COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

CONSIDERATION OF THE TDI FOR DIOXINS AND DIOXIN-LIKE PCBS:

Evaluation of exposure data in the epidemiological studies

1. This paper is a synthesis of COT paper TOX/2001/14 and TOX/2001/24, with additional information as requested by the Committee. It summarises the human data considered by the COT, focussing on how the original data were obtained and processed in order to derive body burdens for exposure in the epidemiology studies. It does not include cancer end-points, which were evaluated by Committee on Carcinogenicity (COC). The paper identifies differences between the various studies with regard to nature of the biological fluid in which the dioxins were measured, expression of the results either for a single compound or as TCDD toxic equivalents (TEQs) and subsequent transformation of the measured levels. The studies identified by the Committee as important for the evaluation are summarised in Table 1.

Study	Reference
A. the Operation Ranch Hand study of US Air Force personnel	Roegner *et al*., NTIS# AD A-237-516 through AD A-237-524, 1991; Grubbs *et al*., Report No AL-TR-920107, 1995,
B. The study of 50 Missouri residents	Webb *et al*., *J Toxicol Environ Health* 28: 183-193, 1989,
C. The evaluation of the BASF accident cohort and	(Ott *et al*., *Int Arch Occup Environ Health* 65: 1-8, 1993;),
D. The cohort of Hamburg chemical workers	Flesch-Janys *et al*., *Environ Health Perspect* 106 (suppl 2): 655-662, 1998
E. The NIOSH study of 281 workers in a plant which produced sodium 2,4,5-trichlorophenate (NaTCP), 2,4,5-trichlorophenoxy acetic acid (2,4,5-T) and 2,4-dichlorophenoxy acetic acid (2,4-D)	Piacitelli *et al*., *Chemosphere* 25: 251-254, 1992, Data taken from Steenland *et al*. J Nat Cancer Ins 91:779-786 (1999) & Sweeney *et al*. *Chemosphere* 20: 993-1000, (1990)
F. Dutch Chemical workers	Hooiveld *et al. Am J Epidemiol* 147:891-901 1998
G. Two Dutch series of studies on developmental effects	Koopman-Esseboom *et al*., *Chemosphere* 29: 2327-2338, 1994; Pluim *et al*., *Acta Paediatr* 83: 583-587.
H. Seveso sex ratio	Mocarelli *et al. Lancet* 355:1858-1863 (2000)

Table 1. Studies used to derive body burdens.

2. The effects observed in one or more human studies and a brief summary of the overall results are summarised in Table 2. The first two studies listed above did not provide adequate detail for full evaluation. Reported odds ratios and derived body burdens are summarised for cardiovascular disease in the worker cohorts and altered sex ratio in Seveso. Relative risks for total cancer in the worker cohorts and derived body burdens are included for comparison.

Effect	**Epidemiological evidence**
Chloracne	Proven association No clear dose relationship
Gastrointestinal effects and liver enzymes	Transient increases in some liver enzymes
Cardiovascular diseases	Positive association in occupational studies but not in airforce veterans exposed to herbicides in Vietnam (Operation Ranch Hand) Dose-response in some studies
Changes in lipid levels	Results not consistent
Diabetes	Overall results not consistent Increased risks of morbidity in Seveso and Ranch Hand
Reproductive hormones	Inconsistent results
Reproductive outcomes	Change in sex ratio of offspring with highly exposed fathers in Seveso No data yet on possible effects such as endometriosis and fertility in women – Seveso endometriosis study on-going
Thyroid function	Results not entirely consistent. Some small differences reported in thyroid hormone uptake levels.
Neurologic / psychological effects	Inconsistent findings. Some effects reported in Ranch Hand and Seveso (polyneuropathies, abnormal co-ordination) No association with depression
Respiratory system	Inconsistent evidence Irritative effects and reduced lung function parameters in some studies
Urinary system	No major renal or bladder dysfunctions observed.
Immunological effects	Inconsistent findings.
Developmental effects	Some observed differences in on-going Dutch studies
Cancer	Regarded as a probable human carcinogen (based on human, animal and mechanistic data) Data suggest that a threshold approach to risk assessment is likely to be appropriate

Table 2. Effects associated with dioxin exposure in human studies.

3. Chloracne is associated with exposure to TCDD but no clear relationship between chloracne and exposure or body burden exists. At a group level,

individuals with severe chloracne tend to have average higher exposures than those with moderate or no chloracne.

A. Operation Ranch Hand studies.

4. Exposure data for the testing of immunological parameters carried out during 1997 examinations in the Air Force Health Study on veterans of Operation Ranch Hand in Vietnam was derived from blood levels of dioxin determined in samples obtained during the1987 or 1992 exams. A total of 2198 of the 2233 participants (952 veterans and 1281 controls) gave blood for determination of dioxin levels. 2154 had quantifiable levels of dioxin (1791 from 1987 and 363 from 1992). After excluding those without measured dioxin levels or whose dioxin levels were non-quantifiable (22 veterans and 57 controls) along with 24 controls with values above 10 ppt or with selected diseases or therapy (16 veterans and 14 controls). The sample size for immunological testing with exposure data was 914 veterans and 1186 controls.

5. Testing consisted of skin tests, immunoglobulin studies & autoantibody panel tests in all participants and total lymphocyte counts & lymphocyte subset investigations on around 40% of veterans randomly selected on final digit of case number (358 veterans and 456 controls). The dioxin levels at the end of service were estimated from the current dioxin value using a half-life of 8.7 years. The half-life was derived from a study on a subset of 278 members of the cohort with 2 measures of dioxin levels, one in either 1982 or 1987 and one in 1992. This half-life was corrected for percent body fat.

6. Measured dioxin levels were assigned to four categories controls (n=1186), background (n=393, <10ppt), low (n=261, 10< <94.1 ppt) & high (n=260, >94.1 ppt). The dividing point between low and high dioxin levels was the median estimated initial dioxin value. All levels were corrected for background by subtraction of 4 ppt and it was assumed that there was no dioxin exposure since the end of military service in Vietnam. No evidence of a consistent relationship between dioxin exposure and the measured immunological parameters was found.

Evaluation of Operation Ranch Hand studies.

7. These exposure data were taken from a single study looking at immunological parameters in order to describe the derivation of the exposure data. Whilst other studies are referenced in the EPA report the cited references are US government reports and it has not been possible to evaluate these. The exposure data were not contemporaneous with the actual exposure but were estimates of levels based on extrapolation from samples taken a number of years (up to 30) after the putative exposure. The assumption of no subsequent dioxin exposure is critical to this extrapolation. There were differences between the three groups of exposed individuals with an over-representation of enlisted ground crew in the high category and officers in the background category. The high category also included more smokers and was younger. These differences

may indicate a systematic difference between this and the other groups. This study was not considered suitable for further evaluation.

B. Missouri residents.

8. Immunological parameters were determined in 51 individuals with measured TCDD levels in adipose tissue following exposure through occupational, recreational or residential exposure. Of these 51 individuals, 2 had died leaving 49 eligible of whom 41 agreed to participate, however one was subsequently excluded from analyses as their blood sample clotted prior to the testing of immune functions. A number of immune function tests were performed on this group who were divided into three sub-groups based on tissue TCDD levels. These groups were 16 individuals with adipose tissue TCDD levels below 20 ppt, 13 individuals with adipose tissue TCDD levels between 20 and 60 ppt (including the excluded individual) and 12 individuals with adipose tissue TCDD levels above 60 ppt.

9. Using regression analyses two of the measured clinical laboratory variables (globulin and albumin/globulin ratio) were significantly related to the adipose tissue TCDD level but the means were still within the normal range. Six variables in the *in vitro* immune tests (IgG, %T3, %T8, number of T8, %T11 & %T4) together with LEU 8 POS were significantly related to the adipose tissue TCDD level. In both cases these were seen after multiple regression analysis after controlling for age and sex. Although 7 of the 9 individuals with low T4 to T8 ratios (below 1.2) had adipose TCDD levels above 60 ppt no clear dose response relationship could be seen.

Evaluation of the Missouri data.

10. The group with measured adipose TCDD levels represented a larger group of exposed individuals in Missouri. There are limited and insufficient data on the sources and duration of exposure. The highest level reported in this study was 750 ppt and there was no control group. Only a single dioxin congener (TCDD) was measured and no information is available on exposure to other dioxins, furans or dioxin-like PCBs. This study was not considered suitable for further evaluation.

C. BASF cohort.

11. This cohort comprised 254 workers at a BASF plant in Ludwigshaven from its contamination in a 1953 accident until demolition of the building in 1968-9. The exposure assessment involved five factors; time period, physical location, nature of work, use of protective equipment & duration of exposure. There were 4 time periods; 22 days after accident, clean up period (~4 months), 5.75 years maintenance etc & final 8 years. There were 3 locations; within enclosed autoclave work area, elsewhere within accident building and outwith the building. Six work groups were identified: production, mechanics & transport, firemen, general construction & maintenance, laboratory workers and

professionals. Protective equipment was varied but only use of gas-proof protective clothing with fresh air supplied masks was used to modify exposure estimates. If such equipment was known to have been worn, work in the autoclave area was downgraded to work within the building for exposure purposes. The duration of exposure was expressed as days and was converted by using each 8 hours of actual exposure as equivalent to 1 day.

12. The total cohort of 254 individuals was subdivided into 3 subcohorts: C1, accident and initial clean-up (n=69); C2, potential exposure 1954-1968 (n=84); C3, involved in toxicology, inspections or demolition (n=94). Blood specimens were collected between 1988 and 1992 (mean date 12/89) from 138 members of the cohort. Blood specimens were deep frozen and transported to the analytical laboratory where 40 ml of whole blood was extracted and analysed for TCDD, 1,2,3,7,8-PnCDD, three HxCDDs, 1,2,3,4,6,7,8-HpCDD and OCDD and corresponding furans using GC/MS. Results were expressed as pg/g extractable lipid (ppt) but were only reported for the dioxin congeners. Both linear and non-linear regression models were used to estimate the contributions of the various exposure situations to the determined dioxin levels. The TCDD levels were extrapolated from the measured values using a 7-year half-life and first-order exponential decay to levels at the exposure date.

13. The current levels were compared to those observed in a previously described external referent group (n=102) without known occupational exposures measured in same analytical laboratory

14. The geometric mean TCDD concentrations for C1, C2 & C3 were 1009.5 ppt, 48.8 ppt & 83.7 ppt but there was a wide distribution of values in these groups e.g. 16% of C2 had values above 1000 ppt. The highest mean dioxin levels were seen in mechanics/transport workers & production workers and associated with the initial post-accident period, having worked in autoclave area and longer working days. For those individuals exposed to dioxin contamination only after 1959, TCDD levels were indistinguishable from background. A similar pattern but with lower amplitude was observed for penta-chlorinated dioxins. There was a disproportionate representation of certain groups in the 138 members of the cohort for whom dioxin levels were determined. In particular production workers, autoclave users, those exposed in the initial period and those exposed for greater than 240 days were under-represented.

Evaluation of BASF cohort studies.

15. Dioxin and furan levels were only determined for 138 of the 254 individuals in the cohort and several groups within the cohort are underrepresented. These discrepancies in representation may result in bias in the exposure estimates. Although several dioxins and furans were measured, only a limited comparison was made with TCDD concentrations.

BASF cohort results

16. Results are summarised in Table 3. There was no increased risk of circulatory diseases generally, or of ischaemic heart disease in particular, in this

relatively small cohort. There was a trend towards increased SMRs with increasing exposure for all cancers, although this was only clearly observed in the highest exposure group with body burdens above 1 µg/kg bw (which equates to a daily intake of 506 pg/kg bw/day).

Cause of death	TCDD Exposure group – body burden cited in paper		
	<0.1 µg/kg bw	0.1-0.99 µg/kg bw	> 1 µg/kg bw
Malignant neoplasms	0.8 (0.4 - 1.6) n = 8	1.2 (0.5 - 2.3) n = 8	1.6 (0.9 - 2.6) n = 15
Diseases of circulatory system	0.8 (0.4 - 1.4) n = 13	1.0 (0.5 - 1.7) n = 11	0.8 (0.4 - 1.3) n = 13
Ischaemic heart disease	0.9 (0.3 - 1.8) n = 7	0.7 (0.2 - 1.7) n = 4	0.6 (0.2 - 1.3) n = 5

Table 3. Standardised mortality ratios (95% CI) and observed deaths for selected causes of death and TCDD dose group, 1953-1992.

D. Hamburg cohort.

17. Flesch-Janys et al. (1998) described further work on a cohort of 1189 German male herbicide and insecticide workers. This work attempted to refine the exposure estimates from their 1995 paper in response to criticisms of the earlier exposure estimates. This analysis considers several covariates and co-exposures, particularly to β-hexachlorocyclohexane (β-HCH) and a previously published estimate of cancer risk at environmental background levels.

18. The levels of dioxins and furans were measured in 320 blood samples or 62 adipose tissue samples from 275 workers (39 females, 236 males) and TEQ values derived. For some workers 2 or 3 samples were available. Only concentrations greater than the 95th percentile of the German population at the relevant time were used to estimate dose rates. Measured concentrations of each congener reduced by the median background concentration were extrapolated to the end of employment using a first order elimination process. A first order kinetic equation was developed linking blood levels and working histories to calculate production department specific dose rates for every congener. These dose rates were used to estimate the concentration of every congener at every point in time for each member of the cohort. The cumulated levels calculated by integration were used in the SMR analysis.

19. The arithmetic mean concentration for TCDD was 101.3 ng/kg (range 2.0-2252 ng/kg) and for higher chlorinated dioxins and furans calculated as I-TEQ the mean was 89.3 (range 5.0-1131.9). The characteristics of the subcohort for whom blood levels were available were compared to the remaining workers. They were slightly younger, started working at the plant later and left the plant later, their duration of employment was longer (9.2 cf. 3 years). The distribution of the subcohort across production departments was generally adequate but was notably inadequate for administration and clean-up

workers and these two categories were combined for the analysis. Estimated dose rates for the various production departments showed that the highest rate (3376.4 ng TCDD/kg blood fat/year) was seen in the trichlorophenol department prior to the change in production process in 1957. After this change levels were comparable for trichlorophenol and 2,4,5- trichlorophenoxyacetic acid production (121.1 & 154.6 ng TCDD/kg blood fat/year respectively). Manual workers were estimated to have 48.2 ng TCDD/kg blood fat/year, whilst the other departments were grouped into 2 groups with 30.2 & 6.7 ng TCDD/kg blood fat/year respectively. One metalworker and two trichlorophenol workers were identified as outliers and excluded from the final model. The dose estimates for metalworkers were decreased from 184 to 48.2 ng TCDD/kg blood fat/year and from 3781.6 to 3376.4 ng TCDD/kg blood fat/year trichlorophenol workers by these exclusions.

20. Estimates of the dose rates for higher chlorinated congeners in the various production departments resulted in highest observed levels of 116.6 ng TEQ/kg/year in the thermic decomposition department. The trichlorophenol department produced estimates of 90.0 and 35.9 ng TEQ/kg/year prior to and after 1957 respectively, the higher value being largely influencd by the high pentadioxin estimate. The estimated dose rate for manual workers was 34.4 ng TEQ/kg/year and estimated dose rates for other departments ranged from 0 to 33.8 ng TEQ/kg/year. Lack of half-life estimates prevented estimation of dose rates for 3 congeners (2,3,7,8-TCDF, 1,2,3,7,8-PnCDF & OCDF), however blood levels of these usually were within the background range and they made no significant contribution to the total TEQ value.

21. The highest β-HCH dose rate was estimated at 46.5 µg/l blood/year for the thermic decomposition department and then the HCH synthesis department at 31.9 µg/l blood/year. Based on only four measurements, a high level (31.6 µg/l blood/year) was estimated for the laundry department. Estimates for other departments ranged from 0 to 17.7 µg/l blood/year.

22. In an earlier report (1995), on the 1189 male workers of this cohort, quantitative estimates of PCDD/F from blood and adipose tissue levels were determined in a smaller subcohort of 190 individuals. These were divided into deciles and quintiles for comparisons.

Evaluation of the Hamburg cohort study.

23. The exposure data were produced from a combination of an exposure matrix with exposure levels estimated from serum or lipid levels determined several years after exposure ended in a subcohort. In 1995 the subcohort comprised 16% of cohort basis but was larger for the 1998 analysis. Although the mortality analyses only involved the 1189 male members of the cohort, the subcohort used for exposure analysis included both male and female members of the total cohort. The exposure data were back-calculated to the end of exposure assuming first order kinetics and a chosen half-life. Any inaccuracies in the chosen parameters will add to several other potential sources of error and bias. These include selection bias from the lack of random sampling of the exposure subgroup and bias in the slope of the estimated regressed tissue

TCDD levels if individuals with long service but low levels are under represented in the subgroup.

Hamburg cohort results.

1995 analysis.

24. Flesch Janys *et al.* (1995) reported on the 1189 male workers of this cohort, for whom a quantitative estimate of PCDD/F exposure was modelled from blood and adipose tissue levels determined in a smaller subcohort of 190 individuals. The total cohort was divided into deciles and quintiles for comparisons but the basis for these divisions is not stated in the paper.

Blood TCDD (ng/kg blood fat)	**Derived body burden** (ng/kg bw)	**Relative risk and 95% confidence limits for**			
		Cancer	**CVD**	**IHD**	**Other CVD**
0-2.8	0-0.56	1.59 (1.01-2.51)	1.22 (0.81-1.83)	1.43 (0.83-2.44)	1.02 (0.54-1.92)
2.81-14.4	0.56-2.88	1.29 (0.75-2.22)	0.88 (0.54-1.44)	0.81 (0. 41-1.61)	0.98 (0.49-1.97)
14.5-49.2	2.9-9.8	1.66 (1.03-2.66)	1.35 (0.91-2.01)	1.18 (0.65-2.16)	1.54 (0.90-2.64)
49.3-156.7	9.8-31.4	1.60 (1.02-2.52)	1.64 (1.12-2.39)	0.90 (0.47-1.75)	2.52 (1.57-4.06)
156.8-344.6*	31.6-69	1.70 (0.99-2.93)	1.53 (0.95-2.44)	1.61 (0.85-3.04)	1.46 (0.72-2.94)
344.7-3890.2*	69-778	3.30 (2.05-5.31)	1.96 (1.15-3.34)	2.48 (1.32-4.66)	1.24 (0.45-3.40)

*Table 4. Risk estimates against estimated TCDD exposure in the Hamburg cohort. Estimated levels are TCDD above German median background at end of exposure. The cohort were divided into quintiles except * which are deciles.*

25. In this study both all cardiovascular disease and ischaemic heart disease showed a dose dependent relationship with both TCDD and all combined dioxins and furans. No increased risk was observed in the two lowest TCDD quintiles whilst a dose-dependent increase was observed in the ninth and tenth deciles. For example ischaemic heart disease had a RR=2.48 in the tenth decile, 95% CI 1.32-4.66 compared to RR=1.61 in the ninth decile, 95% CI 0.85-3.04. The possibility that other established risk factors for ischaemic heart disease (such as serum lipoproteins, blood pressure, diabetes, body mass index) could be confounders cannot be discounted as these were not determined. There was no dose response relationship for other cardiovascular diseases when ischaemic heart disease was excluded.

26. The authors carried out statistical analyses using an external cohort and the two lowest quintiles of exposed workers as the comparison groups. The internal comparison group was perceived to minimise differences which could affect mortality and have exposure above background dioxin levels with the

potential for exposure misclassification was greatest in lower dose ranges. A disadvantage was possible systematic differences in exposure to other substances within the manufacturing facility.

27. The authors also provided these relative risk estimates compared to estimated total TEQ for dioxins and furans, but dioxin-like PCBs were excluded from the estimate (Table 5). An estimated body burden has been included in this table by treating total exposure TEQ as if it were all TCDD. This was necessary due to the lack of data on congener specific composition and half-lives in the original paper. Whilst this probably over-estimates the body burden it provides an approximation of the contribution of other congeners to total dioxin exposure.

Blood TEQ (ng/kg blood fat)	**Derived body burden** (ng/kg bw)	**Relative risk and 95% confidence limits for**			
		Cancer	**CVD**	**IHD**	**Other CVD**
1.0-12.2	0.2-1.44	1.38 (0.93-2.43)	0.93 (0.57-1.50)	1.02 (0.54-1.95)	0.84 (0.40-1.74)
12.3-39.5	1.46-4.9	1.71 (1.07-2.74)	0.92 (0.59-1.46)	0.96 (0. 51-1.82)	0.91 (0.48-1.75)
39.6-98.9	4.92-19.8	1.50 (0.93-2.42)	1.48 (1.01-2.17)	0.97 (0.52-1.81)	2.05 (1.26-3.36)
99.0-278.5	19.8-55.7	1.56 (1.00-2.43)	1.55 (1.07-2.24	1.13 (0.64-2.00)	2.07 (1.27-3.38)
278.6-545.0*	55.7-109	1.71 (0.98-2.98)	1.63 (1.01-2.64)	1.73 (0.92-3.27)	1.53 (0.73-3.20)
545.1-4361.9*	109-872	3.27 (2.04-5.26)	2.06 (1.23-3.45)	2.72 (1.49-4.98)	1.19 (0.44-3.26)

*Table 5. Relative risk and estimated total TEQ levels above German median background at end of exposure in the Hamburg cohort, cohort is divided into quintiles except * which are deciles; derived body burden is as TCDD equivalents since there are insufficient data on congener specific composition and half-lives.*

28. The paper suggests that an increased risk of ischaemic heart disease was clearly observed at derived body burdens above median background of greater than 31.4 ng/kg bw for TCDD and 55.7 ng/kg bw for total TEQ. Unfortunately the median background concentration was not cited by the authors, if the average reported concentration in milk in Berlin (16.55 ng/kg lipid) is used to estimate this, an additional 3.3 ng/kg bw would need to be added to these body burdens.

29. Rearranging the following equation:

body burden = fraction absorbed * intake * $t_{1/2}$ in days/ln 2

these values can be applied to estimate daily intakes of TCDD providing these body burdens. Thus:

intake = body burden / fraction absorbed * $t_{1/2}$ in days/ln 2

for the purposes of this illustrative estimate the fraction absorbed is assumed to be 50% and the half-life of TCDD is assumed to be 2740 days.

30. Thus at the cut-off point above which effects are seen the TCDD body burden is 31.4 + 3.3 ng/kg bw = 34.7 ng/kg bw. The daily intake to derive this body burden is:

34.7 * 0.693 / (0.5 * 2740) = 17.6 pg/kg bw/day

31. However if allowance is made for the contribution of other dioxins and furans measured in the study, the body burden is 55.7+ 3.3 ng TEQ/kg bw = 59 ng TEQ/kg bw. Because there are no congener specific composition data it is not possible to accurately estimate the intake but if this is considered as TCDD for illustrative purposes then the daily intake would be equivalent to 29.8 pg/kg bw/day.

1998 analysis

32. Flesch Janys and colleagues published updated analyses on this cohort in 1998, in the main analysis the cohort was divided into quartiles and the exposure data presented differently. No explanation for the altered subdivision of the cohort is provided. The SMRs for total mortality (1.15, 95% CI 1.05, 1.27) and cancer deaths (1.41, 95% CI 1.17, 1.68) were significantly elevated reflecting elevations at several sites. In these analyses, SMRs for cardiovascular diseases 1.06 (95% CI 0.90-1.24) and ischaemic heart disease 0.97 (95% CI 0.77-1.22) were presented for the whole cohort using the German population SMRs as the comparator. Detailed results were only presented for cancer end-points (shown in table 6).

TCDD (ng/kg blood fat * year)	**Derived body burden**	**Total cancer**	**Lung cancer**	**Haematopoietic & lymphatic cancer**
0-125.2	Not possible to derive body burden from published data	1.24 (0.82-1.79)	1.56 (0.75-2.88)	2.55 (0.69-6.52)
125.2-627.1		1.34 (0.90-1.92)	1.63 (0.78-3.00)	2.21 (0.45-6.45)
627.1-2503.0		1.34 (0.91-1.90)	1.22 (0.53-2.40)	2.90 (0.78-7.42)
>2503.0		1.73 (1.21-2.40)	1.66 (0.79-3.05)	0.80 (0.02-4.46)

*Table 6. Integrated TCDD concentration until the end of follow-up (ng/kg blood fat * year) and cancer risks.*

33. The authors only estimated exposure when blood levels of TCDD were greater than the 95%CI of TCDD blood levels in the German population. The median background value in the German population was subtracted before these blood levels were back-calculated to date of exit from plant for estimation of department specific dose rates. The department specific dose rates were used to estimate blood level at every point and the AUC calculated. The use of

integrated TCDD concentration as the exposure measure cannot be readily converted to body burden.

34. The lack of exposure group analysis for non-cancer end-points in the 1998 analyses of this cohort does not allow confirmation of the previously observed increases in cardiovascular disease amongst the most exposed deciles.

E. NIOSH study.

35. Steenland et al. (1999) described the most recent follow-up through 1993 on a previously characterised cohort of 5172 male workers from 12 US plants manufacturing TCDD contaminated products from 1942 to 1984. All the data were re-reviewed and 40 workers eliminated because they were female, did not work in TCDD exposed jobs or had no recorded date of birth. A further series of exclusions reduced the exposure-level subcohort to 3538 workers (69% of the overall cohort). A further subcohort of 608 workers with documented chloracne in their plant medical records were also analysed.

36. Exposure was estimated using a job-exposure matrix. The job-exposure matrix derived a daily exposure score based on concentration of TCDD in the materials, fraction of the day working on the process and a qualitative contact level to estimate amount reaching the skin or potential for inhalation. The daily scores for each worker were summed to produce a cumulative exposure score.

37. There was marked variation between plants in the median cumulative exposure scores. Whilst cumulative exposure scores permit analyses taking into account duration and level of exposure, there is no standard by which to validate it. The workers with chloracne had markedly higher median cumulative exposure scores and average exposure scores than workers without chloracne. Although serum TCDD levels and cumulative exposure scores were available for one site (described below, *n.b. study actually measures levels at two sites*), the relative poor quality of the work history information made estimation of exposure scores difficult. Many individuals had identical job titles and worked over the same period resulting in similar exposure scores. Spearman correlation coefficients for cumulative exposure score and duration of exposure versus serum dioxin level were 0.70 and 0.71 respectively whilst that for cumulative exposure score versus duration of exposure was 0.91. The life table results for the entire cohort were unremarkable for all cancers combined, ischemic heart disease and diabetes. Whilst heart disease might have been expected to be in deficit due to the healthy worker effect, the slight excess observed could be related to TCDD toxicity or diminution of the healthy worker effect from following most of the cohort past retirement age.

38. Sweeney and colleagues determined TCDD levels at two (Newark NJ & Verona Mo) of the 13 plants in the NIOSH study. This study attempted to provide quantitative values for the qualitative exposure score. The serum TCDD levels in workers were compared with unexposed age matched referents (+/- 5 years). Company records were used to determine total number of days in all areas exposed to TCDD contaminated processes (not adjusted for overtime or

vacation) converted to exposure years by division by 365.25. The steady state background serum dioxin level of 6.37 ppt was the median of the referent group. The half-life extrapolated TCDD dioxin levels at the end of exposure were calculated for 135 workers assuming a log linear one-compartment model and half-life of 7 years with no other occupational TCDD exposure.

39. Serum samples were obtained from 103 workers, 8 office staff & 41 referents from Newark and 32 workers & 12 referents from Verona. Both workers and referents had similar race, sex & age profiles. The mean year of final exposure in Newark was 1963 (median 1964) and the average number of years exposure was 3.9 years. The equivalent figures for Verona were 1970 (1971) and 10 months respectively.

40. Extrapolated serum TCDD levels were not calculated for 10 workers with current levels below the median background level of 6.37 ppt. There was a log normal distribution of both current and half-life extrapolated serum TCDD levels. The relationship between length of exposure with current and half-life extrapolated serum TCDD levels was examined using the Pearson product moment correlation coefficient. Multiple linear regression models were used to examine the relationship between current and half-life extrapolated serum TCDD levels with total years of exposure. These models incorporated confounders (age, race, body mass index & number of days since last exposure) as independent variables to examine their effect on the model. Separate models were necessary due to the large differences in the length of exposure at the two plants.

41. The mean serum TCDD level for 143 workers (*this does not add up from 103 + 32 unless 8 office staff are also included*) was 251.7 ppt whilst that for the 54 referents was 7.8 ppt. This was extrapolated to a mean serum TCDD level at termination of exposure of 2240 ppt. The mean serum TCDD level for Newark workers was 293.4 (geometric mean 76.8) and office workers was 12.5 ppt, both were significantly different from the 8.0 ppt (geometric mean 6.9 ppt) in the Newark referent group. The distribution of serum TCDD levels was from 2 to 3390 ppt in these workers with 65/103 having values above 100 ppt. The mean serum TCDD level for Verona workers was 177.2 ppt (geometric mean 80.7) with a range from 3.4 to 1290 ppt compared to mean levels of 7.1 ppt (geometric mean 6.2) in the referent group. There was no difference between mean serum TCDD level in the Newark and Verona referent groups.

Evaluation of NIOSH TCDD data.

42. Although the current and extrapolated mean serum TCDD levels correlated with the number of years of exposure to TCDD containing materials, it was not possible to directly correlate levels with the more detailed job-exposure matrix in the overall NIOSH study. In addition a marked variability between sites and the details of the individual sites job exposure matrices prevent extrapolation of the measured serum TCDD levels from 2 sites to all 13 sites. The job exposure matrix used provides the best estimate of the exposure for the entire cohort but is only a semi-quantitative measure that cannot be directly related to serum TCDD levels. The subcohort with measured serum

TCDD levels represent a small proportion of the total cohort and it is also unclear whether they represent all employees at these 2 sites. Only a single dioxin congener (TCDD) was measured and no information is available on exposure to other dioxins, furans or dioxin-like PCBs. There are discrepancies amongst the numbers quoted at various points in the paper. The total number of workers at the two sites was 143 but 8 of these were office workers whose values were close to background levels and were therefore discounted in some of the data giving a reported group size of 135. Similarly the total number of sites in the NIOSH has been variously reported as 12 or 13 in different papers.

NIOSH cohort results.

43. The data were analysed by seven cumulative exposure cut points because this was the maximum permitted by the life table program. The selection of cut points before analysis was based on septiles of cumulative exposure for all related deaths and aimed to create similar variances in resulting cause specific SMRs across the septiles (Table 7). The number of observed deaths for diabetes in each septile ranged from 0 to 5.

44. Although the risk of ischaemic heart disease appeared to increase slightly in the highest septiles compared to the US population standardised mortality ratio, this effect is more clearly seen in the Cox regression analysis with marked increases in the rate ratio for the two highest septiles. However the cumulative exposure scores for these cannot be converted to actual dioxin exposures and it is therefore not possible to estimate body burdens or daily intakes associated with these increased risks.

Group (cumulative exposure score)	**Standardised mortality ratio (US population as referent)**			
	All cancers	Lung cancer	Ischaemic heart disease	Diabetes (underlying cause)
Septile 1 (0-<19)	1.14	1.06	0.93	1.87
Septile 2 (19-<139)	1.15	1.07	1.00	2.17
Septile 3 (139-<581)	0.85	0.82	1.05	1.36
Septile 4 (581-<1650)	1.10	0.78	0.97	0.92
Septile 5 (1650-<5740)	1.15	1.12	1.10	1.33
Septile 6 (5740-<20200)	1.34	1.47	1.20	1.10
Septile 7 (>20200)	1.60	1.65	1.28	0

Table 7. Standardised mortality ratio by septile cumulative exposure score in the NIOSH cohort.

45. The authors also carried out Cox regression analysis for death with ischaemic heart disease and diabetes as underlying causes (Table 8). These internal analyses, using the low exposure group as the reference group, permitted more flexible modelling of the exposure response curve, exploration of lag times and possible interactions. The authors suggested that these analyses may also help avoid potential confounding by unmeasured variables and avoid possible healthy worker effects. Age was used as the time variable in the Cox regression analysis.

Group (cumulative exposure score)	Cox regression - rate ratios (95% CI)		
	All cancers	Ischaemic heart disease	Diabetes (multiple causes)
Septile 1 (0-<19)	1.00	1.00	1.00
Septile 2 (19-<139)	0.99 (0.62-1.58)	1.23 (0.75-2.00)	1.27 (0.49-3.33)
Septile 3 (139-<581)	0.71 (0.43-1.19)	1.34 (0.83-2.18)	0.92 (0.33-2.53)
Septile 4 (581-<1650)	0.93 (0.57-1.51)	1.30 (0.79-2.13)	0.81 (0.28-2.30)
Septile 5 (1650-<5740)	0.96 (0.60-1.53)	1.39 (0.86-2.24)	0.98 (0.36-2.65)
Septile 6 (5740-<20200)	1.12 (0.69-1.81)	1.57 (0.96-2.56)	0.72 (0.23-2.21)
Septile 7 (>20200)	1.33 (0.82-2.13)	1.75 (1.07-2.87)	0.54 (0.15-1.89)

Table 8. Cox regression analysis using septile 1 as referent, models controlled for year of birth (quartiles) and age (time variable). The data shown were analysed without a lag period.

F. Dutch occupational exposure.

46. Hooiveld and colleagues (1998) reported a second follow-up on a cohort of workers at a Dutch chemical factory where workers were exposed to phenoxy herbicides, chlorophenols and contaminants. This was one of two Dutch factories included in the IARC multinational study. This study described a further six and a half years of follow-up to the end of 1991. The results were also included (company 9) in the joint analysis of the IARC international cohort.

47. The factory produced chlorophenoxy herbicides from 1950, mainly 2,4,5-trichlorophenoxyacetic acid by a condensation reaction using 2,4,5-trichlorophenol. Synthesis ceased in 1969 but formulation continued until 1976. In addition to exposure to dioxins during normal production, an accident in March 1963 resulted in an explosion and release of dioxins. The cohort consisted of all employees between 1955, the start of complete personnel records, and June 30 1985. Contract workers involved in cleaning up after the accident were included but others were excluded due to the lack of personnel records. 1167 workers were enrolled into the cohort (including 85 contract

workers), 10 were excluded due to lack of a date of birth and 1 due to missing date of end of follow-up. At the time of assessment mean follow-up was 22.3 years, 78% were alive, 17% dead, 4% had emigrated and 1% were lost to follow-up.

48. Exposure was assessed using a questionnaire, an exposure status was defined for each individual based on departments worked in, actual job and exposure to the accident. Exposed individuals were those working in specific departments, those entering these departments regularly and those exposed by the accident. Within the exposed group a division was made based on whether they ever worked in the main production departments. All other workers were classed as non-exposed. A total of 562 subjects (549 male, 13 female) were classified as exposed, of whom 143 males worked in the main production departments. A total of 567 subjects (482 male, 85 female) were classified as non-exposed and a further 27 males were classified as unknown exposure due to the lack of available information. The non-exposed group started working at the plant at a younger age and at a later time.

49. Serum levels of PCDDs, PCDFs and PCBs were measured in a sample of surviving cohort members. These had a minimum of 1 year's employment and were first employed before January 1 1975. These subjects were stratified by department ever or never in main production) and duration of employment (less than or greater than 10 years). Each selected exposed worker was matched with two non-exposed workers by sex, date of birth (within 5 years), date of first employment and length of service (both within 2 years). All survivors exposed during the accident and all female exposed workers were selected. A random sample of the exposed workers who never worked in the main production department was selected as this group in total was too large. A total of 144 subjects were invited to provide blood samples comprising 21 exposed (main production), 24 exposed (never main production), 75 matching non-exposed, 18 exposed due to the accident and 6 women. The samples were analysed for PCDDs, PCDFs and PCBs but the paper only reported relationships with TCDD concentrations. Maximum TCDD levels for exposed workers were extrapolated from the measured levels using a one compartment first order kinetic model with a half-life estimate of 7.1 years. The lag time was defined as years since last exposure or the accident and additional exposure assumed to be negligible. Maximum TCDD levels for non-exposed workers were assumed to be the measured levels. Regression equations derived using maximum TCDD levels and information on independent variables were used to predict maximum TCDD levels for all workers. However 44 of these were excluded leaving 50 eligible for blood sampling. 3 of the eligible subjects were excluded leaving 47 (14 exposed by the accident, 17 exposed workers and 16 non-exposed workers). A wide range of extrapolated TCDD levels were obtained with a non-normal skewed distribution. Estimates of the effects of the accident, years in production and employment before 1970 on the predicted background TCDD level.

50. Overall mortality compared to the general population was greater than expected in males and females, although the latter resulted from a single death. Amongst the 140 workers exposed as a result of the 1963 accident increased

risks were observed for overall mortality (SMR 1.4, 95% CI 1.0, 1.8) and cancer mortality (SMR 1.7, 95% CI 1.1, 2.7). The subgroup with chloracne had an elevated risk of mortality from ischaemic heart disease (SMR 3.7, 95% CI 1.4, 8.1). When the exposed workers were compared with non-exposed workers, increased relative risks were observed for mortality due to diseases of the respiratory system (RR 1.8), particularly ischaemic heart disease (RR 2.1). These increases remained, albeit somewhat reduced, after adjustment for age, calendar year and time since first exposure.

51. Using the extrapolated TCDD levels, exposed workers were divided into exposure groups, relative risks for the medium and high exposure groups were compared to the referent group. The referent group was all workers with baseline predicted TCDD levels (no exposure from accident, never worked in main production, not exposed to phenoxyherbicides or chlorophenols before 1970).

Evaluation of Dutch occupational study.

52. The exposure data was derived from a combination of work diaries with the measured values from a subset of 31 exposed individuals and 16 controls. These 31 exposed individuals were further sub-divided with 14 exposed both through work and an accident and 17 through work alone. Whilst this allows the higher acute data from the accident to be incorporated in the model, it is only used to estimate exposure levels and there are no analyses of whether the different modes of exposure (continuous versus high acute) affected the risk. Although several congeners were measured the data, were only analysed in respect of TCDD exposure.

Dutch occupational cohort results

53. Three exposure groups based on the predicted TCDD level were described for the cohort (low, medium and high). These tertiles were not of equal size as they were based on similar levels in the predicted TCDD values. There was an increased risk of circulatory disease when the two highest exposure groups were compared to the lowest (Table 9). The reference group comprised 530 subjects with estimated TCDD max = 7.1 ppt (body burden 1.42 ng/kg bw).

Exposure group		**Medium**	**High**
Number		259	242
TCDDmax (ppt)		7.7-124.1	124.2-7307.5
Derived body burden (ng/kg bw)		1.54-24.8	24.8-1462
Relative risk (95% CI) (ICD-9)	All cancers (ICD 140-208)	5.0 (2.2-11.5)	5.6 (2.5-12.7)
	Circulatory system (ICD 390-459)	1.8 (1.0-3.6)	2.3 (1.2-4.3)
	Ischaemic heart disease (ICD 410-414)	1.8 (0.8-4.1)	3.1 (1.4-6.5)
	Cerebrovascular disease (ICD 430-438)	2.9 (0.8-11.6)	1.5 (0.3-7.1)

	Other heart diseases (ICD 415-429)	1.5 (0.2-10.4)	0.7 (0.1-7.9)

Table 9. Relative risks compared to the low exposure group in the medium and high exposure groups.

54. The authors also adjusted these ratios for age, end of follow up and time from first exposure and reported that the relative risks for ischaemic heart disease remained elevated (1.5 for the medium (95% CI 0.7-3.6) and 2.3 for the high (95% CI 1.0-5.0) respectively). Using the clear increase in the high exposure group at body burdens above 24.8 ng/kg bw would (using the equations above) equate to a daily intake of 12.5 pg/kg bw/day.

G. Dutch developmental studies.

55. The cohorts were recruited in Rotterdam to represent highly industrialised regions and Groningen to represent less industrialised, more rural areas. Each of these cohorts was further sub-divided between breast feeding for a minimum of six weeks and formula fed using a single batch of one commercial formula. Inclusion criteria were: absence of serious illness or complications during pregnancy or delivery, caucasian, first or second child born at term (37-42 weeks), delivered without caesarian section, forceps or vacuum extraction and availability of samples of maternal blood from last month of gestation and cord blood.

56. In addition to plasma from maternal and cord blood samples, milk samples were obtained at two and six weeks and, where possible, three months. Total 24-hour volume was measured and 10% aliquots pooled for analysis, the remainder was fed to infants by bottle. Plasma was only analysed for four non-planar PCB congeners, whereas milk was analysed for 17 2,3,7,8-substituted PCDDs and PCDFs, 3 planar PCBs and 23 non-planar PCB congeners. A study population of 418 pairs was available from an initial size of 489, the majority of the losses were due to difficulties in maintaining breast feeding. These divided into 104 breast-fed and 107 formula-fed in Groningen and 105 breast-fed and 102 formula-fed in Rotterdam

57. Pluim and colleagues (1994) recruited 67 healthy pregnant Caucasian women for the study, 24 withdrew after delivery due to problems either with the commencement of breast feeding or insufficient breast milk production. Five infants were excluded due to asphyxia or perinatal infection and 3 more due to maternal use of medicines with possible fetal effects. A total of 35 mother infant pairs remained, 32 with hospital delivery and 3 with midwife supervised home delivery. All the infants were full-term and healthy, birth weights were greater than 2500 g and APGAR scores were normal. Mean maternal age was 28.9 years (range 21-38). All planned to breast feed for a minimum of 12 weeks but 7 switched to bottle feeding at 5 weeks, 2 at 6 weeks and 2 at 8 weeks.

58. The calculated cumulative intake of dioxins via breast milk at 11 weeks of age was calculated using concentration of dioxins in milk fat, amount of fat in

milk and amount of milk consumed by the infant. It was assumed that the fat content averaged 2.5% over the entire period and that consumption was 700 g of milk per day when exclusively breast fed and half that until breast feeding ceased. The authors considered that use of bottle-fed infants as controls was inappropriate due to differences in maternal social status, inability to obtain representative breast milk samples to estimate prenatal exposure and the inability (due to their very long half-lives) to exclude long-term effects of intrauterine exposure.

59. Maternal and cord blood were sampled immediately after birth. Blood samples were obtained from infants at 1 and 11 weeks between 10:30 and 14:00 shortly after feeding. The haematological profile, including leucocytes, platelets and differential, was determined along with plasma levels of cholesterol, total and conjugated bilirubin and γ-glutamyltransferase (GGT), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities. One sample could not be obtained at each time *(presumably different individuals but not stated).* Two milk samples were obtained on the same day 3 weeks post delivery by emptying one breast with an electric breast pump whilst the infant suckled the other breast. The 2 samples were pooled and stored for analysis of the 17 most toxic PCDD and PCDF congeners.

Evaluation of Dutch developmental cohorts.

60. These two series of studies represent the only series with measured exposure data for all individuals in the cohort. Comparisons are performed on all individuals for whom data exists leading to variations in the actual mean levels from comparison to comparison.

61. It was difficult to determine whether the observed differences were due to dioxin exposure or to confounding factors. It was also not possible to determine whether any cognitive changes represented temporarily delayed milestones of development or a persistent decrement and that follow-up studies were needed. Complex correlations were found between dioxin and PCB levels and confounding factors, such as breast-feeding, smoking and maternal education. Linear regression analysis had been used to assess the influence of confounding factors. It was not clear whether this was appropriate, and the data were insufficient to determine whether the statistical approach might result in over- or under-correction. Concerns over the known and potential confounders made it impossible to reach firm conclusions.

H. Seveso.

62. Following an explosion at the ICMESA factory at Seveso in 1976, chemicals including TCDD were deposited over an area of 2.8 km^2. The contaminated area was initially divided into 3 major zones (A, B & R) based on surface soil TCDD concentrations. Zone A was the most heavily contaminated zone, there was an immediate 25% mortality rate in animals but the 736 residents were not evacuated until 15 days after the accident. The 4737 residents of zone B, the next most heavily contaminated, were not evacuated

but were warned of the risk of consuming locally-grown food. The 31800 residents of zone R were neither evacuated nor did they receive any warnings on food consumption.

63. Plasma level data are available for at least a subcohort of the Seveso accident cohort. However there are differences in the representability of available samples between exposure areas and given the nature of exposure the relevance of body burden as the dose metric needs to be considered. The principal end-points with exposure data are mortality, cancer incidence and sex ratios of children after paternal exposure. An on-going study is looking at endometriosis but so far only details of the case definition have been published, the authors have been contacted and indicated that a paper is likely to be submitted later this year.

Paternal effects. Sex ratio of offspring

64. Mocarelli and colleagues (2000) reported on the sex distribution of children born between 1 April 1977 and 31 December 1996 with one or both parents exposed to measured concentrations of TCDD in the Seveso incident. The study population of 452 families comprised 3 groups; 130 males with measured TCDD concentrations married to females from outside the exposure area (zones A, B & R), 154 females with measured TCDD concentrations married to males from outside the exposure area in 1976 and 168 couples with both partners living in the exposure area in 1976. The group with both partners exposed was made up of individuals with measured (142 females, 109 males) and estimated (27 females, 59 males) TCDD concentrations (*total number of females in this group appears to be 169*). The exposed individuals were aged between 3 and 45 years old in 1976. The unexposed population were defined as those from outside the exposure area or who were from the exposure area but had a TCDD value of 15 ppt or less on a serum lipid basis in 1976.

65. Demographic data consisted of date of birth, town of residence in 1976, date of marriage, date of children's birth and sex of live births. The towns where the study population resided from 1971 to 1996 were recorded. Children born to unmarried parents were included in the study (*no ascertainment that the putative father was the actual father*). Demographic information from 1966 to 1996 was used to identify all residents of zone A between 1 January 1971 and 10 July 1976, this was used to estimate the sex distribution of children in zone A before the incident. TCDD concentrations were determined from serum samples collected and frozen in 1976. The 81 non-detectable measurements were assigned a value of half the limit of detection. The year on year TCDD serum concentrations for individuals were determined using Filser's model.

66. A chi-squared test was used to compare observed to expected sex distribution in children. Investigation of independent variables on the probability of male birth used univariate and multivariate logistic regressions. TCDD serum concentrations were included in analyses as either a dichotomous variable (outside or less than 15 ppt) or a three level variable (greater than 80 ppt, between 15 and 80 ppt, less than 15 ppt). The tertiles were based on the distribution of TCDD concentrations among parents. Paternal and maternal age

at time of conception were included in the model (dichotomised at 35 years). A separate model was used to assess links between sex distribution and age of father when exposed to TCDD.

67. Two approaches were used to estimate TCDD exposure for the 86 individuals for whom no samples were available (in each case a sample was available for the other parent). As TCDD values of married couples living in the same zone in 1976 were highly correlated for the 76 couples (3A, 27B, 46R) where TCDD concentrations were only available for 1 parent the same value was used for the other parent. For the 10 couples, all from zone R, not married in 1976 the missing parental value was assigned the median concentration for adults in zone R (about 25 ppt). These substitutions allowed incorporation of all 674 children in the analysis. The effects of these substitutions were assessed by comparing only those with actual measurements and the unexposed group.

68. A total of 535 serum samples from 296 mothers and 239 fathers were available (plus 86 with no sample), of these 54 females and 130 males had partners from outside the exposure zone. A total of 452 families were included (*there appears to be an error somewhere as there is an odd number of individuals*). The distribution of samples by zone and as a percentage of total zone parents is shown in Table 10.

Zone	Number of blood samples		Percentage of zone total	
	mothers	fathers	mothers	fathers
A	86	88	78	76
Seveso B	46	47	37	34
Meda R	86	59	24	17
Cesano Maderno B	78	45	19	11

Table 10. Available blood samples for assessing paternal and maternal TCDD exposure.

69. The median paternal serum concentration was 96.5 ppt (range 2.8-26400 ppt; 25th & 75th percentiles 37.5 & 225 ppt) whilst median maternal serum concentration was 62.75 ppt (range 6.45-12300 ppt; 25th & 75th percentiles 29.25 & 162.5 ppt). The overall sex ratio of 0.487 was not significantly different from the expected value of 0.514. However analysis by parental exposure status revealed significant differences from the expected value when either both parents or the father alone had serum TCDD concentrations greater than 15 ppt. These results are shown in Table 11.

Father's serum TCDD	Unexposed	>15 ppt	>15 ppt	Unexposed
Mother's serum TCDD	**Unexposed**	**>15 ppt**	**Unexposed**	**>15 ppt**
Number of males	31	96	81	120
Number of females	20	121	108	100
Total children	51	217	186	220
Sex ratio	0.608	0.442*	0.438*	0.545

95% CI	0.47-0.74	0.38-0.51	0.36-0.61	0.48-0.61

Table 11. Sex ratio of children based on parental TCDD exposure.

70. The data were further subjected to univariate and multivariate analyses to explore the effect of parental TCDD exposure on the probability of a male birth. Using the chi-squared test increasing paternal serum TCDD concentrations were associated with a decrease in the proportion of male births (Table 12). In the univariate analysis, paternal exposure was a significant predictor for male birth and the probability was significantly lower (odds ratio 0.555, CI 0.38-0.88) than in the unexposed group.

Father's serum TCDD (ppt)	**Number of males**	**Number of females**	**Total children**	**Sex ratio**	**95% CI**
Unexposed (<15)	151	120	271	0.557	0.49-0.61
15.1-31.8	35	45	80	0.438	0.33-0.55
31.9-60.7	41	40	81	0.506	0.40-0.81
61-117.0	38	43	81	0.469	0.36-0.58
118.0-264.0	32	48	80	0.400*	0.29-0.61
281.0-26400	31	50	81	0.383*	0.28-0.49

Table 12. Sex ratio of children at differing paternal TCDD concentrations.

71. Results were similar when repeated only with those for whom measured TCDD serum concentrations existed and those from outside the exposure zone. There was no effect of either maternal or paternal age at conception on the sex distribution of children. The paternal exposure remained significant when paternal and maternal dichotomised exposure were included in the model. The effect of paternal age at the time of TCDD exposure was examined by dividing the male subjects by age in 1976 into two groups; less than 19 years old and 19 years or older. For unexposed fathers the sex ratio of children was similar in both groups (0.535 (95% CI 0.448-0.622) and 0.577 (95% CI 0.494-0.660) respectively). Both the exposed groups differed significantly from the age equivalent unexposed group (0.41 (95% CI 0.23-0.72) and 0.61 (95% CI 0.38-0.97) respectively). When compared to the expected sex ratio only males exposed at less than 19 years old differed significantly. This group sired children from 1984 onwards and the median year for births was 1991. Even in 1991 they fathered significantly more daughters than sons.

72. The distribution of sex ratio over time from 1966 in zone A was examined. The ratio was normal until 1972 but there was a clear increase in females born in the periods 1973-1976 and 1977-1984 when parents had lived in zone A for some period of time between 1971 and 1976. The parental ages at conception were comparable over the time period. Based on census records a comparator town (Seregno, pop 39000), located outwith the contaminated area but of similar types of industrial, environmental and socioeconomic conditions, had the expected normal sex ratio (0.514) between 1966 and 1996.

73. The effect in offspring appears to commence at initial TCDD concentrations below 80 ppt, which according to the authors is equivalent to less than 16 ng/kg body-weight (*note this is presumed to be the extrapolation using Filser's model to derive year on year exposure data*).

Overall evaluation of exposure data.

74. The Dutch developmental studies are unusual in being based both on background environmental levels of exposure and having contemporaneous measured dioxin values for all individuals. Almost all the other studies reported the effects of high level occupational exposure or the results of accidental release and, with the exception of the Ranch Hand and Missouri resident studies, exposure estimates were either based on extrapolation from current values combined with work diaries or qualitative work diaries alone. The NIOSH study had measured values for only a small subset of workers but there were no health effect data in this small (less than 2% of total) subgroup. In the other occupational studies the only non-cancer end-points measured were cardiovascular diseases. The Ranch Hand studies used extrapolation from current values to estimate exposure but there were systematic differences in the exposed groups and the possibility of significant confounders from both military service and post service occupations. Whilst the retrospective measures and work histories in the worker cohorts represent the best available assessments of exposure, the inherent compromises make it difficult to determine a value associated with an effect level. Some effects were only significantly elevated in around 10-20% of the cohort with the highest exposure.

75. In addition sudden high level exposures may not be representative of steady-state conditions resulting from continual low level dietary exposure and the use of body burdens from these studies may not be the most appropriate dose metric. There are also difficulties in comparing these with animal studies.

76. The limitations of the exposure assessments were discussed. It has been possible from some of these studies to estimate body burdens associated with increased risks of ischaemic heart disease and alterations in the sex ratio of children following paternal exposure. Using the equations in the SCF opinion these derived body burdens can be converted to daily intakes.

77. Using TCDD alone the risk of ischaemic heart disease was increased at body burdens of 24.8-34.7 ng/kg bw (daily intakes of 12.5-17.6 pg/kg bw/day). One study measured co-exposure to other dioxins and furans which resulted in the body burden rising from 34.7 ng/kg bw to 59 ng TEQ/kg bw (equivalent to a

daily intake of 29.8 pg/kg bw/day if treated as TCDD). The alteration in sex ratio was observed at a TCDD body burden of 23.67 ng/kg bw (daily intake of 11.9 pg/kg bw/day). No study has included measurement of dioxin-like PCB exposure and estimated its contribution to the body burden.

Appendix 1. Summary table of exposure data from epidemiology studies.

Study	Total cohort	Sampled	Exposure measured by	Extrapolation assumptions	Tissue levels (range) (pg TCDD/g lipid)	Derived body burden [a] (ng/kg bw)	End-points studied	Notes
A. Ranch Hand study of US Air Force personnel	952 (exposed only, 1281 controls)	914 (exposed only, 1186 controls)	Extrapolation of end of service dioxin from measured levels up to 30 years after	Half-life of 8.7 years from study on subset, corrected for percent body fat First order decay No post service exposure to dioxin	initial < 10 10 < <143 10 < >143	< 2 2< <28 >28	Selected enzymes+ Cholesterol levels Triglyceride levels Thyroid hormones Immunological parameters+ Circulatory diseases Reproductive outcomes Cancer Diabetes*	current levels < 10 > 10 > 10 ρg TCDD/g lipid Total exposure during military service
B. Study of 50 Missouri residents	51	40	Measured adipose tissue dioxin levels	Not relevant	<20 20-60 >60	< 4 4-12 > 12	Selected enzymes+ Cholesterol levels Triglyceride levels Immunological parameters+	Total exposure from all sources Post contamination levels
C. BASF accident cohort	254	138	Extrapolated levels from current measurement to validate work diary Non-linear regression for contribution of each job	half-life of 7 years first-order exponential decay Work diaries used to estimate exposure of remaining members Corrected for body fat	C1 1009.5 C2 48.8 C3 83.7 (geometric mean levels)	100 100-990 > 1000 µg TCDD/ kg bw (cited in paper)	Selected enzymes Diseases of circulatory system * Ischaemic heart disease * Non-cancer deaths Thyroid hormones Immunological parameters + Cancer*	Other dioxin/ furan congeners measured but only TCDD used for comparisons Total exposure through work plus accident In factory

D. Hamburg chemical workers	1589 (1189 males reported in paper)	320 blood and 62 adipose tissue samples from 275 workers both male and female	Extrapolated levels from current measurement to validate work diary	half-life 7.1 years 1st order kinetic model Work histories used to estimate exposure of remaining members	TCDD 2.0-2252.0 (arithmetic mean 101.3) TEQ + TCDD 11.7-2985.8 (arithmetic mean 89.3) TEQ – TCDD 9.7-1263.4	0.4-450.4 (arithmetic mean 20.2)	Cardiovascular disease* Non-malignant respiratory disease Unnatural causes* Cancer*	Background subtracted Excess exposure due to work Levels extrapolated to end of employment N.B. Exposure modelling refined in later papers in response to criticisms
E. NIOSH study of workers in a plant	5172 @ 13 plants	143 + 54 referents @ 2 plants	Measured levels showed marked plant to plant variability and unable to correlate with qualitative measures	half-life 7 years log-linear one-compartment open model no exposure after ceasing work at plant steady state background equal to median of referents	Extrapolated Newark Workers 2-30900 Missouri Workers 3-6100	0.4-6180 0.6-122	No end points assessed separately at these two sites	Current Newark Workers 2-3390 Office staff 7-26 Referents 2-20 Missouri Workers 3-1290 Referents 2-17 End of work extrapolated levels
E. NIOSH study	5172 @ 13 plants	None	Qualitative work diaries	Not relevant	Not relevant Qualitative exposure	Not calculable from qualitative exposure	Cancer* Cardiovascular disease*	Estimate of exposure due to work activities only
F. Dutch occupational series	1167	48 Further subdivided into Accident	Extrapolated levels from current measurement to validate work diary	half-life 7.1 years one-compartment 1st order kinetic model	Extrapolated dioxin levels Accident 904.7-2272.3	 18.1-454	Diseases of circulatory system* Ischaemic heart disease* Cerebrovascular disease	Measured dioxin levels Accident 47.5-118.7 Exposed not accident 5.0-15.1

		n=14 Exposed not accident n=17 Unexposed n=16			Exposed not accident 29.9-175.1 Unexposed 4.0-8.3	5.8-35 0.8-1.6	Cancer*	Unexposed 4.0-8.3 All dioxin and furan congeners and PCBs measured but only TCDD used for comparisons Extrapolated to maximum exposure due to work
G(1). Dutch series of studies on developmental effects – Rotterdam	Up to 418 mother-baby pairs [b] Half breast fed and half formula fed	Up to 418 mother baby pairs	Measured maternal and cord plasma and milk levels in all members[a] (maternal PCB/dioxin) for breast-fed, maternal PCB only for formula fed	Not relevant	Plasma: Maternal 0.6-7.4 Cord 0.1-2.1 42 months 0.1-1.5 (5-95 percentile) *Milk Total TEQ 28-155 Dioxin TEQ 11-76 ng TEQ/kg milk fat*	2.2 – 15.2 (maternal body burden – dioxin only)	Growth, physical and mental development Thyroid hormones Number of statistically significant differences reported, values within normal ranges, further studies to assess whether these are persistent effects	17 dioxin and furan congeners and PCBs measured in breast milk 4 PCBs only in plasma Total exposure from all sources
G(2). Dutch series of studies on developmental effects – Amsterdam	35 mother baby pairs	35 mother baby pairs	Measured maternal and cord plasma and milk levels in all members	Not relevant	*Concentration 8.7-62.5 ng TEQ/kg milk fat Cumulative intake at 11 weeks 5.7-125.7 ng TEQ*	1.7 – 12.5 (maternal body burden)	As for G(1)	17 dioxin and furan congeners measured in breast milk Total exposure from all sources
a) Body burden derived as described below, unless otherwise indicated. b) Actual cohort size varies in studies using part or whole cohort, levels are calculated on studied mother baby pairs in each case so vary slightly								

Body burden derivation

Based upon information provided in the EPA review, the following assumptions have been made in deriving the body burden approximations:

- At steady state in humans, 90% of the body load of dioxins has been shown to be localised in body fat; for the purposes of the crude estimations in this paper, 100% localisation in body fat is assumed.
- The body load of dioxins is equilibrated throughout all body fat (data are derived from adipose tissue, blood fat or breast milk fat)
- Fat constitutes 20% of bodyweight
- Average bodyweight of 70kg

Thus a dioxin content of *x* pg/g lipid correlates with a body burden of 200*x* pg/kg bw.

Blood levels are given in ρg TCDD/g lipid, where the original values were expressed differently (e.g. ppt) it has been confirmed

* significant differences reported with exposure
+ no consistent pattern of changes

UK body burden calculated from 1997 TDS data for average (1.8 pg TEQ/kg bw/day) and high-level consumers (3.1 pg TEQ/kg bw/day) by assuming all exposure is TCDD; average 3.6 ng/kg bw, high-level 6.1 ng/kg bw.