50–Y89)					
Background (reference)	≤0.4	139	Ref (–)	122	Ref (–)
Low	0.4–1.9	68	1.02 (0.76 to 1.36)	94	1.05 (0.80 to 1.38)
Medium	1.9–9.9	78	1.34 (1.01 to 1.77)	89	1.39 (1.06 to 1.82)
High	≥9.9	64	1.52 (1.12 to 2.05)	44	1.87 (1.33 to 2.64)
All cancers (C00–D48)					
Background (reference)	≤0.4	101	Ref (–)	80	Ref (–)
Low	0.4–4.1	30	0.67 (0.44 to 1.01)	56	0.79 (0.55 to 1.12)
Medium	4.1–20.1	30	0.76 (0.50 to 1.15)	33	1.07 (0.70 to 1.61)
High	≥20.1	31	1.32 (0.88 to 1.96)	23	1.80 (1.16 to 2.82)
Stomach cancer (C16)					
Background (reference)	≤0.4	8	Ref (–)	8	Ref (–)
Low	0.4–4.1	2	0.50 (0.11 to 2.33)	1	0.13 (0.02 to 1.03)
Medium	4.1–20.1	1	0.34 (0.04 to 2.66)	2	0.54 (0.11 to 2.58)
High	≥20.1	3	1.33 (0.32 to 5.51)	3	2.46 (0.66 to 9.16)
Trachea, bronchus and lung cancer (C33–C34)					
Background (reference)	≤0.4	27	Ref (–)	24	Ref (–)
Low	0.4–4.1	7	0.53 (0.24 to 1.17)	11	0.52 (0.26 to 1.05)
Medium	4.1–20.1	10	0.89 (0.42 to 1.85)	12	1.15 (0.57 to 2.34)
High	≥20.1	8	1.07 (0.48 to 2.37)	5	1.20 (0.46 to 3.13)
Urinary cancers (C64–C68)					
Background (reference)	≤0.4	9	Ref (–)	4	Ref (–)
Low	0.4–4.1	4	0.76 (0.23 to 2.57)	10	2.49 (0.75 to 8.29)
Medium	4.1 to 20.1	5	1.32 (0.46 to 3.81)	7	3.95 (1.09 to 14.34)
High	≥20.1	5	2.04 (0.70 to 5.95)	2	3.14 (0.58 to 17.01)
Non-Hodgkin's lymphoma (C82–C83, C85)					
Background (reference)	≤0.4	2	Ref (–)	1	Ref (–)
Low	0.4–4.1	1	2.99 (0.21 to 43.29)	3	3.77 (0.42 to 34.27)
Medium	4.1–20.1	2	5.28 (0.48 to 58.06)	2	7.78 (0.68 to 89.27)
High	≥20.1	2	10.28 (1.05 to 100.4)	1	8.09 (0.44 to 149.07)
Benign and unspecified cancers (D00–D48)					
Background (reference)	≤0.4	4	Ref (–)	4	Ref (–)
Low	0.4–4.1	0	–	2	0.61 (0.11 to 3.40)
Medium	4.1–20.1	2	1.26 (0.23 to 6.92)	0	–
High	≥20.1	1	0.89 (0.11 to 7.09)	1	1.37 (0.15 to 12.06)
Ischaemic heart disease (I20–I25)					
Background (reference)	≤0.4	35	Ref (–)	33	Ref (–)
Low	0.4–1.9	16	1.17 (0.65 to 2.09)	21	1.02 (0.60 to 1.76)
Medium	1.9–9.9	14	1.00 (0.54 to 1.85)	20	1.25 (0.72 to 2.18)
High	≥9.9	28	2.60 (1.57 to 4.31)	19	2.78 (1.57 to 4.91)
Accidents, poisoning and violence (S00–Y09)					
Background (reference)	≤0.4	11	Ref (–)	11	Ref (–)
Low	0.4–1.9	2	0.70 (0.15 to 3.30)	6	0.93 (0.35 to 2.51)
Medium	1.9–9.9	7	1.64 (0.63 to 4.28)	6	1.54 (0.59 to 4.02)
High	≥9.9	10	2.80 (1.21 to 6.47)	7	2.72 (1.02 to 7.23)

HRs are adjusted for age.
*Based on occupational history including periods of employment in different departments.
†Based on a priori assumed exposure status.
ICD-10, International Classification of Diseases, Tenth revision; n, number of deaths; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

showed no evidence of strong non-linear effects. Risk estimates from these analyses were, however, imprecise due to low case numbers in the analyses and/or reference categories.

Our finding of an increased risk of mortality from IHD is consistent with a recent review[18] and with results from the second follow-up in this cohort,[5] but was not found during the first follow-up and when the present data were analysed using qualitative exposure estimates.[10 15] Alterations in lipid metabolism after TCDD exposure may lead to cardiovascular disease, but other mechanisms, such as inflammatory effects leading to atherothrombosis, also have been suggested.[4 6] We did not have information on other well known risk factors (ie, body mass index (BMI), smoking) for IHD for most workers in our study. However, confounding by BMI and smoking was unlikely as these factors were not associated with plasma TCDD levels in the 187 workers selected for blood sampling. The association between plasma TCDD levels and mortality from accidents, poisoning and violence indicates that there may be residual confounding by as yet unidentified risk factors.

Previous analyses in this cohort reported increased risks of mortality from NHL for workers exposed to chlorophenoxy herbicides.[5 10] However, these results were based on a small number of cases and could not be confirmed in the latest, third follow-up (HR 0.92, n=7).[15] The current analysis did show

a significant increased risk for NHL in the total cohort (HR 1.36), with a consistent exposure–response relationship (low HR 2.99; medium HR 5.28; high HR 10.28, compared to background). However, the elevated risk was reduced (HR 1.27) and no longer significant when workers from factory B were excluded from the analysis. Given the small number of cases (n=7), these estimates are imprecise and should be interpreted with considerable caution, but as other larger studies have also reported elevated exposure–response relationships with NHL,[3 6 19 20] these findings may still be noteworthy.

An association between plasma TCDD and mortality from stomach cancer and mortality from benign and unspecified cancers was found only within factory A and should also be interpreted with caution. Increased mortality from stomach cancer among workers exposed to TCDD has been reported before,[19 21 22] although not consistently.[3 19]

We recently reported an increased risk of urinary cancers for workers from factory A.[15] This finding could not be attributed to any specific exposure using the available qualitative exposure proxies. In the present analysis, no relationship was found between TCDD plasma levels and mortality from urinary cancers in the full cohort, although there was a suggestion of an exposure–response relationship in the stratified analysis.

Several cohorts have reported excess risk of lung cancer and/or respiratory cancers in workers exposed to chlorophenoxy herbicides, although not always in relation to TCDD exposure.[3 14 19 22] In a pooled study, increased standardised mortality ratios were reported for both TCDD exposure and exposure to any chlorophenoxy herbicide or chlorophenol.[3] In the present investigation, TCDD plasma levels were not associated with lung cancer mortality, and there was no evidence of an exposure–response relationship.

Exposure assessment for this study was based on regression modelling of plasma TCDD levels. As these estimates are inherently uncertain, we developed a range of alternative models for sensitivity analysis (not shown). Exposure estimates from the majority of these models were strongly correlated and differed mainly in the absolute levels. One model, based on earlier a priori exposure classification, also resulted in a slightly different rank ordering of workers. We therefore re-analysed exposure–response relationships for a few selected outcomes using this alternative model. Estimated trends for most of the outcomes were very similar, with the possible exception of urinary cancers where an exposure–response relationship was suggested for the alternative model. The number of cases in most of these analyses was small, and therefore does not allow firm conclusions.

In summary, we found associations between modelled plasma levels of TCDD and mortality from all causes, IHD and possibly NHL. TCDD levels were also associated with mortality from accidents, poisoning and violence, suggesting that these associations may be partly confounded by differences in risk profiles between exposed and non-exposed workers. With the exception of NHL, these associations remained when workers from factory B were excluded or when alternative exposure estimates for TCDD were used. We did not confirm earlier reported risks for mortality from all cancers and respiratory cancers and also did not find a clear association between TCDD exposure and mortality from urinary cancers.

The number of cases for some of the more interesting outcomes (NHL, respiratory cancers) remained rather small, which calls for cautious interpretation of our findings. Given the long time-span since last exposure to TCDD, we do not expect more precise estimates from longer follow-up. We therefore advocate pooled analyses of occupational cohorts exposed to TCDD or studying large populations environmentally exposed to TCDD to further elucidate the potential adverse health effects associated with TCDD exposure.

Competing interests None.

Ethics approval This study was conducted with the approval of the Utrecht Medical Center.

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