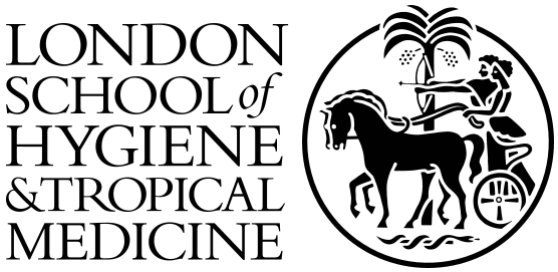

Sex ratio and reported health of the offspring of New Zealand phenoxy herbicide producers exposed to 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin

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Abstract (max. 250 words)

Background. In 1996 it was first reported that parental exposure to 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) was associated with the birth of less boys than girls. Only a handful of studies have reported on this association since.

Objectives. To study the offspring sex ratio of men and women employed in a New Zealand phenoxy herbicide production plant between 1969 and 1984, in relation to their individual TCDD serum concentrations determined in 2007/8.

Methods. A total of 127 men and 21 women reported 355 children conceived after starting employment at the plant. The association between their TCDD serum concentrations back-calculated to the time of birth and the probability of a male birth was estimated through logistic regression, adjusting for the age of the exposed parent at birth, current body mass index and smoking.

Results. The overall sex ratio was 0.55 (197 boys, 158 girls). For fathers with serum TCDD concentrations ≥20 pg/g lipid at time of birth the sex ratio was 0.47, while 0.60 for <20 pg/g (Odds Ratio (OR) 0.49; 95% Confidence Interval (CI) 0.30-0.79). For exposed mothers the corresponding sex ratios were 0.68 and 0.53. The probability of a male birth decreased with higher paternal serum TCDD at time of birth (<4; 4-20; 20-100; >=100 pg/g lipid), with ORs of 1.00 (reference); 1.00 (95%CI 0.50-2.02); 0.52 (0.29-0.92); 0.45 (0.23-0.89), p-trend: 0.007.

Conclusions. This study supports earlier findings indicating that paternal serum TCDD concentrations in excess of 20 pg/g lipid are associated with a reduced sex ratio, also when exposure starts in adulthood.

Introduction

Dioxins, and in particular 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD), have been associated with a wide range of medium to long term health effects (EPA 1994). In 1996 it was first reported that fewer boys than girls were born to parents accidentally exposed to TCDD in an industrial accident in 1976 in Seveso, Italy (Mocarelli et al. 1996). Since then, this association has been examined in both animal and human studies, aiming to: (i) replicate this finding in other populations; (ii) find a mechanistic explanation; and (iii) determine the relevant dose and time window of exposure. The number of studies in humans has been limited, however, mainly due to the small number of exposed cohorts for which individual TCDD serum measurements are available.

Sex ratios are typically calculated as the number of male births divided by the total number of births. In 1998, sex ratios for children fathered by U.S. veterans of operation Ranch Hand, the unit responsible for spraying dioxin-contaminated agent orange in Vietnam from 1962 to 1971 (Michalek et al. 1998), were reported. The authors did not observe a lower sex ratio related to serum TCDD concentrations at time of conception (the sex ratio of children born post service from Ranch Hand veterans with serum TCDD of <10 pg/g at time of conception was 0.51, compared to 0.51 for fathers with TCDD of >=10 pg/g).

In 2000, further findings of the Seveso study were reported (Mocarelli et al. 2000), indicating that a significantly lower sex ratio was present if fathers had been exposed to dioxin (the sex ratio of children born post-accident from unexposed fathers with serum concentrations <=15pg/g was 0.56, compared to 0.44 for fathers with serum concentrations >15pg/g), particularly if they were younger than 19 years at the time of exposure (sex ratio 0.38). TCDD concentrations in serum samples from mothers were not a significant predictor of the

probability of a male birth. This suggested that the effect was male-mediated, and particularly strong if the exposure occurred before or during puberty of the future fathers.

In response to this finding, the sex ratio of the offspring of the male Austrian chloracne cohort exposed to TCDD during production of 245T in the early 1970s (n=157) was reported (Moshammer and Neuberger 2000). The finding of a sex ratio of 0.46 for 56 children born after fathers' onset of exposure was consistent with the Seveso study, although based on small numbers.

In 2001, the sex ratio of offspring of 281 male workers from two plants producing phenoxy herbicides (including 2,4,5-T), which was part of the NIOSH study, were reported (Schnorr et al. 2001). The sex ratio among offspring, including children conceived both before and after start of employment, was not markedly associated with adult TCDD exposure at time of conception (0.51 for children from fathers with estimated <20 pg/g serum TCDD concentrations, compared to 0.56 for fathers with >=20 pg/g serum TCDD).

In 2002, a report on Russian phenoxy herbicide producers from Ufa was published (Ryan et al. 2002), indicating a decrease in the number of boys for exposed fathers (sex ratio 0.38 for children born after the start of exposure), while no decrease was observed for exposed mothers (sex ratio 0.51 for 39 children born after start exposure), in line with the Seveso findings, although fathers were older than 20 at the time of exposure.

In summary, offspring sex ratios have now been reported for four sizable populations with known exposure to TCDD. For two (US-Ranch Hand, US-NIOSH) no association was

reported and for two (Italy-Seveso, Russia-Ufa), a lower sex ratio was reported to be associated with exposure in the fathers but not for the mothers.

Here we report on the sex ratio of the offspring of male and female workers employed at a phenoxy herbicide production plant in New Plymouth, New Zealand between 1969 and 1984, for whom individual TCDD serum concentration were determined in 2007/8.

Methods

The pesticide producers included in this study were part of the New Zealand component of the IARC international cohort of producers and sprayers of phenoxy herbicides (t Mannetje et al. 2005), and had worked for at least 1 month between 1969 and 1984 in the pesticide production plant in New Plymouth, New Zealand. Of the 1025 original production cohort members, 631 were known to be alive, living in New Zealand, and aged less than 80 years on 01/01/2006. Of these 430 were randomly selected and invited to participate in a morbidity survey, and 244 completed the survey. During 2007/2008 the participants provided blood for the determination of TCDD serum concentrations of which detailed results have been reported elsewhere ('t Mannetje et al. 20xx). During a face-to-face interview, completed at the time of phlebotomy, participants were asked to provide details on all their live born or still born biological children, including gender, name, date, and place of birth. The questionnaire did not ask about specific health outcomes, but a free text comments box was available for comments regarding the health of the child. Based on responses, dichotomous variables of specific health outcomes were constructed for those that were repeatedly reported.

Serum samples taken at time of interview were analyzed for concentrations of TCDD, 6 other chlorinated dibenzo-dioxins, 10 chlorinated dibenzofurans and 15 PCBs, using gas

chromatography-high-resolution mass spectrometry (GC-HRMS). Individual serum concentrations of TCDD at the time of phlebotomy were back-calculated to the time of the birth of the child by using a first order elimination model('t Mannetje et al. 20xx). A half-life of 7.6 years was used which was based on the results from a 15 year follow-up of U.S. Vietnam war veterans of operation Ranch Hand (Michalek and Tripathi 1999).
Different exposure variables were constructed, based on (i) employment in a job that was associated with high TCDD exposure ('t Mannetje et al. 20xx) (ii) serum TCDD concentration at time of phlebotomy and (iii) estimated serum TCDD concentration at the time of birth.

Sex ratios for exposure groups were calculated as the number of male births divided by the total number of births. The association between exposure and the probability of a male birth was estimated through logistic regression of correlated outcome data with children from the same parent being correlated (using SAS proc genmod with repeated statement) (2011), crude, and adjusting for the age of exposed parent at year of birth, current BMI and smoking status of the parent. The association between the natural logarithm of the estimated serum TCDD concentration at birth (ln-TCDD), and the probability of a parent- reported health problem in the offspring was estimated through logistic regression of correlated outcome data, adjusting for sex of the exposed parent, age of exposed parent at time of birth, and sex of the child.

This study received ethical approval the Central Regional Ethics Committee (ref: CEN/06/02/002) on the 19th of May 2006. All study participants provided informed consent.

Results

Of the 244 participants, 32 did not report having biological children. A total of 212 participants reported a total of 622 births (175 fathers with 509 births, 2.9 births on average; 37 mothers with 113 births, 3.1 births on average). For 10 of the 622 births, gender was not reported and these were excluded from analyses. A further 257 were excluded from the analyses because they were conceived before the parent started employment at the plant (assuming conception 0.75 year before birth), leaving 355 births for the analyses. The overall sex-ratio was 0.55. The sex ratio was lower for births in the 1970s compared to births in the 1990s (table 1).

Table 2 presents the results of the logistic regressions, modelling the probability of a male birth by sex of the exposed parent. For fathers employed in a highly exposed job within the plant, a significantly different sex ratio was not observed (OR 1.11; 95%CI 0.72-1.70), nor was a longer duration in a highly exposed job associated with a significantly altered sex ratio. Only 3 children (1 girl, 3 boys) were born to mothers who worked in a highly exposed job before conception.

Having a serum TCDD concentration ≥4 picogram per gram (pg/g) lipid at the time of phlebotomy was associated with a decreased probability of a male birth for exposed fathers (OR 0.46, 95%CI 0.29-0.73), but not for exposed mothers (OR 1.40; 95%CI 0.55-2.97). For exposed fathers, there was no clear dose-response association between serum TCDD at the time of phlebotomy and the sex ratio of the offspring (table 2).

When looking at categories of estimated serum TCDD concentration at the time of birth, a dose-response association was observed for exposed fathers (p-trend=0.007). There was no decreased probability of a male birth for fathers with serum TCDD levels of 4-20 pg/g at time

of birth, while 20-100 pg/g TCDD at birth was associated with an OR of 0.52 (95%CI 0.29-0.92) and >=100 pg/g with an OR of 0.45 (95%CI 0.23-0.89). For exposed mothers, the opposite pattern to that of exposed fathers was observed (i.e. higher probability of a male birth with higher maternal TCDD), but ORs were not statistically significant and based on a small number of births.

We investigated which of the variables included as confounders resulted in the largest change from the crude ORs for the association between TCDD exposure categories at the time of birth and the probability of a male birth for exposed fathers (table 3). The only variable resulting in an appreciable change of the crude OR was current BMI of the father, which itself was associated with a higher probability of a male birth. This association became statistically significant when adjusted for TCDD at the time of conception ($BMI_{<25\ (ref)}$ OR=1; BMI_{25-30} OR=1.5, 95%CI 0.9-2.7; $BMI_{>=30}$ OR=2.3, 95%CI 1.1-4.9).

Table 4 presents the association between the father's estimated serum TCDD concentration at the time of birth and the probability of a male birth stratified by the age of first paternal exposure, assuming exposure started at the start of employment at the plant. The numbers are small, particularly for those fathers who were first exposed before the age of 30, but they are indicative of a stronger association between TCDD exposure and the probability of a male birth if first exposure occurred before the age of 37.

Additional stratifications were also performed for the association between the father's estimated serum TCDD concentration at the time of birth and the probability of a male birth, for the following variables (supplementary tables 1-4): (i) paternal age at birth; (ii) maternal age at birth; (iii) father's current BMI; (iv) year of birth of the child. These analyses indicated

that the negative association between TCDD and the probability of a male birth was observed independently of the age of either parent, while the association was mainly present for fathers with BMI<25 (p-trend 0.019) and for births after 1980 (p-trend <0.001).

Of the 355 births that occurred after the start of employment of the parent at the plant, a health problem was reported for 57 births (table 5). Specific health problems that were repeatedly reported included asthma, birth defects, and thyroid/gland problems. All health problems combined (any health problem reported) was not significantly associated with a serum TCDD of more than 20 pg/g at time of birth (OR 1.33; 95%CI 0.72-2.45), nor was it significantly associated with ln-TCDD (OR 1.10, 95%CI 0.91-1.32). Congenital malformations in the offspring were not more frequently reported by highly exposed parents (OR 0.54; 95%CI 0.16-1.85) and was not associated with ln-TCDD (OR 0.68, 95%CI 0.68-1.16). Asthma was reported for 9 children of exposed parents, but an OR could not be calculated due to the small numbers. None of the parents exposed to less than 20 pg/g TCDD at time of birth reported a thyroid problem in offspring born after the start of employment, whilst of the 137 children born to highly exposed parents, a thyroid problem was reported for 3 (3 children of 2 fathers and 1 mother with serum TCDD at time of birth of 79, 90, and 208 pg/g respectively). Although based on very small numbers, the reporting of a thyroid problem in the offspring was positively associated with ln-TCDD at time of birth (OR 1.85, 95%CI 1.37-2.48).

Discussion

In this study, serum TCDD concentrations above 20 pg/g lipid at the time of birth for occupationally exposed fathers were associated with the birth of relatively fewer boys than girls. For occupationally exposed mothers serum TCDD concentrations were not associated

with a lower sex ratio, although this finding was based on much smaller numbers. These findings support a male-mediated reduction in sex ratio associated with serum TCDD concentration at the time of birth, consistent with the findings of the Seveso study (Mocarelli et al. 2000) and the Russian pesticide producers study (Ryan et al. 2002).

The sex ratio of offspring was 0.47 for fathers with serum TCDD concentrations above 20 pg/g at the time of birth. This is comparable to, but not as low as, the sex ratios reported for Seveso or the Russian pesticide producers (table 6), for which TCDD exposures were generally higher than in the New Zealand producers ('t Mannetje et al. 20xx). Although this population was occupationally exposed to a variety of compounds and pesticides, we consider paternal TCDD exposure at the time of conception to be the most likely explanation for the observed association, given the consistency of our findings with those reported for the Seveso population (where the sole exposure was known to be TCDD), and the presence of a dose-response relationship for TCDD at time of birth.

The mechanism through which paternal TCDD exposure could affect the sex ratio has not yet been established, but recent animal and human studies provide some insight. In a study of TCDD-treated male mice mated with non-treated females (Ishihara et al. 2010), the Y-bearing/X-bearing sperm ratio was not significantly decreased, but the sex ratio of the 2-cell embryos of the TCDD group was significantly lower than that of the control group. In a study in rats (Ikeda et al. 2005), in utero TCDD exposure in male rats significantly decreased the number of male offspring. Another study in rats (Rowlands et al. 2006) did not observe a change in sex ratio, but no distinction between paternal and maternal TCDD exposure was made. Thus, whilst data are limited, studies in rodents are generally supportive of the hypothesis of a male-mediated reduction in sex ratio resulting from TCDD exposure starting

either in utero or at reproductive age. The effect is not explained by changes in the Y-bearing/X-bearing sperm ratio, nor by a disproportionate loss of male embryos of more than 2 cells, and it has been proposed that a decrease in fertility of Y-bearing sperm may be responsible (Ikeda et al. 2005).

Studies of the effects of TCDD on semen in exposed human populations are limited. In the Seveso study, in utero and lactational TCDD exposure of children of exposed mothers was associated with a permanent reduction in sperm quality (lower sperm concentration, count and motility) (Mocarelli et al. 2011). This effect was also observed in rhesus monkeys (Arima et al. 2009). Seveso men exposed to TCDD in infancy also had reduced sperm concentration and motility (Mocarelli et al. 2008), while the opposite effect was seen with exposure during puberty. No effect on semen quality was seen in men exposed to TCDD as adults (Mocarelli et al. 2008). In a study of veterans of operation Ranch Hand, no associations between serum dioxin levels and testicular abnormalities, sperm count, sperm abnormalities, or testicular volume were observed (Henriksen and Michalek 1996). Thus, while there is evidence of negative effects on sperm quality when TCDD exposure occurs at young ages (in utero to puberty), there are currently no studies indicating that adult exposure to TCDD affects sperm quality.

There are several lines of evidence indicating that reduced male fertility is associated with a reduced sex ratio. For example, testicular cancer (Moller 1998), higher scrotal temperatures (Perez-Crespo et al. 2008) and older paternal age (Chahnazarian 1988), have all been associated with a reduction in the proportion of male births. While the exact mechanism remains unclear, animal studies have also linked the production of sons with fertility (Terrell et al. 2011).

Several social conditions affecting sex ratio, possibly through a higher loss of males than females after fertilisation, have also been reported. For example, the life expectancy of a population is positively and deprivation is negatively associated with sex ratio, indicating that mothers in poor conditions are more likely to give birth to daughters (Terrell et al. 2011). There are also several reports of reduced sex ratio after earthquakes (D'Alfonso et al. 2012) and other major disasters, indicating that sudden intense maternal stress around the time of conception results in a lower sex ratio. However, these effects appear to be acute, transient, and likely female-mediated, as opposed to the long-term and male-mediated effects hypothesised for TCDD exposure.

Recent human studies indicate that the effect of male TCDD exposure on their offspring's sex ratio may not only be long-term but also dependent on the timing of male exposure in relation to age and sexual maturation. The Seveso study found that reduction in sex ratio was strongest when exposure occurred under the age of 19, indicating that the time before and during puberty may be a particularly sensitive period for dioxin reproductive toxicity in men (Mocarelli et al. 2000). In occupationally exposed cohorts such as this study and the Russian study, timing of first exposure is difficult to establish, but it is safe to assume that first employment and therefore first exposure would have occurred in adulthood (the youngest age of first employment at the plant in this study was 23). Thus, our findings, as well as those of the Russian study (Ryan et al. 2002), indicate that the effect is not limited to exposure before or during puberty, although we did observe the strongest association for fathers first exposed before the age of 37.

We considered a range of factors that may confound the association between TCDD and sex ratio, of which only paternal BMI was found to alter the TCDD-sex ratio association after inclusion in the model. Paternal BMI was positively associated with a male birth outcome: sex ratio of fathers with normal weight (BMI<25) was 0.47, while for overweight fathers (BMI ≥25) it was 0.60, a pattern which has been observed previously (Abu-Rmeileh et al. 2011). Inclusion of current paternal BMI in the model strengthened the negative association between paternal TCDD and male birth outcome, possibly due to BMI-associated misclassification of exposure. A higher body percentage fat has been associated with slower elimination of TCDD (Michalek and Tripathi 1999), resulting in an over-estimation of historical TCDD exposure based on current TCDD determination in those with a high BMI, which may result in strengthening of the TCDD-sex ratio association when adjusting for BMI such as observed here. When stratifying the results by current paternal BMI, the strongest negative association between serum TCDD and sex-ratio was observed for the group with BMI<25, which is in line with our assumption that for this group estimated TCDD at time of birth would be less misclassified compared to the overweight group.

In this population, the overall sex ratio was higher (0.55) than expected for the general population (generally 0.51) with higher sex ratios for more recent years of birth, for which the reasons are not clear. Although sex ratio has slightly increased over time in New Zealand, in trend with increased life expectancy (Dixson et al. 2013), this cannot explain the magnitude observed here. Also, the effect is contrary to a parental age cohort effect that could be expected: the earlier births are more likely to be from younger parents and have lower birth order, which have both been associated with a higher sex ratio (Terrell et al. 2011). A paternal BMI related cohort effect would be consistent with the observed increase in sex ratio over time, given that higher paternal BMI has been associated with a higher sex ratio (Abu-

Rmeileh et al. 2011), but this would assume an increase in the fathers' BMI over time, on which we do not have data. Alternatively, it could in part reflect a real effect of TCDD exposure, with the highest TCDD exposure and therefore lowest sex ratio to be expected in the earlier years. Recall bias may be also involved; given that information on offspring was self-reported many years after birth, there is a possibility that recall of early losses of births, which are more common for male births, may be worse for births that occurred longer ago, resulting in a more pronounced undercount of male births for earlier years. When stratifying by year of birth, a strong dose-response association was observed for births after 1980 (p-trend<0.001), but not for births before 1980 (p-trend=0.6). This may be due to misclassification of TCDD serum concentration at time of birth, which can be expected to be more substantial for births that occurred in earlier years. In particular, when back-calculating paternal TCDD levels to the time of birth, exposure was assumed to start when employment started, which may not be the case for all participants, potentially resulting in a substantial misclassification of TCDD exposure at time of birth for those births that occurred close to the start of employment, obscuring the dose-response association for the earlier births.

We could not evaluate the effect of other potential confounders such as maternal stress, but maternal stress around conception is unlikely to be associated with paternal TCDD concentrations in this occupationally exposed population. However, stress could be a factor in some study populations where mother's stress is indirectly associated with the father's TCDD exposure, such as is the case for the Ranch Hand cohort (Michalek et al. 1998), and may be a possible explanation for the absence of an association between paternal TCDD exposure and alteration in the sex ratio in the offspring of the veterans.

In this study we did not observe that a higher serum TCDD concentration was associated with a more frequent report of health problems or congenital malformations in the offspring. Most evidence of health effects in offspring is based on studies involving in utero or perinatal TCDD exposure, with the mother as the main route of exposure. In the current study, TCDD serum concentrations were available mainly for fathers (we did not determine the mothers TCDD serum concentration of male cohort members) and the number of female cohort members was insufficient to study the association between maternal TCDD exposure and reported health outcomes in the offspring. In addition, the study used the parent's self-report of health problems in the offspring based on an open ended question, which is likely to be subject to substantial misclassification and lacks clinical verification. Notwithstanding these limitations, we did observe an association between TCDD (ln(TCDD) at time of birth) and reported thyroid problems in the offspring. Although this association is based on very small numbers, it is statistically significant and is noteworthy in the light of toxicological and mechanistic data indicating that dioxin may impair thyroid function in the offspring (Giacomini et al. 2006). Evidence in human populations is very limited, but it has been reported that children born from mothers exposed to TCDD in the Seveso incident had higher neonatal blood thyroid-stimulating hormone (b-TSH, a sensitive marker of subclinical primary hypothyroidism) than the reference population, and maternal TCDD levels estimated at the date of delivery were positively associated with neonatal b-TSH (Baccarelli et al. 2008). After further testing, two children from the contaminated areas and none from the reference were diagnosed with primary hypothyroidism (Baccarelli et al. 2008). Thus, thyroid effects in the offspring associated with parental TCDD exposure may be of clinical significance and warrants further investigation.

Conclusions

373 This study lends further support to a second generation effect of TCDD exposure that started
374 in adulthood, with paternal TCDD serum concentrations in excess of 20 pg/g lipid at time of
375 birth associated with the birth of relatively fewer boys than girls.
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Table 1. Study population characteristics: number of births by plant employees conceived after commencing employment, by parents' demographic characteristics

	fathers employed at plant (n=127)			mothers employed at plant (n=21)		
	girls (n)	**boys (n)**	**sex ratio**	**girls (n)**	**boys (n)**	**sex ratio**
	137	167	0.55	21	30	0.59
year of birth						
<1970	21	14	0.40			
70-80	54	61	0.53	5	14	0.74
80-90	42	61	0.59	13	12	0.48
>1990	20	31	0.61	3	4	0.57
age parent (employed at plant) at birth						
<25	19	20	0.51	3	11	0.79
25-30	47	56	0.54	7	6	0.46
30-40	59	80	0.58	9	11	0.55
≥40	12	11	0.48	2	2	0.50
age other parent at birth						
<25	33	44	0.57			
25-30	47	51	0.52			
30-40	30	43	0.59			
≥40	3	1	0.25			
unknown	24	28	0.54	21	30	0.59
age at start employment						
23-30	26	39	0.60	7	6	0.46
30-37	47	66	0.58	8	11	0.58
>37	64	62	0.49	6	13	0.68

Table 2. Association between three indicators of TCDD exposure of parent and male birth outcome through logistic regression of correlated outcome data

	Fathers employed at plant (n=127)							Mothers employed at plant (n=21)				
	girls (n)	***boys (n)***	***sex ratio***	***Crude OR***	***95% CI***	***OR[1)]***	***95% CI***	***girls (n)***	***boys (n)***	***sex ratio***	***Crude OR***	***95% CI***
Employment in highly exposed job												
no	77	87	0.53	1.00	ref	1.00	ref	20	27	0.57	1.00	ref
yes	60	80	0.57	1.19	0.76-1.85	1.11	0.72-1.70	1	3	0.75	2.01	0.43-9.46
No	77	87	0.53	1.00	ref	1.00	ref	20	27	0.57		
0.1-1.5 years	20	34	0.63	1.52	0.76-3.03	1.37	0.67-2.82	1	2	0.67		
1.5-5 years	28	29	0.51	0.92	0.51-1.67	0.86	0.47-1.57	0	0	-		
≥5 years	12	17	0.59	1.27	0.76-2.11	1.33	0.81-2.16	0	1	-		
				p-trend	*0.685*	*p-trend*	*0.775*					
Serum TCDD of parent at time of phlebotomy												
<4 pg/g lipid	76	114	0.60	1.00	ref	1.00	ref	14	18	0.56	1.00	ref
≥4 pg/g lipid	61	53	0.46	0.58	0.37-0.91	0.46	0.29-0.73	7	12	0.63	1.40	0.66-2.97
<4 pg/g lipid	76	114	0.60	1.00	ref	1.00	ref	14	18	0.56		
4-10 pg/g lipid	49	37	0.43	0.50	0.32-0.79	0.43	0.27-0.67	7	10	0.59		
10-25 pg/g lipid	5	9	0.64	1.27	0.38-4.23	0.90	0.29-2.79	0	1	-		
≥25 pg/g lipid	7	7	0.50	0.70	0.28-1.76	0.52	0.17-1.64	0	1	-		
				p-trend	*0.183*	*p-trend*	*0.064*					
Estimated serum TCDD of parent at time of birth												
<20 pg/g lipid	74	112	0.60	1.00	ref	1.00	ref	15	17	0.53	1.00	ref
≥20 pg/g lipid	63	55	0.47	0.58	0.37-0.90	0.49	0.30-0.79	6	13	0.68	1.90	0.84-4.31
<4 pg/g lipid	59	88	0.60	1.00	ref	1.00	ref	9	9	0.50		
4-20 pg/g lipid	15	24	0.62	1.08	0.53-2.18	1.00	0.50-2.02	6	8	0.57		
20-100 pg/g lipid	30	27	0.47	0.60	0.35-1.02	0.52	0.29-0.92	6	12	0.67		
≥100 pg/g lipid	33	28	0.46	0.58	0.30-1.09	0.45	0.23-0.89	0	1	-		
				p-trend	*0.037*	*p-trend*	*0.007*					

1) OR= Odds Ratio (modelling the probability of a male birth), adjusted for: age of parent at year of birth, current BMI parent, smoking status parent

458 **Table 3.** Effect of adjustment for potential confounders on the association between estimated

459 paternal serum TCDD concentration at time of birth and the probability of a male offspring.

TCDD[1)] *(pg/g)*	girls	boys	crude		father's current BMI		smoking		age father at birth		age mother at birth		year birth child	
			OR	95%CI	OR 1	95%CI	OR 2	95%CI	OR 3	95%CI	OR 4	95%CI	OR 5	95%CI
<4	59	88	1.0	ref	1.0	ref	1.0	ref	1.0	ref	1.0	ref	1.0	ref
4-20	15	24	1.1	0.5-2.2	1.0	0.5-2.0	1.1	0.5-2.2	1.1	0.5-2.2	1.1	0.6-2.3	1.1	0.5-2.5
20-100	30	27	0.6	0.4-1.0	0.5	0.3-0.9	0.6	0.4-1.0	0.6	0.3-1.0	0.6	0.4-1.0	0.6	0.3-1.0
≥100	33	28	0.6	0.3-1.1	0.5	0.2-0.9	0.6	0.3-1.1	0.6	0.3-1.1	0.6	0.3-1.1	0.7	0.4-1.4
ORs associated with categories (see footnote) of the potential confounders: (adjusted for paternal TCDD at birth)					1.0	(BMI<25)	1.0	(never)	1.0	(age<25)	1.0	(age<25)	1.0	(<1970)
					1.5	0.9-2.7	1.1	0.7-1.8	1.2	0.6-2.5	0.8	0.4-1.4	1.9	0.8-4.2
					2.3	1.1-4.9	1.1	0.5-2.4	1.2	0.6-2.5	0.9	0.5-1.7	2.2	0.9-5.1
									0.8	0.3-2.2	0.2	0.0-2.0	2.0	0.8-5.1

460

461 1) serum TCDD of father at time of birth

462 OR: crude Odds Ratio

463 OR1 adjusted for BMI: 4 categories: <25; 25-30; >=30; missing (for missing BMI the OR is

464 not reported)

465 OR2 adjusted for Smoking: 3 categories: never; ex; current

466 OR3 adjusted for Age father at birth: 4 categories: <25; 25-30; 30-40; >=40

467 OR4 adjusted for Age mother at birth: 5 categories: <25; 25-30; 30-40; >=40; missing (for

468 missing mother's age at birth the OR is not reported)

469 OR5 adjusted for the year of birth of the child: 4 categories: <1970, 1970-80, 1980-90, >1990

470

Table 4. Association between estimated paternal serum TCDD concentration at time of birth and the probability of a male offspring, by father's age of first exposure.

fathers employed at plant (n=127) **age father at start employment**	**girls (n)**	**boys (n)**	**sex ratio**	**Crude OR**	**95% confidence interval**	**OR[1)]**	**95% confidence interval**
23-30							
<4 pg/g lipid	15	24	0.62	**1.00**	ref	**1.00**	ref
4-20 pg/g lipid	4	9	0.69	**1.37**	0.36-5.31	**2.00**	0.35-11.4
20-100 pg/g lipid	4	5	0.56	**0.51**	0.16-1.68	**0.17**	0.04-0.68
≥100 pg/g lipid	3	1	0.25	**0.20**	0.03-1.37	**0.04**	0.01-0.26
				p-trend	*0.128*	*p-trend*	*0.016*
30-37							
<4 pg/g lipid	24	43	0.64	**1.00**	ref	**1.00**	ref
4-20 pg/g lipid	3	5	0.63	**0.94**	0.19-4.75	**1.26**	0.20-8.08
20-100 pg/g lipid	12	11	0.48	**0.51**	0.25-1.07	**0.37**	0.16-0.85
≥100 pg/g lipid	8	7	0.47	**0.49**	0.12-2.09	**0.59**	0.18-1.95
				p-trend	*0.148*	*p-trend*	*0.062*
>37							
<4 pg/g lipid	20	21	0.51	**1.00**	ref	**1.00**	ref
4-20 pg/g lipid	8	10	0.56	**1.20**	0.42-3.48	**2.02**	0.78-5.21
20-100 pg/g lipid	14	11	0.44	**0.75**	0.28-2.05	**0.87**	0.34-2.25
≥100 pg/g lipid	22	20	0.48	**0.86**	0.35-2.12	**1.22**	0.48-3.13
				p-trend	*0.625*	*p-trend*	*0.934*

1) OR= Odds Ratio (modelling the probability of a male birth), adjusted for: age of parent at year of birth, current BMI parent, smoking status parent

Table 5. Association between an estimated parental >=20 pg/g TCDD serum concentration at time of birth and the probability of a parent-reported health problem in the offspring.

355 births after start employment	TCDD <20 (218)		TCDD ≥20 (137)		OR[1)]	95% confidence interval	OR for ln(TCDD) continuous	95% confidence interval
any health problem reported	33	15%	24	18%	1.33	(0.72-2.45)	1.10	(0.91-1.32)
congenital malformation	13	6%	5	4%	0.54	(0.16-1.85)	0.89	(0.68-1.16)
thyroid problem [2)]	0	0%	3	2%	-	-	1.85	(1.37-2.48)

1) OR= Odds Ratio, adjusted for: age of parent at year of birth, sex of exposed parent, sex of child

2) For none of the offspring with reported thyroid problem, the parent reported a thyroid problem. Of the 3 children reported to have thyroid problems, 2 were from an exposed father and 1 from an exposed mother. The OR could not be adjusted for age of exposed parent at year of birth.

487 **Table 6.** Sex ratios reported for the offspring of TCDD exposed populations.

	Non-exposed group		***TCDD exposed group***		
	Children (n)	Sex ratio	Paternal TCDD at the time of conception	Children (n)	Sex ratio
US, Ranch Hand (Michalek et al. 1998)	346	0.51	≥10 pg/g	557	0.51
Italy, Seveso (Mocarelli et al. 2000)	271	0.56	>15 pg/g	403	0.44
US, NIOSH (Schnorr et al. 2001)	292	0.51	≥20 pg/g	252	0.56
Russia, Ufa (Ryan et al. 2002)	Ufa city	0.51	not reported	188	0.38
New Zealand (this study)	186	0.60	≥20 pg/g	118	0.47

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