DIOXIN (TCDD) EXPOSURE-RESPONSE ANALYSIS

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Introduction
The classification of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) as a human carcinogen is based on mechanistic arguments, sufficient evidence of carcinogenicity in animals and limited epidemiological evidence in humans[1]. Limitations of the existing research on humans include the retrospective nature of exposure estimation, the proper exposure metric to use in examining exposure-response, possible confounding by other exposures and lack of consistency of results[2-7]. We used data from the National Institute for Occupational Safety and Health (NIOSH) multi-plant cohort mortality study of chemical workers with occupational exposure to TCDD[8] to evaluate the relation between new estimates of cumulative exposure to TCDD and cancer, to determine if the relation between TCDD and cancer is consistent across plants and to assess the potential for confounding by smoking and other occupational exposures.

Materials and Methods
We obtained demographic, vital status and cause of death data and a job-exposure matrix (JEM) from the NIOSH study[8] of 3,538 men who were employed in the production of chemicals potentially contaminated with TCDD at eight plants in the US and who had sufficient data to estimate their exposure to TCDD[9]. We linked the work histories to the JEM to obtain time-dependent TCDD cumulative exposure scores[10]. These scores were used to estimate the individual histories of serum lipid concentrations in parts per trillion (ppt)-years, using a concentration- and age-dependent model (CADM)[4,5], as well as a previously used 8.7-year half-life model[11,12]. Analyses used standardized mortality ratio (SMR) and Cox regression procedures with age as the time variable. To determine the importance of recent compared to more remote exposure to TCDD, some analyses used an exposure lag period of 15 years[8,12]. To address confounding and internal consistency, we examined the relation between TCDD and cancer by plant and separately for smoking-related cancers and other cancers. Analysis of variation in the exposure-response across the exposure range using penalized smoothing splines indicated possible distortion by extreme values of cumulative TCDD ppt-years. To explore the influence of such values further, we restricted some analyses to exposure below the 95th percentile or used logarithmic (ln) transformed exposure values. Except where noted, results pertain to CADM-based exposure estimates.

Results and Discussion
The CADM model yielded cumulative TCDD serum lipid estimates about five times higher than those from the age-dependent only, 8.7-year half-life model. The estimates from the two models were highly correlated, and the shape of the exposure-response relation was similar using the exposure estimates from the two models.

Compared to US general population, TCDD-exposed workers had a 17% increase in mortality from all cancers, a 22% increase in mortality from smoking-related cancers and a 12% increase in mortality from other cancers (Table 1). Average cumulative exposure varied among the eight plants in a manner not consistent with patterns of cancer excesses. Four of the eight plants had an excess of other cancers, with the highest SMR among the four occurring at the plant with the lowest TCDD average exposure. Two of these four plants also had an excess of smoking-related cancer. Workers at three other plants had an excess of smoking-related cancer but not other cancer; at two of these plants there was an excess of nonmalignant respiratory disease, and workers at one plant had been exposed to a bladder carcinogen and had a large excess of bladder cancer (included in the smoking-related cancer category). The last plant had fewer than expected deaths in both cancer categories. These inconsistent results underscore the fact that workers may have been exposed to carcinogens other than TCDD, and, at the least, suggest that TCDD was not responsible for all of the excess cancer deaths that were observed.

Analyses using penalized splines indicated a positive exposure-response relation up to about 1,400,000 ppt-years for unlagged exposure and up to about 4,500,000 ppt-years for 15-year lagged exposure. Only 2.2% and 0.2% of observations, respectively, had TCDD levels above these two values. Accordingly, some of our further analyses removed observations with TCDD ppt-years falling in the upper 5% of the exposure distribution. The restricted data on unlagged TCDD ppt-years included 240 total cancer decedents and 3,351 subjects. The restricted data on 15-year lagged TCDD ppt-years included 235 cancer decedents and 3,282 subjects. The p-splines based on the restricted date were reasonably consistent with linear exposure-response relations.

Cox regression analysis of cancer mortality by approximate septile of TCDD ppt-years, without an exposure lag, did not reveal any statistically significant results, although the largest rate ratio (RR) occurred in the highest exposure group (RR, 1.5; 95% confidence interval, 1.0-2.4). When exposure was lagged by 15 years, RRs for all cancers combined were statistically significant in the two highest exposure septiles (Table 2). Further Cox regression models used continuous rather than categorical TCDD exposure variables. Analyses of the full exposure range (i.e., not excluding observations with extreme exposure estimates) indicated that there was no statistically significantly exposure-response association between untransformed TCDD ppt-years and cancer rates, regardless of the lag period (Table 3). Analyses restricted to observations with exposure below the 95th percentile (or unrestricted but ln-transformed) indicated that TCDD exposure was associated positively with cancer rates. These associations were strengthened when a 15-year lag was applied. Plant-specific analyses indicated that the association between ln-transformed, unlagged TCDD ppt-years and cancer was positive at six of the eight plants and inverse at two, while the association between ln-transformed, 15-year lagged TCDD ppt-years and cancer was positive at all plants; however, none of the plant-specific exposure-response relations was statistically significant. The association between TCDD ppt-years and cancer mortality was similar for smoking-related cancers (ln-transformation and a 15-year lag: beta = 0.084, s.e. = 0.042) and for other cancers (beta = 0.078, s.e. = 0.040). Removing one plant at a time from analyses of TCDD ppt-years (ln-transformed, lagged 15 years) had little impact. The regression coefficients for TCDD ranged from 0.054 after excluding plant 10 to 0.098 after excluding plant 9 or plant 13, and all coefficients were statistically significant or borderline statistically significant (Table 4).

Based on the patterns of results by type of cancer and plant, we conclude that confounding by smoking and/or occupational exposure other than TCDD could account for some of the apparent association between TCDD and cancer. However, confounding is unlikely to account for all of the TCDD-cancer association, as a positive relation persisted in analyses of cancers that are not smoking related and in analyses that remove various plants. We further conclude that the association between TCDD and cancer was absent or weak unless a lag period of 15 years or ln-transformation or restrictions was applied to the exposure data. This finding has several possible explanations: 1) “recent" exposure could have been particularly poorly estimated, a rather implausible explanation given that all of the exposure estimates are back-extrapolations from recent serum estimates; 2) recent cumulative exposure might not contribute biologically to the association between TCDD and cancer, if the initiation and promotion phases for cancer were completed some years before subjects’ deaths; or TCDD could be acting primarily as a cancer initiator rather than a promoter, an explanation inconsistent with the finding that TCDD is not genotoxic[13]; 3) sustained high-level exposure, experienced in earlier time periods and emphasized by use of a 15-year lag period, could be more likely to contribute to cancer development than recent exposure, consistent with the idea that a promoting agent must be present for a sustained period to accelerate tumor development[14]. We observed a considerable strengthening of the association between TCDD and cancer when the upper 5% of the observations were excluded or when TCDD was ln-transformed. Both approaches reduce the influence of extremely high exposure values. The strengthening could be due to exposure misclassification, to statistical instability resulting from sparse data in the high end of the exposure range[15] or to both of these.

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Table 1. Observed (Obs) number of deaths, standardized mortality ratio (SMR) and 95% confidence interval (CI) for all cancer, smoking-related cancers (oral cavity and pharynx, esophagus, larynx, lung and bladder), all other cancer and non-malignant respiratory disease (NMRD) for the eight plants

	All cancer	Smoking-related cancer	All other cancer	NMRD
Plant (TCDD*)	Obs, **SMR**, CI	Obs, **SMR**, CI	Obs, **SMR**, CI	Obs, **SMR**, CI
1 (51,669)	42, **111**, 80-150	17, **99**, 58-159	25, **121**, 78-179	10, **94**, 45-172
3 (4,365)	21, **116**, 72-178	12, **157**, 81-275	9, **86**, 40-164	5, **93**, 30-217
4 (9,656)	13, **90**, 48-153	6, **97**, 36-211	7, **84**, 34-173	1, **22**, 1-124
7 (11,442)	2, **62**, 8-223	2, **133**, 16-481	0, **0**, 0-212	0, **0**, 0-479
8 (170,642)	22, **125**, 78-189	18, **224**, 133-353	4, **42**, 11-107	9, **191**, 87-363
9 (140,292)	99, **101**, 82-124	35, **80**, 56-111	64, **119**, 92-152	13, **49**, 26-83
10 (135,931)	43, **187**, 135-252	24, **225**, 144-335	19, **154**, 93-240	12, **200**, 103-349
13 (975)	14, **180**, 98-301	6, **157**, 58-342	8, **185**, 80-364	2, **106**, 13-384
Total (84,057)	256, **117**, 103-132	120, **122**, 101-145	136, **112**, 94-133	52, **86**, 64-112

* Mean value of cumulative TCDD ppt-years, lagged 15 years, among all subjects at each plant.

Table 2. Rate ratio* (RR) and 95% confidence interval (CI) for all cancer, smoking-related cancer and other cancer by level of cumulative exposure to TCDD, lagged 15 years (ppt-years, approximate septiles)

Exposure decile (range, ppt-years)	All cancer RR (95% CI)	Smoking-related cancer RR (95% CI)	Other cancer RR (95% CI)
1 (<324)	1.0	1.0	1.0
2 (324-<1,091)	1.0 (0.6-1.9)	1.2 (0.5-2.8)	0.9 (0.4-2.2)
3 (1,091-<3,835)	1.7 (1.0-3.1)	1.3 (0.5-3.1)	2.1 (1.0-4.7)
4 (3,835-<11,220)	1.4 (0.8-2.6)	1.1 (0.4-2.7)	1.8 (0.8-4.0)
5 (11,220-<35,041)	1.4 (0.7-2.5)	1.7 (0.7-4.0)	1.1 (0.4-2.6)
6 (35,041-<106,715)	2.1 (1.2-3.9)	2.1 (0.9-4.9)	2.2 (1.0-5.0)
7 (106,715+)	2.0 (1.1-3.9)	2.2 (0.9-5.3)	1.8 (0.8-4.3)

*Estimated from Cox regression models that included race and year of birth in addition to TCDD.

Table 3. Results of Cox regression models of the relation between TCDD ppt-years and all cancer; models included race (white, nonwhite) and year of birth (quartiles)

Exposure lag period, TCDD exposure variable & model	Coefficient estimate for TCDD (beta)	Standard error of beta
TCDD ppt-years, unlagged		
1. Full data, untransformed (range: 130-22,932,289 ppt-years)	-8.9×10^{-9}	5.8×10^{-8}
2. Full data, ln-transformed	5.3×10^{-2}	3.1×10^{-2}
3. Excluding exposure above 95 percentile (>539,340 ppt-years)	1.6×10^{-6}	6.2×10^{-7}*
TCDD ppt-years, lagged 15 years		
1. Full data, untransformed (range: 6-17,284,554 ppt-years)	1.7×10^{-8}	9.1×10^{-8}
2. Full data, ln-transformed	8.1×10^{-2}	2.9×10^{-2}*
3. Excluding exposure above 95 percentile (>252,950 ppt-years)	3.3×10^{-6}	1.4×10^{-6}*

* $p<0.05$.

Table 4. Results of Cox regression models* of the relation between exposure to TCDD (ln-transformed, lagged 15 years) and mortality rates for all cancers, excluding one plant at a time

Plant ***excluded***	Beta†	Standard error	Rate ratio†	95% CI‡
Plant 1	0.082	0.030	1.09	1.02-1.15
Plant 3	0.085	0.030	1.09	1.03-1.15
Plant 4	0.074	0.029	1.08	1.02-1.14
Plant 7	0.078	0.029	1.08	1.02-1.14
Plant 8	0.081	0.031	1.09	1.02-1.15
Plant 9	0.098	0.038	1.10	1.02-1.19
Plant 10	0.054	0.032	1.06	0.99-1.12
Plant 13	0.098	0.030	1.10	1.04-1.17

* All models adjusted for race (white or nonwhite) and year of birth quartiles.

† Regression parameter or rate ratio estimate for 1 ppt-year TCDD.

‡ 95% CI, 95% confidence interval of the rate ratio.