# LONG-TERM ENDOCRINE RELATED OUTCOMES OF 2,3,7,8-TCDD EXPOSURE: THE SEVESO MORTALITY STUDY

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**Introduction**

In vitro and animal studies strongly support the hypothesis that 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) may disrupt several endocrine pathways.

First of all, TCDD itself behaves as a hormone, binding to the Aryl Hydrocarbon (Ah) Receptor, a nuclear receptor similar to those of glucocorticoids, mineralcorticoids, androgens, estrogens, progesterone, and vitamin D.

Ah receptor exists as a cytoplasmic, multimeric complex that includes heat shoch protein 90 and association of TCDD with the receptor results in dissociation from heat shock proteins. This in turn initiates transport of the receptor into the nucleus where it associated with the dioxin/xenobiotics responsive elements (DRE) which is a classic DNA recognition element modulating transcriptional activity (1).

Although TCDD does not bind the estrogen receptor, it can affect estrogenic actions through Ah receptor binding, as it is clearly demonstrated by in vitro and animal studies (1).

Effects on thyroid gland function and morphology (2), pituitary hormone secretion and biological activity (3), pineal release of melatonin (4) and adrenal function (5) have been described.

Nevertheless, little is known about endocrine effects of TCDD in humans.

We report on the potentially endocrine related health effects observed in the population exposed to TCDD after the Seveso accident.

**Material and Methods**

The Seveso accident in 1976 exposed the population of a large residential area to substantial amounts of TCDD. The accident occurred in a chemical plant near the town of Seveso, Italy, where an uncontrolled exothermic reaction during the manufacture of trichlorophenol produced the sudden release of a cloud of chemical substances, including TCDD (6).

Long-term effects were investigated with mortality and cancer incidence studies comparing the experience of the exposed population in three contamination areas (A-zone, very high contamination, around 800 inhabitants, B-zone, high contamination, nearly 6,000 inhabitants; R-zone, low-scanty contamination, 38,000 inhabitants) to a non exposed population of nearly 230,000 subjects living in the surrounding area. This zone categorization was well correlated to blood concentrations of TCCD measured in specimens taken at the time of the accident (7) and nearly 20 years after (8,9).

**Results and discussion**

In order to ascertain anti-estrogenic effects of TCDD (1) in Seveso population we analyzed 20 years mortality from estrogen promoted cancer.

Table 1 shows a consistent, but statistically non-significant and only suggestive, decrease in mortality from uterus, ovary and breast cancer when zones A and B are analyzed jointly, whereas liver cancer mortality was increased. These findings could suggest a selective estrogen receptor modulator action of TCCD in humans, or, in alternative, the presence of a direct cancer promoting effects on liver due to higher hepatic concentrations of TCDD subsequent to gastrointestinal absorption and, at the opposite, a prevalent hormone-mediated anti-neoplastic effect on uterus, ovary and breast.

Taking into account the bimodal distribution of breast cancer mortality by age, with an early peak mainly depending on genetic factors, and a later peak strongly linked to hormonal factors, we separately evaluated breast cancer mortality occurred before and after 50 years of age. Table 2 shows that the decrease in mortality occurred essentially in the older age-group. Again, the RR estimates are statistically non-significant and suggestive only. These results would confirm those obtained in animals by Kociba et al. who first reported a dose-dependent decrease in spontaneous mammary and uterine tumors in long-term feeding studies with TCDD (10)

Although in female subjects we found a tendency toward a decrease in uterus, ovary, and breast cancer mortality, the reasonable hypothesis that this phenomenon derived from a decreased estrogen action implies serious and substantial health consequences, namely in post-menopausal women.

We know that most women in western societies die of coronary atherosclerosis, which kills more women than all forms of cancer combined (11). Since epidemiological studies indicate that effects of estrogens on vascular diseases are highly beneficial (12), the overall balance of the exposure to an anti-estrogen such as TCDD may be negative.

Moreover, the potential effect on bone metabolism is to be remarked. In fact, estrogen is often considered the main factor affecting the incidence of osteoporosis and fractures (13). This is also an extensive problem for it has been estimated that 30% of all postmenopausal white women sustain an osteoporotic fracture (14) and most of fractures in older females result from osteoporosis among women who experience accelerated bone loss secondary to estrogen deficiency (15).

Finally, there are plausible biological and neurophysiologic mechanisms that might account for a beneficial effect of estrogen on cognition, reducing risk for developing Alzheimer disease or other forms of dementia (16).

Thyroid cancer was increased in highly exposed subjects, but only 2 cases were observed (1 M, 1 F). It is known that TCDD may cause a reduction of circulating thyroid hormone levels, probably inducing liver microsomal enzymes and accelerating thyroxin and triiodotironine catabolism (17). The decrease in thyroid hormone blood concentration is associated with a compensatory increased secretion of pituitary Thyroid Stimulating Hormone (TSH). TCDD could indirectly force thyroid gland, through TSH hypersecretion, toward the development of hypertrophy, hyperplasia, adenomas and - less frequently – carcinomas (2).

In humans, it has been suggested that thyroid hyperstimulation by TSH could induce cancer only when acting in conjunction with other metabolic or immunologic abnormalities (17), as it could have occurred in TCDD exposed subjects.

Deaths from diabetes were elevated among women in high exposure zones (RR=1.7; CI 95% 1.1-2.7), in agreement with diabetes prevalence studies carried out among U.S. Air Force veterans exposed to Agent Orange (highly contaminated by TCDD) during the Vietnam war (18).
Finally, endocrine factors may be involved in the generation of the observed systematic difference in TCDD blood levels between males and females (Table 3).
In conclusion, our data seem to be in aggrement with the hypothesis of substantial multiple endocrine disrupting actions of TCDD in humans.

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TABLE 1 - MORTALITY FROM OESTROGEN PROMOTED NEOPLASMS, 1976-1996, BY LATENCY AND LENGTH OF STAY IN THE CONTAMINATED AREA AMONG HIGHLY EXPOSED WOMEN IN SEVESO

| STUDY VARIABLE | LIVER | | UTERUS | | OVARY | | BREAST | |
|---|---|---|---|---|---|---|---|---|
| | RR | 95% CI | RR | 95% CI | RR | 95% CI | RR | 95% CI |
| **TOTAL** | 1.3 | 0.6-2.9 | 0.4 | 0.1-1.7 | 0.6 | 0.2-1.9 | 0.7 | 0.4-1.2 |
| **LATENCY** | | | | | | | | |
| ≤10 yr. | 0.9 | 0.2-3.7 | 0.4 | 0.1-2.9 | 0 | 0 | 0.8 | 0.4-1.6 |
| >10 yr. | 1.6 | 0.6-4.4 | 0.5 | 0.1-3.9 | 1.2 | 0.4-3.8 | 0.7 | 0.3-1.5 |
| **LENGTH OF STAY** | | | | | | | | |
| < 1 yr. | 4.0 | 0.5-31.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1-5 yr. | 0 | 0 | 0 | 0 | 0 | 0 | 0.9 | 0.4-2.2 |
| 6-10 yr. | 1.7 | 0.4-6.9 | 0.8 | 0.1-5.8 | 0.8 | 0.1-5.8 | 0.6 | 0.2-1.9 |
| > 10 yr. | 1.5 | 0.5-4.7 | 0.6 | 0.3-1.5 | 1.0 | 0.3-4.2 | 0.7 | 0.3-1.6 |

TABLE 2 - MORTALITY FROM BREAST CANCER, 1976-1996, BY AGE GROUP, LATENCY, AND LENGTH OF STAY IN THE CONTAMINATED AREA AMONG HIGHLY EXPOSED FEMALES IN SEVESO

| STUDY | < 50 YEARS | | | ≥ 50 YEARS | | |
|---|---|---|---|---|---|---|
| VARIABLE | OBS | RR | 95% CI | OBS | RR | 95% CI |
| **LATENCY** | | | | | | |
| ≤10 years | 3 | 0.9 | 0.3-2.8 | 4 | 0.7 | 0.3-1.9 |
| >10 years | 3 | 1.2 | 0.4-3.8 | 4 | 0.5 | 0.2-1.5 |
| **LENGTH OF STAY** | | | | | | |
| < 1 year | 0 | 0 | - | 0 | 0 | - |
| 1-5 years | 3 | 1.4 | 0.4-4.5 | 2 | 0.6 | 0.2-2.6 |
| 6-10 years | 0 | 0 | - | 3 | 1.0 | 0.3-3.1 |
| > 10 years | 3 | 1.8 | 0.6-5.6 | 3 | 0.5 | 0.2-1.5 |

TABLE 3 - LIPID ADJUSTED TCDD LEVELS (PPT) IN THE SEVESO POPULATION IN 1992-93 BY ZONE AND GENDER

| ZONE | GENDER | NO. | GM | MEDIAN | RANGE |
|---|---|---|---|---|---|
| **ZONE A** | F | 2 | 60.5 | 63.0 | 45.3-80.7 |
| | M | 5 | 50.5 | 73.3 | 9.8-89.9 |
| | | | *(p=0.81)* | *(p=0.99)* | |
| **ZONE B** | F | 26 | 17.6 | 16.8 | 1.3-62.6 |
| | M | 25 | 6.7 | 6.5 | 3.5-44.7 |
| | | | *(p=0.0003)* | *(p=0.0003)* | |
| **REFERENCE AREA** | F | 28 | 6.1 | 6.6 | 1.8-18.1 |
| | M | 24 | 3.7 | 4.4 | 1.0-13.8 |
| | | | *(p=0.007)* | *(p=0.005)* | |