Meta-Analysis of Dioxin Cancer Dose-Response for Three Occupational Cohorts

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List of Abbreviations

TCDD - 2,3,7,8-tetrachlorobenzo-p-dioxin

USEPA – United States Environmental Protection Agency

CI – Statistical confidence interval

NIOSH – National Institute for Occupational Safety and Health

SMR – Standardized Mortality Ratio

CSLC – Cumulative serum lipid concentration

TEQ –the amount of TCDD that would produce the same toxicity as a mixture of TCDD-like compounds (unit of measurement for TCDD-like compounds)

ED_{05} – Effective dose 05, exposure predicted to result in an increase in the lifetime probability of cancer of 0.05.

Outline of Section Headers

Abstract

This paper presents a meta analysis of data from three cohorts occupationally exposed to TCDD and related compounds. A statistically significant ($p = 0.02$) trend was found in total cancer mortality with increasing dioxin exposure. The trend tests show an increase in total cancer at cumulative TEQ serum levels that would result from lifetime intake of 7 pg TEQ/kg body wt/day, with no increase at 6 pg/kg/day. A linear dose response provided a good fit to the combined data, and predicted an ED_{01} (dioxin exposure resulting in a 0.01 increase in lifetime risk of cancer mortality) of 45 pg/kg/day (95% CI: 21, 324). USEPA estimates that current lifetime human exposures to dioxin average approximately 1 pg/kg/day (99% percentile 3 pg/kg/day). Although it appears unlikely that current exposures through foods would reach either 7 pg/kg/day or the ED_{01}, our analysis argues for careful consideration of the upper ranges of long-term average exposures for dioxins..

Introduction

In 2000 the United States Environmental Protection Agency (USEPA) released a draft risk assessment for 2,3,7,8-tetrachlorobenzo-p-dioxin (TCDD) and other dioxin-like compounds that evaluated the current state of knowledge regarding exposures and health effects of these compounds (USEPA, 2000). Included in this assessment was an estimate derived from epidemiological data of the 1% "effective dose", ED_{01}, defined as the lifetime average body burden of TCDD that would increase the lifetime risk of cancer (all kinds) mortality by 1%. Exposures to other dioxin-like compounds were accounted for by using "toxicity equivalent factors" to express the amount of all dioxin-like compounds in a mixture in TEQ units, defined as the amount of TCDD that would produce the same toxicity. The USEPA's risk assessment was criticized by Starr (2001), who showed that the epidemiological data used by USEPA was consistent with an elevated background cancer risk of about 32% relative to comparison populations, and no dioxin effect.

The USEPA's ED_{01} estimate was based on data from three occupational cohorts: 5,172 workers from 12 U.S. chemical plants studied by the National Institute for Occupational Safety and Health – the "NIOSH cohort" (Fingerhut et al. 1991; Aylward et al. 1996), 1,189 workers at a chemical plant in Hamburg, Germany -- the "Hamburg cohort" (Flesch-Janys et al. 1998), and 243 workers exposed as a result of an uncontrolled release in 1953 of TCDD from an autoclave being used for trichlorophenol production at a BASF AG plant in Ludwigshafen, Germany -- the "BASF cohort" (Ott and Zober 1996). The NIOSH cohort was by far the largest of the three, both in terms of the number of workers exposed and the number of cancers observed during followup.

Recently, an additional six years of followup was conducted for the NIOSH cohort (Steenland et al. 1999) and, more importantly, a new exposure assessment was developed that allowed an estimation of TCDD exposure for all members of the cohort (Steenland et al. 2001). This information was not available in time to be incorporated into the USEPA (2000) assessment. The present paper incorporates this new information from the NIOSH cohort, along with the information previously available for the Hamburg and BASF cohorts, into a risk assessment similar to that conducted by the USEPA (2000). Results of this risk assessment are compared to those obtained by USEPA and Starr (2001). They are also compared to risks estimates based only on the NIOSH cohort (Steenland et al. 2001) and only on the Hamburg cohort (Becher et al. 1998). In addition, we also applied an analysis to determine the lowest exposures for which there were statistically significant associations between dioxin exposure and cancer mortality.

Review of New Data for NIOSH Cohort

The data for the NIOSH cohort utilized in the USEPA (2000) risk assessment were from the Fingerhut et al. (1991) study, which included followup through 1987. Steenland et al. (1999) extended followup of this cohort for an additional six years. They also developed a cumulative exposure score for each member of a subcohort of 3538 workers (69% of total cohort) obtained by eliminating workers with inadequate exposure information or who were exposed to pentachlorophenol in addition to dioxin. This exposure score was based on work history, the concentration of TCDD in process materials, and a qualitative evaluation of the potential for dermal and inhalation

exposure to TCDD-contaminated materials. More recently, Steenland et al. (2001) derived estimates of cumulative TCDD serum levels for this subcohort based on a regression analysis of exposure scores and serum lipid TCDD concentrations available for 170 workers at one of the NIOSH-studied facilities. This relationship was then used to estimate the cumulative serum lipid TCDD concentrations for all 3538 workers, based on the assumption that TCDD uptake and elimination obeys first-order pharmacokinetics. Steenland et al. (2001) observed a significant positive trend ($p = 0.003$) between all cancer mortality and the logarithm of cumulative serum lipid TCDD lagged 15 years.

Whereas Steenland et al. (2001) used worker-specific data on both the plant and the specific process, such information was not available to USEPA (2000) or Starr (2001). Instead, the exposure analysis used by USEPA and Starr assigned all workers in broad categories of duration of exposure the same cumulative serum level, regardless of the plant or job assignment within a plant, or when exposure took place in relation to the followup period. In the following sections we incorporate the new Steenland et al. (1999, 2001) data for the NIOSH cohort into a meta-analysis of the epidemiological data for dioxin.

Meta-Analysis Methods

Our meta-analysis is based upon the same three occupationally-exposed cohorts as the USEPA (2000) analysis: the NIOSH cohort, the BASF cohort and the Hamburg cohort. Standardized mortality ratio (SMR, the ratio of observed to expected cancer deaths, multiplied by 100) was the response measure used in these studies. Mortality

data for specific kinds of cancer were not evaluated, and the term “cancer” refers to mortality from all cancers. Cancer incidence data were not available, and consequently risks from non-fatal cancers were not reflected in the data, and risks from rapidly fatal cancers carried more weight than those from cancers with better survival rates.

Exposure Assessment: In order to make comparisons among different epidemiological studies, it is useful to have exposure quantified in a common metric that is plausibly related to risk. Since our analysis was based on published data, various aspects of the analysis, including selection of the dose metric, was constrained by the way in which data were presented in the published reports. Cumulative serum lipid concentration (CSLC, ppt-years), or "area under the serum lipid concentration curve", was selected as the exposure metric to relate to risk. By comparison, USEPA used average lifetime serum lipid concentration as the exposure measure in its analysis (USEPA, 2000). A time dependent exposure such as CSLC allows one to distinguish potential differences in risks from different exposure patterns that result in the same lifetime average exposure.

Flesch-Janys (1998, Table 5) categorized observed and expected cancer deaths in the Hamburg cohort (n=1189) by quartiles of TEQ CSLC (ppt-years), reduced by the cumulative TEQ contributed by background. Since only exposure ranges were provided in Flesch-Janys (1998), we specified average values within these ranges -- the mid-point for bounded ranges, and twice the lower bound for the highest (unbounded) range. Observed and expected numbers of cancer deaths, relative risks, and the estimated exposures for the Hamburg cohort are shown in Table 1.

Ott and Zober (1996, Table 1) categorized cancer deaths and SMRs in the BASF cohort (n=247) by total intake of TCDD (μg/kg body weight) as a result of the autoclave incident, estimated from detailed work activity analysis and from serum lipid TCDD concentrations measured in a subset of workers. Dr. Zober (Zober MA. Personal communication) provided arithmetic average total doses for each of the four exposure categories (0.015, 0.485, 1.38 and 3.72 μg/kg body weight, respectively). To convert these total intakes to TCDD CSLC (ppt-years), we divided them by 0.25 (based on an assumed average percent body fat of 25%) and by the decay rate (0.099/yr, corresponding to a half-life of seven years, as assumed by Ott and Zober). The resulting data are shown in Table 1.

Steenland *et al*. (2001, Table 2) computed risk ratios categorized by septiles of TCDD CSLC, including the contribution by background exposures to TCDD, using a 15-year lag (i.e., defined so that exposures in the most recent 15 years did not contribute). These risk ratios used the low exposure group as the reference group, and consequently are not appropriate for our meta-analysis, which needs the risks relative to the normal background uncontaminated by occupational dioxin exposure. Also, these risk ratios depend upon the observed risk in the low exposure group, which might involve considerable uncertainty. However, Steenland *et al*. (1999, Table 2) categorized observed cancer deaths and expected deaths for the NIOSH cohort by septiles of the cumulative exposure score, also using a 15-year lag. Since there was a high correlation between the cumulative exposure score and CSLC (Spearman correlation of 0.9, Steenland *et al*. 2001), the CSLC for the groups defined by septiles of cumulative exposure (Steenland *et al*. 2001) should be good approximations of

exposures in the comparable groups defined by the septiles of the exposure index (Steenland *et al*. 1999). Consequently, in our meta-analysis the CSLC (ppt-years, lagged 15 years, TCDD half-life of 8.7 years assumed) from Steenland et al. (2001) were applied to the cancer mortality data in Steenland et al. (1999). Central values for the exposure ranges (anti-logs of medians of log-transformed values) were provided by Dr. Steenland (Steenland, K. Personal communication). The resulting dose-response data are shown in Table 1.

Dose-Response Modeling: The dose-response data in Table 1 were modeled assuming that the SMR depends linearly on cumulative serum lipid concentration (CSLC, in units of ppt-y),

$$SMR = 100*\alpha*(1 + \beta*CSLC), \qquad (1)$$

where $100*\alpha$ is the baseline SMR and β is a parameter that gauges the carcinogenic potency of dioxin. This model was fit both with the baseline SMR fixed at 100 ($\alpha \equiv 1$) and with variable baseline SMR (α estimated). The fitting was accomplished using maximum likelihood, assuming that the observed cancers in each exposure group were realizations of independent Poisson variables, each with a mean equal to the expected number of cancer deaths derived from the comparison population used by the original authors, times the SMR predicted by equation (1), divided by 100. The meta analysis of the combined data from the three studies was accomplished via the combined likelihood

of the three data sets in Table 1. Likelihood ratio tests were used to test hypotheses, and confidence intervals were calculated using the profile likelihood method (Venzon and Moolgavkar, 1988; Kodell and West, 1993; Crump, 1995). All hypothesis tests of individual parameters are two-sided.

Two types of analyses were used to evaluate the cancer dose-response. First, a series of trend tests were applied to the data to determine the lowest dose for which there was a statistically significant trend in SMR using data from this dose and all lower doses, and the highest dose for which there was no statistically significant trend using data from this dose and all lower doses (Tukey, 1985). Second, estimates were made of ED_{10}, ED_{05} and ED_{01}, the lifetime average daily TEQ intakes (pg/kg/day) corresponding to an increase of 0.1, 0.05 and 0.01, respectively, in the lifetime probability of mortality from cancer.

To develop the trend analyses, the data in Table 1 were ordered with respect to CSLC, and a likelihood ratio test for a significant exposure-related trend (i.e., test for β in equation (1) being significantly different from zero with α estimated) was applied to the data. Then the data at the highest exposure were omitted and the trend test was reapplied to the remaining data. This procedure was applied repeatedly until only the data for the lowest dose group remained.

To estimate ED_{10}, ED_{05} and ED_{01}, the cumulative lipid concentration (ppt-years), lagged 15 years, from a constant daily intake was computed as a function of age, assuming a first-order elimination process with a 7.6 year half-life, a 50% systemic uptake of ingested dioxin, dioxin concentration in serum lipid is an appropriate surrogate for dioxin concentration in total lipid, and all dioxin is sequestered in lipid, which

comprises 25% of body weight (EPA, 2000). For a posited long-term average daily intake, the resulting cumulative age-specific lipid concentrations were applied in conjunction with the model (1) to predict the age-specific mortality rates in the presence of dioxin exposure. These were then applied in a life-table analysis to predict the lifetime risk of cancer in the presence of dioxin exposure (Crump 1994). The additional risk posed by dioxin exposure was calculated by subtracting from this lifetime risk the corresponding risk assuming no additional exposure to dioxin above background. To calculate an ED_{01}, the long-term average daily intake was adjusted to make the additional lifetime risk equal to 0.01. This calculation used, as baseline mortality rates for all-cause mortality and all-cancer mortality, U.S. rates (both sexes and all races combined) for the years 1985-1990.

Whereas background exposures are not included in the exposures estimated for the Hamburg and BASF cohorts (Table 1), the NIOSH exposures include the contribution of an assumed background of 5 ppt TCDD in serum lipid. In the trend analyses, a background contribution of 3000 ppt-years (e.g., 50 ppt for 60 years, as USEPA (2000) reported that background TEQ lipid levels in North America were about 55 ppt in the late 1980s) was added to the Hamburg and BASF exposures in Table 1, and 2700 ppt-years (45 ppt for 60 years, considering the 5 ppt already included in the NIOSH estimates) was added to the NIOSH exposures. Because the background mortality rates used to calculate ED_{01} already include any contribution to cancer mortality from background dioxin exposure, this adjustment for background was not made in the ED_{01} calculations. Consequently, the ED_{01} determined from our analysis are best interpreted as long-term average daily intakes of TCDD or TEQ above the

current TEQ background that are predicted to increase the lifetime probability of cancer mortality by 0.01 above the current baseline probability. The latter was estimated as 0.125 in our analysis and includes any contribution by background levels of dioxin.

Meta-Analysis Results

Table 2 summarizes results of fitting model (1) to the data in Table 1. The hypothesis of no dioxin effect ($\beta = 0$) was rejected ($p = 0.00007$) when the baseline SMR was fixed at 100, and also when the baseline SMR was estimated ($p = 0.02$). Because the hypothesis that the baseline SMR = 100 ($\alpha = 1$) was rejected ($p = 0.008$), the results in Table 2 obtained with baseline SMR variable (α estimated, bottom half of Table 2) are preferred. The linear model provided an adequate fit to the data (goodness of fit $p = 0.29$), produced a baseline SMR estimate of $100\alpha = 117$ (95% CI:104, 130), and predicted that each ppt-year of cumulative lipid concentration increased the relative risk by $\beta = 6.3 \times 10^{-6}$ (95%CI: 8.8×10^{-7}, 1.3×10^{-5}).

To test for potential nonlinearity in the dose response, model (1) was expanded by replacing CSLC by $CSLC^K$, $K \geq 1$. This expanded model is linear if K = 1 and sublinear (threshold-like) if K>1. The best estimate of K was 1, indicating there was no evidence of sublinearity in the dose response. Although models with K<1 (supralinear models) provided even better fits (and higher risks), these were discounted because they produce an infinite slope to the exposure-response curve at zero exposure, which is not considered biologically plausible.

Figure 1 shows the SMRs from the three studies and corresponding 95%

confidence intervals, plotted against CSLC (log scale) in Table 1, after adjusting as described earlier to include background TEQ CSLC. The fit of the linear model (1) with variable baseline SMR is also displayed. This figure provides a visual confirmation of the adequacy of the linear model to describe these data.

Figure 1 suggests possible non-homogeneity in the dose responses of the three studies, since all four data points from Ott and Zober (1996) are below the predicted curve, and three of four data points from Flesch-Janys et al. (1998) are above the predicted curve. A likelihood ratio test of whether separate β for each study provided a better fit to the data was non-significant ($p = 0.13$, 2 df). A test of whether both separate β and separate α provided a better fit was also non-significant ($p = 0.17$, 4 df). These results suggest that perhaps some, but not extreme, heterogeneity exists among studies, and consequently supports a combined analysis of data from all three studies using a common model.

Table 2 provides ED_{01}, ED_{05} and ED_{10}, calculated with both the baseline SMR fixed at 100 and with the baseline SMR variable. As noted above, results from the latter model are preferred because the hypothesis that the baseline SMR = 100 was rejected ($p = 0.008$) and the model with the baseline variable provided a good fit to the data ($p = 0.29$). The model with variable baseline predicted ED_{10} = 475 pg/kg/day (95% CI: 223, 3401), ED_{05} = 231 pg/kg/day (95% CI: 109, 1653) and ED_{01} = 45 pg/kg/day (95% CI: 21, 324).

It was noted earlier that exposures were lagged 15 years in the Steenland et al. (1999, 2001) study, but not lagged in the remaining studies. In our calculations of ED_{10}, ED_{05} and ED_{01} we used a 15-year lag. Some lag seemed appropriate, because

exposures immediately prior to death are not likely to influence the cancer response. Since Steenland et al. (1999, 2001) employed a 15 year lag, and the majority of the data were from this study, it was decided to use a 15-year lag in the calculations. When no lag was employed (results not shown), the estimated ED_{10}, ED_{05} and ED_{01} were smaller by roughly 40%.

Table 3 gives the results from the series of trend tests. The trend was significant ($p = 0.02$) when all the data were included. When the data at the highest exposure were omitted, the trend remained significant ($p = 0.04$) and the slope, β, increased. As the 9 highest dose groups were successively omitted, the dose-response slope, β, increased at each step, until only doses of 3988 ppt-years or less remained. Also, as successive data points were omitted, the trend remained significant ($p \leq 0.05$) through the step at which only the data corresponding to a cumulative serum level of 7120 ppt-years or less were left. When the 7120 ppt-years data point was omitted, leaving 6416 ppt-years as the highest dose, the trend became barely non-significant ($p = 0.07$) and remained so as the next data point was omitted. However, the trend again became significant ($p = 0.04$) when the highest exposure remaining was 3988 ppt-years. Statistical significance was not obtained when the 3988 ppt-years group was omitted (leaving 3853 ppt-years as the highest remaining exposure), or when subsequent dose groups were omitted. Thus, there is consistent statistical evidence of an exposure effect at 7120 ppt-years and above. There is, however, also statistical support for an effect at 3988 ppt-years and above.

Discussion of Dioxin Risk Assessments

USEPA (2000): The dose-response assessment methodology applied to epidemiology data in the USEPA draft health effects dioxin document differed from our analysis in mainly three ways. First, rather than using Steenland et al. (1999, 2001), the USEPA used Fingerhut et al. (1991), which included six fewer years of followup and a less detailed exposure assessment. Second, the USEPA used average body burden as the exposure metric, whereas we used cumulative serum lipid concentration. Third, USEPA assumed a baseline SMR of 100, whereas we allowed the baseline SMR to increase above 100 because the hypothesis that SMR =100 could be rejected. USEPA did not conduct a formal test of the hypothesis that SMR =100, nor did they report on the fit of the model to the data. However, Starr (2001) reproduced the USEPA analysis, and concluded that the model did not fit adequately. Based on their meta-analysis, USEPA estimated an ED_{01} = 47 ng/kg body burden (95% lower bound: 30 ng/kg). This body burden is estimated (see footnote to Table 4) to correspond to a daily intake of 27 pg/kg/day (95% lower bound: 18 pg/kg/day).

Starr (2001): Starr conducted a critique of the USEPA (2000) risk assessment for dioxin that included a meta-analytic evaluation of the same dose-response data from the NIOSH, Hamburg and BASF studies as was used by USEPA in its meta-analysis. Starr concluded that the data from these three studies were consistent (goodness of fit p-value = 0.31) with an elevated background SMR = 132 and no exposure effect. By contrast, our comparable analysis, based upon the updated NIOSH data, found a significant dose response trend (p = 0.01).

Applying the same linear model for relative risk as the USEPA (constraining the

background SMR = 100), Starr (2001) estimated an ED_{01} ppt lipid concentration of 47 ppt (95% lower bound: 28 ppt), which agrees with USEPA's results. However, Starr noted that this model did not describe the data adequately (goodness of fit p-value = 0.0003). When the background SMR was estimated, the linear model provided an adequate fit (goodness of fit p-value = 0.31) and an ED_{01} = 145 ppt (95% lower bound: 49 ppt). This lipid concentration is estimated (see footnote to Table 4) to correspond to a daily intake of 72 pg/kg/day (95% lower bound: 24 pg/kg/day). However, the fit of the intercept-only model was equally as good as that of the linear model.

Steenland et al. (2001): Steenland et al. conducted a quantitative risk assessment using only the updated data from the NIOSH study. A significant (p=0.003) positive dose-response trend was found between estimated log cumulative TCDD serum level and all-cancer mortality. Steenland et al. estimated additional lifetime risk of cancer from TCDD exposure using two models. One model assumed that relative risk was a linear function of log TCDD CSLC lagged 15 years, and the second assumed that relative risk was a piece-wise linear function of (untransformed) TCDD CSLC with no lag.

The piecewise linear model selected by Steenland et al. had a change in slope at a cumulative serum level of 40,000 ppt-years – this break-point being determined by "a process of elimination". A threshold model was found not to significantly improve the fit. The use of the piecewise model caused the risk estimates to be larger than what would be obtained using a purely linear model.

Based on the piecewise linear model, Steenland et al. estimated an increased

lifetime risk of 0.0005 in males, and 0.0004 in females, from an incremental exposure of 1 pg/kg/day TCDD over the risk at a background exposure to 0.5 pg/kg/day. Using the same model, they estimated an increased lifetime risk of 0.0071 in males, and 0.0060 in females, from an incremental exposure of 10 pg/kg/day TEQ over the risk at a background exposure to 5 pg/kg/day. Because the background TEQ exposure of 5 pg/kg/day is the more realistic scenario, we will focus on the average risk in males and females under this scenario. Since the model is linear in this exposure range, an additional lifetime risk of 0.0065 from an additional exposure to 5 pg/kg/day is equivalent to an ED_{01} = 5*0.01/0.0065 = 7.7 pg/kg/day (95% CI: 5.0, 19).

The log-linear model employed by Steenland et al. predicted risks of up to 20-fold higher than those predicted by the piece-wise linear model. However, Steenland et al. noted that this model may be unrealistic and expressed a preference for the piecewise linear model. Results from the log-linear model are not considered further herein.

Becher et al. (1998): Becher et al. conducted a quantitative dose-response assessment using only the data from the Hamburg study. Cumulative lipid concentration over time, with a lag of either 0 of 10 years was used as the exposure variable. A number of Poisson and Cox regressions were used to investigate dose response relations, and in each analysis TCDD and TEQ exposures were significantly related to total cancer.

To evaluate the shape of the dose response, Becher et al. considered three mathematical forms for relative risk: the “multiplicative model”, $RR = e^{\beta d}$, where d is cumulative TCDD or TEQ exposure; the “additive model”, $RR = 1 + \beta d$ (equivalent to our linear model, eq. 1); and the “power model”, $RR = (1 + \beta d)^k$, which is an extension of

the additive model. In the basis of each of these models, a linear relationship between exposure and lifetime risk was assumed by Becher et al. in the low dose range. Very similar risks were estimated for males and females separately, and combined, and both for no exposure lag and for a ten year lag; consequently, only results for males and females combined using a ten year lag will be discussed here. Using these models, Becher et al. estimated the additional lifetime risk of mortality from total cancer from lifetime daily intake of 1 pg/kg dioxin to be 0.0012 (multiplicative model), 0.0022 (additive model) and 0.0052 (power model).

The additive model provided a slightly better fit (higher likelihood) than the multiplicative model, and the power model predicted a supralinear dose response that provided only a very minor, statistically insignificant improvement over the fit provided by the additive model. The lifetime risk of 0.0022 from intake of 1 pg/kg dioxin per day (additive model) is equivalent to an ED_{01} = 0.01/0.0022 = 4.5 pg/kg/day. Although this analysis was based on TCDD serum levels, the slope Becher et al. obtained (β = 0.018 ppt^{-1}) was very similar to the slope they obtained using TEQ serum levels (β = 0.0175 ppt^{-1}). Consequently, it appears that a similar ED_{01} would have been obtained using TEQ serum levels.

The Present Analysis: The new NIOSH data (Steenland et al. 1999, 2001), which incorporates six additional years of followup and a detailed exposure analysis, provides new information on the potential carcinogenicity of dioxin. Based on a meta-analysis of data from three epidemiological cohorts, including the old NIOSH data, Starr (2001) did not find a statistically significant relationship between dioxin exposure and total cancer. However, using the data from the same three cohorts, but incorporating

the new NIOSH data, we did find a statistically significant relationship between dioxin exposure and cancer ($p = 0.02$).

Because we lacked the necessary data, we were not able to evaluate the likelihood that confounding with lifestyle factors or occupational exposures to other chemicals may have been responsible for the observed responses in the individual studies. However, fitting model (1) with the background SMR as an estimated parameter effectively compared the responses of workers exposed to different amounts of dioxin. Thus, confounding as an explanation for the association is less of a concern for the comparison in the present analysis than it would be if direct comparison were made of exposed workers to an external comparison group. Similarly, Steenland et al., using internal comparisons based on Cox regression, found significant trends in cancer in the NIOSH data with logarithm of cumulative exposure score, cumulative exposure score after omitting the highest 1% of exposure scores (Steenland et al. 1999), and logarithm of cumulative serum level (Steenland et al. 2001).

The trend analysis (Table 3) demonstrates statistical evidence of an association between dioxin TEQ exposure and cancer mortality for TEQ CSLCs of 3,988 ppt-years and higher. The highest dose where a trend was not supported by the analysis is a TEQ CSLC of 3,605 ppt-years. In addition this analysis does not support the frequently quoted observation that the human evidence for dioxin carcinogenicity is limited to populations with very high exposures. If anything, our analysis suggests the contrary, since the slope of the dose-response curve increased as higher doses were successively omitted (Table 3). The lack of statistical significance at the lowest doses does not necessarily indicate the absence of a dioxin effect in this dose range, since

this could be the result of a reduction in statistical power as higher doses are omitted.

The estimated long-term average daily intake corresponding to a cumulative lifetime (to age 70) exposure of 3,988 ppt-years is 7 pg/kg/day. By comparison, based on combined analysis of fat intake, estimates of average dioxin intake, and variation in serum dioxin levels, current average human daily intake is estimated to be about 1 pg/kg/day TEQ, with a 99% percentile of 3 pg/kg/day (USEPA, 2000). Thus, while current US food-borne exposures are not likely to range up to the levels where our analysis found significant associations with cancer mortality, our analysis provides some evidence that TEQ exposures near current background levels are carcinogenic.

The linear dose-response model based on cumulative exposure described the data well (goodness of fit p-value = 0.29), despite the fact that the cohort members experienced patterns of exposure ranging from acute (e.g., from the autoclave accident in the BASF plant) to longer term exposures. Moreover, there was no statistical evidence of a sublinear dose response or threshold. Our trend analysis (Table 3), taken at face value, indicates that, if a threshold for the carcinogenicity of dioxin exists, it is likely below a cumulative serum level of 4,000 ppt-years.

Despite the statistical significance of the test for dose-response trend in our meta-analysis (p = 0.02), the data were marginally consistent, according to a goodness of fit test, with no effect of exposure and a background SMR of 124 (goodness-of-fit p-value of 0.08). However, a goodness of fit test does not specifically evaluate the hypothesis of increasing response with increasing exposure to dioxin. In contrast, a trend test provides a specific, and statistically more powerful, evaluation of this hypothesis.

There are several differences in the exposure estimates for the three epidemiological studies used in our meta-analysis. First, the NIOSH estimates employed a 15-year lag, whereas no lag was used with the other cohorts. Given that followup in the Steenland et al. cohort extended for many years past the time at which exposures were most significant, results based upon cumulative exposure lagged 15 years should not differ greatly from those based upon unlagged exposure.

A second difference in the exposure estimates is that those for the Hamburg cohort included total TEQ (Flesch-Janys et al. 1998), whereas estimates for the NIOSH (Steenland et al. 1999, 2001) and BASF (Ott and Zober, 1996) cohorts quantified only TCDD. Based on the available lipid samples from workers, total TEQ exposures in the Hamburg cohort appears to have been primarily a result of exposure to TCDD (Piacitelli et al. 1992; Ott and Zober 1996; Ott et al. 1993), whereas total lipid TEQ in the Hamburg cohort were estimated to be about twice that resulting from TCDD alone (Flesch-Janys et al. 1998). Steenland et al. omitted (and consequently so does this analysis) all workers in the NIOSH cohort who were exposed to pentachlorophenol, which is contaminated with dioxins other than TCDD. Thus, it appears that the exposure estimates available for each cohort are reasonable estimates of total TEQ exposures.

A third difference in the exposure estimates is that the exposures for the NIOSH cohort included 2,3,7,8-TCDD background exposures, whereas the exposures for the Hamburg and BASF cohorts did not include any background. In the trend analysis (Table 3 and Figure 1) the exposures in Table 1 were modified (2,700 ppt-years added to NIOSH exposures and 3,000 ppt-years to Hamburg and BASF exposures) to include

TEQ contributions to background. However, we obtained similar results (not shown) in the trend analysis when no background adjustment was made, and also when 300 ppt-years was subtracted from NIOSH exposures. No adjustment for background was made in the calculations of ED_{01}, and these are best interpreted as pertaining to additional risk over any that may exist from background exposures. However, these estimates are based on a linear model, and consequently will be insensitive to how background exposures are handled so long as the CSLC background is small relative to $1/\beta$, which is the case here. As a verification of this, the analysis leading to our ED_{01} of 45 pg/kg/day (Table 2 and 4) was repeated using the exposures adjusted for background (Table 3 and Figure 1); the resulting change in the ED_{01} was less than 3%.

There is some evidence that at high exposures liver enzymes are induced that serve to increase the elimination rate of dioxin compounds (Carrier et al. 1995). Such an effect was not accounted for in the analyses discussed herein, but rather in each case first-order pharmacokinetics were assumed. Based on the estimated maximum body burdens in these studies, the amount of under-estimation of the cumulative exposures from not accounting for enzyme induction is expected to be at most a factor of 1.5 for the upper dose levels (Zeilmaker *et al*. 1998; Van der Molen *et al*. 2000).

Table 4 summarizes ED_{01} estimates derived from linear (or piecewise linear) models. The USEPA ED_{01} estimate and the estimate by Starr with baseline SMR = 100 agree very closely (23-24 pg/kg/day), as expected since Starr's calculation is intended as a reproduction of USEPA's. However, Starr showed that this model provided an inadequate fit to the data ($p < 0.003$). It is interesting that our meta-analysis with SMR = 100 also predicted a very similar ED_{01} (25 pg/kg/day), as there are a number of

differences between our calculations and those of USEPA and Starr. We used the updated followup and exposure data for the NIOSH cohort, and used cumulative lipid serum concentration as the exposure measure, whereas USEPA and Starr used the earlier NIOSH data and employed average body burden as the exposure metric.

Since the hypothesis that background SMR = 100 was rejected ($p = 0.008$), the model with background SMR estimated is the preferred one from our meta-analysis. This model predicted an ED_{01} = 45 pg/kg/day, and was based upon a statistically significant linear trend ($p = 0.02$). This estimate is also preferred over Starr's estimate of 72 pg/kg/day because it reflects the updated followup and more precise exposure estimates for the NIOSH cohort.

The estimate, ED_{01} = 4.5 pg/kg/day, from the linear model applied by Becher et al. (1998) to the Hamburg data is 10-fold smaller than our preferred estimate of ED_{01} = 45 pg/kg/day based on data from all three cohorts. This difference is mainly attributable to differences in the underlying data. When we restricted our analysis to just the Hamburg data, we obtained an ED_{01} = 11 pg/kg/day, and when we repeated this analysis using Hamburg TCDD exposures rather than TEQ exposures, we obtained an ED_{01} = 4 pg/kg/day.

Our preferred ED_{01} of 45 pg/kg/day is six times higher than the Steenland et al. estimate of 7.7 pg/kg/day. We have not determined the full basis for this difference, although contributing factors are known. Both analyses estimate cancer risk above that of an unexposed worker population rather than of an external comparison population. Effects of different assumptions regarding pharmacokinetic parameters (uptake fraction, half-life, and percent lipid) and background exposures appear minor. Part of the

difference is attributable to the fact that the Steenland et al. analysis was based only on the NIOSH cohort and our analysis also incorporated the data from the BASF and Hamburg cohorts. However, when we repeated our analysis using only the NIOSH data, our ED_{01} estimate only decreased from 45 pg/kg/day to 32 pg/kg/day. The most likely reason for the remaining difference is that, whereas we used a purely linear model, Steenland et al. used a piecewise linear model with a break in the slope at a CSLC of 40,000 ppt. Use of the piecewise linear model resulted in a smaller ED_{01} than would have been obtained using a linear model. However, it should be kept in mind that none of these models can be verified at low exposure levels.

At present we do not see a clear choice between our ED_{01} estimate of 45 pg/kg/day and the Steenland et al. estimate of 7.7 pg/kg/day. Our estimate has the advantage of drawing from three different studies. On the other hand, the Steenland et al. estimate has the advantage of being based on individual worker data from the largest of the three studies rather than summarized data. If the different policy implications of the two estimates are large, it could be worthwhile to conduct an analysis that combine the best features of each and perhaps include data from other cohorts with extensive TCDD exposure evaluation, such as the Dutch accident cohort (Hooiveld et al. 1998).

Overall, the available dose-response assessments for dioxin and cancer indicate that dioxin TEQ exposures within roughly 3-fold of current background levels may be carcinogenic. The proximity of food-borne dioxin exposure levels to those associated with cancer argues for careful consideration of both the cancer mechanism and the upper ranges of long-term average exposures for dioxins.

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Table 1
Dose Response Data from Three Epidemiological Studies

Cum. Lipid TCDD or TEQ (ppt-y)	Cancer deaths Obs	Exp	SMR
Flesch-Janys (1998)			
180	25	23.3	107
988	34	20.8	164
3416	31	23.3	133
10425	34	20.8	164
Ott and Zober (1996)			
605	8	10.0	80
19614	8	6.7	120
55645	8	5.7	140
150454	7	3.5	200
Steenland et al. (1999, 2001)			
260	67	68.4	98
402	27	30.0	90
853	31	27.2	114
1895	30	25.4	118
4420	34	25.6	133
12125	33	19.5	169
59838	34	22.1	154

Table 2
Results of Fitting Model (1) to Data in Table 1

		(95% CI)
Baseline SMR = 100 (α = 1)		
β (ppt-years)$^{-1}$ (x106)	11	(5.1,19)
P-value for test of β = 0 (no dioxin effect)	0.00007	
Goodness of fit p-value	0.05	
ED_{10} (pg/kg/day)	266	(161,587)
ED_{05}	129	(78,285)
ED_{01}	25	(15,56)
Baseline SMR variable		
α	1.17	(1.04,1.30)
P-value for test of α = 1	0.008	
β (ppt-years)$^{-1}$ (x106)	6.3	(0.88,13)
P-value for test of β= 0 (no dioxin effect)	0.02	
Goodness of fit p-value	0.29	
ED_{10} (pg/kg/day)	475	(223,3401)
ED_{05}	231	(109,1653)
ED_{01}	45	(21,324)

Table 3
Tests Results for Dose-Response Trend Applied to Data Ranked by Cumulative Serum Lipid Concentration (CSLC), Adjusted to Include Background TEQ.

CSLC (ppt-y)	Cancer deaths Obs	Exp	SMR	Study	β(Slope)[a] (ppt-y)$^{-1}$	trend p-value[a]
153,434	7	3.5	200	Ott and Zober (1996)	5.7E-06	0.02**
62,538	34	22.1	154	Steenland et al. (1999, 2000)	7.6E-06	0.04**
58,645	8	5.7	140	Ott and Zober (1996)	1.6E-5	0.05**
22,614	8	6.7	120	Ott and Zober (1996)	4.6E-05	0.005***
14,825	33	19.5	169	Steenland et al. (1999, 2000)	6.7E-05	0.001***
13,435	34	20.8	164	Flesch-Janys (1998)	7.8E-05	0.008***
7,120	34	25.6	133	Steenland et al. (1999, 2000)	1.2E-04	0.05**
6,416	31	23.3	133	Flesch-Janys (1998)	1.9E-04	0.07*
4,595	30	25.4	118	Steenland et al. (1999, 2000)	6.4E-04	0.08*
3,988	34	20.8	164	Flesch-Janys (1998)	1.7E-01	0.04**
3,605	8	10.0	80	Ott and Zober (1996)	4.8E-5	0.78
3,553	31	27.2	114	Steenland et al. (1999, 2000)	4.0E-04	0.49
3,180	25	23.3	107	Flesch-Janys (1998)	0	1
3,102	27	30.0	90	Steenland et al. (1999, 2000)	0	1
2,960	67	68.4	98	Steenland et al. (1999, 2000)		

[a] Slope and two-sided p-value for dose-response trend obtained using data from given exposure group and all groups with lower CSLC.

* $p \leq 0.1$
** $p \leq 0.05$
*** $p \leq 0.01$

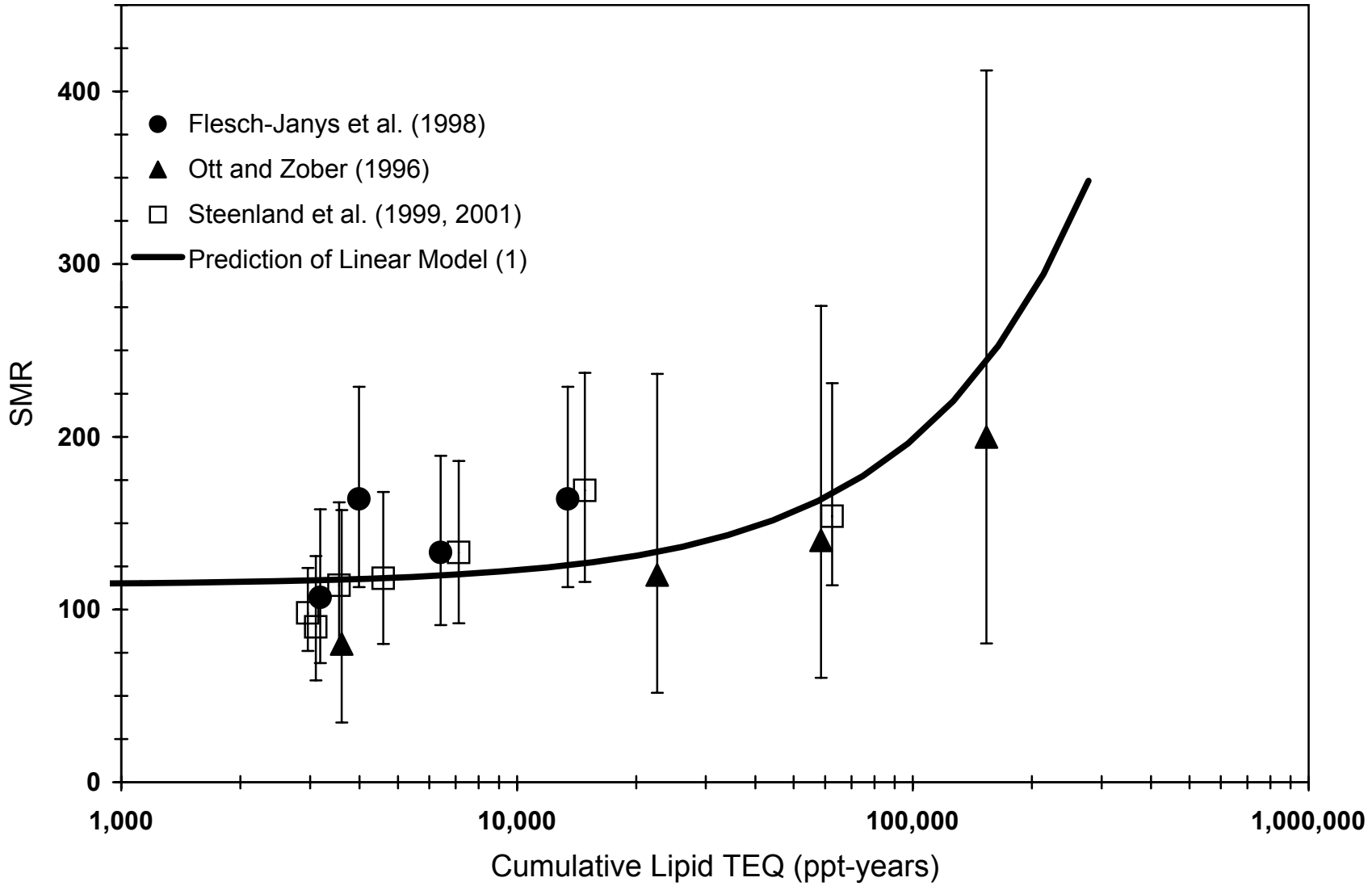
Table 4
Summary of ED_{01} Estimated from Linear or Piecewise Linear Dose-Response Models

Study and Model	Background SMR	pg/kg/day intake ED_{01}	95% LB	95% UB	ppt, steady-state serum ED_{01}	95% LB	95% UB	g-o-f p-value
Becher et al. (1998)	(effectively	4.5	NR	NR	9.1	NR	NR	NR
EPA (2000) (linear)	fixed (=100)	23	15	NR	47	30	NR	NR
Starr (2001) (linear)	fixed (=100)	24	14	NR	47	28	NR	0.003
	estimated	72	24	infinite	145	49	infinite	0.31
Steenland et al. (2001) (piece-wise linear)	(effectively estimated)	7.7	5.0[a]	19[a]	15[a]	10[a]	37[a]	NR
Present study (linear)	fixed (=100)	25	16	47	51	33	95	0.08
	estimated	45	23	173	91	47	346	0.29

The relationship between daily intake and steady-state serum concentration was determined assuming first order pharmacokinetics, half-life of 7.6 years, 50% systemic uptake of TCDD, TCDD sequestered only, and homogeneously, in lipid, which forms 25% of human body by weight.
[a] 97.5% bounds, rather than 95%.

Figure Legends

Figure 1: SMRs (with 95% Confidence Bounds) from Three Studies Categorized by Cumulative Lipid Concentration (Table 1), Adjusted to Include Background TEQ.


Flesch-Janys et al. (1998)
Ott and Zober (1996)
Steenland et al. (1999, 2001)
Prediction of Linear Model (1)
SMR
400
300
200
100
0
1,000
10,000
100,000
1,000,000
Cumulative Lipid TEQ (ppt-years)