# ANALYSIS OF SEX RATIOS FROM SEVESO AND RANCH HAND COHORTS USING BINOMIAL PROBABILITY

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## Introduction

Currently, there is a discussion regarding the potential effects of dioxin exposure and the subsequent sex ratio of children born to those exposed. Two of the most thoroughly documented and analyzed of the dioxin exposure cohorts are the Seveso and Ranch Hand cohorts. [1,2] These two studies provide contrasting results regarding the mediation (male or female parent) of the sex ratio differences, as well as the magnitude of the potential effect.

Many of the conclusions published about the Seveso cohort strongly implicate serum TCDD concentrations near current and historic background concentrations (10 to 20 ppt), with a subtle alteration of sex ratios among the offspring of exposed parents. Additionally, the results published for Seveso conclude the difference in sex ratios is male mediated, while the Ranch Hand analysis suggests that the difference is mediated by the female.

We present a method to reconcile the differences in conclusions between these two studies using probability theory and to provide more evidence of the effect that dioxin exposure on the live birth sex ratio. The original results of each study are compared to results of the reanalysis and conclusions are drawn.

## Methods and Materials

Part of the problem with comparing the Seveso cohort to the Ranch Hand cohort is that the analyses are fundamentally different and not directly comparable. Mocarelli et al present sex ratios and confidence intervals in terms of the male birth ratio, while Michalek et al present the sex ratios and confidence intervals based on the female birth ratio. Additionally, the Seveso data include odds ratios for various analyses and sub-analyses.

The data contained in each paper are reanalyzed using the binomial distribution, without the calculation of odds ratios, confidence intervals or confidence levels. These statistical measures, while applicable and useful, may add a layer of complexity to a relatively staightforward problem. In this study, we calculate the probability of occurrence of the sex ratios noted in several Seveso and Ranch Hand analyses and sub-analyses.

To help illustrate the method employed, consider the probability associated with flipping a coin to be analogous to the determination of the sex of a child. If the chance is equally divided between male (tails) and female (heads), for one coin flip the $P(F)=0.5$ and the $P(M)=0.5$. For three independent coin flips, the probability of having exactly one male [tails, $P(M=1)$] is 0.375, while the probability of having one or fewer males [tails, $P(M<=1)$] is 0.50. A concise interpretation of this result is that if an infinite number of 3 coin-flip experiments were performed, 37.5 % of the time you would have exactly one male (tails) and 50 % of the time you would have either 0 or 1 male (tails). However, the probability associated with each type of birth is not 50-50. For the general population it is assumed that the probability of a male birth is 51.4 % and the probability of a female birth is 48.6 %.[3] These male and female birth probabilities are used in all further probability analysis.

**Results and Discussion**

**Table 1.** Male sex ratio and probability of occurrence for Seveso cohort.

| Fathers's TEQ Concentration (ppt) | Mother's TEQ Concentration (ppt) | Number of Male Children | Number of Female Children | Male Sex Ratio and CI ($95^{th}$)[1] | Probability of Occurrence of Male Birth Ratio |
|---|---|---|---|---|---|
| <=15 | <=15 | 31 | 20 | 0.608 (0.47-0.74) | 93.1 % |
| >15 | >15 | 96 | 121 | 0.442 (0.38-0.51) | 2.1 % |
| >15 | <=15 | 81 | 105 | 0.436 (0.36-0.51) | 1.9 % |
| <=15 | >15 | 120 | 100 | 0.545 (0.48-0.61) | 84.2 % |
| All | All | 328 | 346 | 0.487 | 8.3 % |

The original Seveso analysis indicates a correlation between TCDD concentration in excess of background in males and a decrease in the likelihood of male off-spring (sex ratio and $95^{th}$ CI). However, the confidence intervals include the expected male birth ratio (0.51), weakening the strength of the conclusion. The above conclusion is more decisively supported with the binomial probability results, which indicate very low probabilities of occurrence (2.1 % and 1.9 %) for the male birth ratios observed when the father's measured blood serum TCDD levels were greater than 15 ppt in 1976. The probability analysis also indicates that there is little likelihood that maternal exposure is related to the sex ratios observed.

**Table 2.** Female sex ratio and probabilities of birth occurrence for Ranch Hand cohort.

| Fathers's TEQ Concentration (ppt) | Mother's TEQ Concentration (ppt) | Number of Male Children | Number of Female Children | Male Sex Ratio and CI ($95^{th}$)[1] | Probability of Occurrence of Male Birth Ratio |
|---|---|---|---|---|---|
| Comparison (<10) | 642 | 612 | 0.488 (0.46-0.5160 | 56.3 % | 46.2 % |
| Background (<10) | 176 | 170 | 0.491 (0.438-0.545) | 60.0 % | 44.2 % |
| Low (10-79) | 135 | 142 | 0.513 (0.452-0.573) | 82.8 % | 20.4 % |
| High (>79) | 149 | 131 | 0.468 (0.408-0.528) | 29.2 % | 74.8 % |

The authors of the Ranch Hand analysis conclude that the birth ratios observed imply that the process that controls the sex ratio may be mediated by the female. The analysis does not support this conclusion. In fact, the binomial probability results indicate that for the low exposure group, it is possible that the male sex ratio is dependent on the fathers' exposure, since the probability of occurrence of the observed male sex ratio is relatively low (20.4 %).

The original analysis concludes that increasing serum TCDD levels in fathers was associated with decreasing male births. However, this result is confounded by the fact that the confidence intervals associated with the male sex ratio include the expected level, except for the most highly exposed fathers. The original conclusion is supported and clarified with the binomial probability analysis, which quantifies the rapidly decreasing probabilities of observed male birth ratios with increasing serum TCDD levels in the father. It is interesting to note that the decrease appears to be bi-modal, centered on paternal TCDD levels of from 15.1 to 31.3 ppt and levels greater than 118 ppt.

**Table 3.** Probabilities of occurrence of male sex ratios for different TCDD serum levels in fathers.

| Fathers's TCDD Concentration (ppt) | Number of Male Children | Number of Female Children | Male Sex ratio and CI ($95^{th}$)[1] | Probability of Occurrence of Male Birth Ratio |
|---|---|---|---|---|
| 0-15 | 151 | 120 | 0.557 (0.49-0.61) | 93.1 % |
| 15.1-31.3 | 35 | 45 | 0.438 (0.33-0.55) | 10.4 % |
| 31.9-60.7 | 41 | 40 | 0.506 (0.40-0.61) | 48.8 % |
| 61.4-117.0 | 38 | 43 | 0.469 (0.36-0.58) | 24.3 % |
| 118.0-264.0 | 32 | 48 | 0.400 (0.29-0.51) | 2.7 % |
| 281.0-26400.0 | 31 | 50 | 0.383 (0.28-0.49) | 1.2 % |

**Table 4.** Probabilities of occurrence of male sex ratios for different ages at exposure.

| Fathers Exposure Status | Number of Male Children | Number of Female Children | Male Sex ratio and CI ($95^{th}$)[1] | Probability of Occurrence of Male Birth Ratio |
|---|---|---|---|---|
| Unexposed (all ages) | 151 | 120 | 0.557 (0.49-0.61) | 93.1 % |
| Exposed (<19 years old) | 50 | 81 | 0.382 (0.30-0.47) | 0.2 % |
| Exposed (>19 years old) | 127 | 144 | 0.469 (0.41-0.53) | 7.9 % |

The original conclusions indicate that there is an increased risk of altering the male sex ratio for fathers who exhibit >15 ppt serum TCDD concentration. However, it is unclear if fathers exposed when they were older (> 19 years of age) produced male offspring at a ratio significantly different from the historical average (i.e. CI overlaps 0.514). The binomial probability analysis indicates that the male birth ratios for both exposed groups are relatively unlikely to occur (0.2 % and 7.9 %), and are very likely related to paternal dioxin exposure.

The binomial probability analysis presented here provides a basis for intuitively understanding the degree to which Seveso and Ranch Hand cohort live birth sex data vary from expected ratios and serves as a possible first step for quantifying the dose response relationship between dioxin TEQ exposure and live birth sex ratio.

## References


1. Mocarelli P., Gerthoux P.M., Ferrari E, Patterson D.G., Kieszk S.M., Brambilla P., Vincoli N., Signorini S., Tramacere P., Sampson E.J., Turner W.E., and Needham L.L. (2000) Lancet. 355:1858-1863.
2. Michalek J.E., Rahe A.J., and Boyle C.A. (1998) Epidemiology. 9, 4:474-475.
3. Mocarelli P., Brambilla P., Gerthoux P.M., Patterson D.G., and Needham L.L. (1996) Lancet. 348:409.