

Serum dioxin concentrations and endometriosis: a cohort study in Seveso, Italy

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Dioxin, a ubiquitous contaminant of industrial combustion processes, including medical waste incineration, has been implicated in the etiology of endometriosis in animals. We sought to determine if there is an association of dioxin exposure and endometriosis in humans. We conducted a population-based historical cohort study 20 years after the 1976 factory explosion in Seveso, Italy, which resulted in the highest known population exposure to 2,3,7,8-tetrachlorodibenzo-*para*-dioxin (TCDD). Participants were 601 female residents of the Seveso area who were ≤ 30 years old in 1976 with adequate stored sera. Endometriosis disease status was defined by pelvic surgery, current transvaginal ultrasound, pelvic examination, and interview (for history of infertility and pelvic pain). “Cases” had surgically-confirmed disease or an ultrasound consistent with endometriosis. “Non-diseased” had surgery with no evidence of endometriosis, or no signs or symptoms. Other women had “uncertain” status. In order to assess TCDD exposure, individual levels of TCDD were measured in stored sera collected soon after the accident. We identified 19 “cases” and 277 “non-diseased” women. Relative to women with levels ≤ 20 ppt, the relative risk ratios (RRR) for women with serum TCDD levels of 20.1 to 100 ppt and >100 ppt were 1.2 (90% CI=0.3-4.5) and 2.1 (90% CI=0.5-8.0) respectively. Tests for trend using the above exposure categories and continuous logTCDD were non-significant.

Validation Study of the CALUX Bioassay in Human Serum as a Measure of Dioxin Toxic Equivalents

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We examined the validity of using the CALUXTM (chemical-activated luciferase gene expression) bioassay to measure the total dioxin-like toxic equivalents (TEQ) of mixtures of polyhalogenated aromatic hydrocarbons in human serum from a background-exposed population. Polychlorinated dibenzo-*p*-dioxins (PCDD), polychlorinated dibenzofurans (PCDF), and polychlorinated biphenyls (PCB) congeners were quantified by high-resolution gas chromatography/ mass spectrometry (GC/MS) in archived serum from 78 Italian women (20 to 49 years) attending the gynecologic clinic at Desio Hospital. International toxicity equivalents were calculated for PCDDs (PCDD-TEQ), PCDFs (PCDF-TEQ), PCBs (PCB-TEQ), and Total TEQ. A sample of 40 archived plasma specimens was then selected for the CALUXTM bioassay, based on the distribution of Total TEQ derived from GC/MS data. The CALUXTM bioassay was performed blind to the GC/MS results.

Descriptive statistics of individual PCDD, PCDF, PCB congener data as well as Total TEQ data will be presented. The relation of PCDD-TEQ, PCDF-TEQ, PCB-TEQ, and Total TEQ with CALUX-TEQ will be presented. Potential confounding effects of age and other covariates will be considered. The results of this study will help determine the feasibility of using the CALUX bioassay to conduct large-scale epidemiologic study of the health effects of dioxin and dioxin-like compounds.

Serum dioxin levels and chronic disease risk in women of Seveso, Italy

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Dioxin (2,3,7,8-tetrachlorodibenzo-*para*-dioxin or TCDD), a widespread environmental contaminant, has been shown to disrupt multiple endocrine pathways. In 1997, IARC classified TCDD as a known human carcinogen. More recently, TCDD has been associated with chronic diseases including ischemic heart disease and diabetes, primarily based on results of mortality studies of occupationally exposed men. To our knowledge this association has not been investigated in a female TCDD-exposed cohort.

We examined the relation of serum TCDD level and occurrence of chronic diseases including cancer, heart disease, diabetes, and thyroid disease from 1976 to 1996 among women in the Seveso Women's Health Study (SWHS). The SWHS is a comprehensive reproductive epidemiologic study of a female population exposed to very high levels of TCDD as a result of an explosion in Seveso, Italy in 1976. The SWHS cohort comprises 981 women who were 40 years or younger in 1976, resided in the most contaminated areas at the time of the explosion, and had stored sera that was collected soon after the explosion. As part of the study a questionnaire including demographic, lifestyle, reproductive, pregnancy and medical histories, and exposure information was administered. Self-report diagnosis of chronic diseases was confirmed with medical records and individual-level TCDD concentration in serum was measured by high-resolution mass spectrometry. In the SWHS cohort, chronic disease cases included: 21 (2.1%) cancers; 68 (7%) heart disease, 25 (2.6%) diabetes, and 77 (8%) thyroid disease. The relation between individual serum TCDD levels and each of the chronic diseases will be presented.

10689 - Soft tissue sarcoma and dioxins– A case-control study

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INTRODUCTION: Dioxins are persistent chlorinated environmental contaminants. The most potent dioxin, TCDD, is known to cause cancer in laboratory animals after high doses. Soft tissue sarcoma (STS) has been proposed to be a candidate for a dioxin-induced cancer in humans. However, previous epidemiological studies have suffered from poor exposure data and mixed chemical exposures. The objective was to test this association in a large prospective case-control study with individual estimates of dioxin exposure. **METHODS:** A multicenter setting was used with 4 university hospitals and 12 other hospitals in southern Finland. Participants included 109 patients with STS (cases) and 224 matched controls. Controls were patients operated for appendicitis. To estimate the risk of STS in a general population exposed to relatively low levels of dioxins via food, individual dioxin concentrations were analysed from subcutaneous fat samples by gas chromatography-mass spectrometry. **RESULTS:** The average (range) exposure to dioxins was 33.4 (4.4 to 145.5) ng/kg (WHO-TEq in fat). No increased risk associated with increased dioxin concentration was observed. In contrast, the highest risk of STS was found at low levels of dioxin. Odds ratios (compared with the lowest quintile of dioxin, median 11.5 ng/kg WHO-TEq in fat) varied from 0.39 (95 % CI 0.16 – 0.98) to 0.66 (95 % CI 0.22 – 1.99). The result was unaffected by studied confounders and the findings were similar for different STS subtypes, age groups and study areas. **CONCLUSION:** The results imply that STS risk is not increased by higher dioxin concentrations, and there may even be a negative association between dioxins and STS risk at the present population levels. If dioxins cause STS at high occupational doses, the result challenges the use of linear extrapolation models from high to low doses in dioxin carcinogenicity. The study was funded by the European Commission, Contract No. ENV-CT96-0336.

10922 - Organochlorine Levels in Adipose Tissue and Non-Hodgkin's Lymphoma.

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Serum or adipose tissue organochlorine (OC) levels (PCBs, DDT, and chlordanes) have been associated with the risk of non-Hodgkin's lymphoma (NHL) in several recent, but small, case-control studies. We conducted a case-cohort analysis of the hypothesized OC-NHL association using data from the U.S. EPA's National Human Adipose Tissue Survey (NHATS), a national survey of autopsy diagnoses and specimens assembled from pathologists in 47 urban areas. Specifically, from 1969 to 1983, postmortem diagnoses were recorded for approximately 15,000 individuals (infants, children, and adults) and their adipose tissue OC levels were measured using packed column gas chromatography/electron capture detection analysis. We identified individuals with measures of adipose PCBs, DDTs, or chlordanes. After excluding those with postmortem diagnoses of wasting diseases, severe malnutrition, or acute pesticide poisoning, adults (≥ 20 years) with ICD codes at autopsy for NHL were identified (n=203). Controls (n=8,688) were identified by excluding adults with other cancers. Associations were assessed with logistic regression modeling after adjustment for age at death and gender. The median ages for cases and controls were 58 and 56 years, respectively. There was no significant association of either PCBs or DDT-related compounds with increased risk of NHL. Adipose tissue levels of heptachlor were largely nondetectable but other chlordanes were significantly associated with NHL risk. Specifically, compared to adults with nondetectable levels, those with adipose transnonachlor concentrations greater than the median (>0.09 ppm wet weight) had 1.8 (95% CI = 1.3, 2.7) increased odds of NHL. Similarly, compared to adults with nondetectable oxychlordanes levels, those with adipose tissue concentrations greater than the median (>0.09 ppm wet weight) had 2.0 (95% CI = 1.4, 3.0) increased odds of NHL. These results support an association of exposure to chlordanes, but not PCBs or DDT, with significant increased odds of NHL among a general population sample of deaths for which autopsies are performed. Chlordanes were used in U.S. agriculture until 1983, with continued extensive use as termiticides until their final U.S. ban in 1988. Chlordanes are resistant to environmental degradation as a consequence of which the general population continues to be at risk for exposure via contaminated soils and treated homes. Their potential association with an increasingly common group of cancers, NHL, is notable and warrants further study.

Temporal Trends in Human TCDD Body Burden: Decreases Over Three Decades and Implications for Exposure Levels

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The objective of this work was to determine whether conclusions could be reached about the temporal trends in exposures to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) from the data on lipid levels of TCDD in the general population. Data were compiled from the literature for the United States, Canada, Germany, and France over the past 30 years. Mean lipid levels of TCDD exhibited a steady decrease by nearly a factor of 10 over this time period, and current lipid-adjusted TCDD levels are about 2 ppt. A simple one-compartment pharmacokinetic model was used to simulate what temporal trends in exposure would result in the observed temporal trends in lipid levels of TCDD in humans over the past thirty years. The results indicated that absorbed intake levels of TCDD must have decreased by more than 95 percent from levels in 1970 to result in the observed decrease in human lipid levels, with the bulk of this decrease occurring before 1980. Based on this modeling and the pharmacokinetic properties of TCDD in humans, we conclude that mean levels of TCDD in the general population are likely to decrease further over the next 15 years, to between 0.5 and 1 ppt, even if intake levels do not decrease further. Fewer data over a shorter time period are available for other dioxin and furan congeners in human lipid, but these data indicate substantial decreases as well. Food sampling data support these trends. If the trend in general population levels for other dioxin and furan congeners is similar to that of TCDD, general population toxic equivalency (TEQ) lipid levels may currently be 10-fold or more lower than in 1970, and still decreasing.

A CASE-CONTROL STUDY OF BIRTH DEFECTS, CANCER AND DIOXIN EXPOSURE

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Background: Dioxin is a ubiquitous contaminant that persists in the environment and accumulates in the food chain. In the family of dioxin compounds, 2,3,7,8 tetrachloro-dibenzo-*p*-dioxin (TCDD) is the most toxic member. In Vietnam, between 1962 and 1971, the U.S. military used approximately 72 million liters of herbicides for destruction of crops and defoliation. A review of epidemiological studies associating health outcomes with TCDD exposure suggests an increased risk of cancer, diabetes, cardiovascular disease, chloracne and unfavorable pregnancy outcomes (e.g., neural tube defects or NTDs in offspring of exposed fathers).

Hypothesis: This study hypothesizes that the birth of an NTD-affected offspring implies increased susceptibility to TCDD toxicity in Vietnam veterans. Thus, a Vietnam veteran father of an NTD-affected offspring may be at increased risk for cancer.

Methodology: Over 40 U.S. vital statistics offices provided a list of fathers of NTD offspring (including father's name, age, date of birth, state of residence) abstracted from death, fetal death and birth certificates. To identify potential Vietnam veteran fathers of NTD offspring, we cross-linked the vital statistics list of fathers with a nation-wide computer file of 2.8 million Vietnam veterans. We sent surveys of medical and military histories to over 4,000 matching veterans. Male Vietnam veterans who reported the birth of at least one NTD offspring and a personal history of one or more medically diagnosed cancers served as cases. Male Vietnam veterans who reported at least one offspring but no personal cancer history served as controls. We developed an exposure index to TCDD-contaminated herbicides based on survey responses regarding branch and unit of service, dates of service, Military Occupational Specialty Code, rank, and locations of service in Vietnam. We are also analyzing blood samples for TCDD levels. We are examining the reported birth of an NTD-affected offspring as a potential modifier of the association between TCDD exposure and cancer.

Analysis: A multiple logistic regression model is being constructed to calculate the log odds of cancer against the index level of exposure to TCDD-contaminated herbicides, the birth of an NTD-affected offspring, and a TCDD*NTD offspring interaction term. The model adjusts for potential confounding variables including age, race/ethnicity, smoking, and family history of cancer.

Preliminary Results: Thus far, 15 of the 129 veteran fathers of an NTD-affected offspring reported a personal history of a cancer diagnosis. Of the 1,592 veteran fathers of unaffected offspring, 157 reported a cancer diagnosis. TCDD exposure measures (including blood TCDD levels) and potential confounders are being incorporated into the logistic modeling to derive more valid estimates of the independent and joint effects of TCDD exposure and birth of NTD-affected offspring on cancer risk.

**SPERM CHANGES IN HUMAN EXPOSED TO POLYCHLORINATED
BIPHENYLS AND DIBENZOFURANS**

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Exposure to the ubiquitous environmental contaminants of certain dioxins, polychlorinated biphenyls (PCBs) and similar chemicals is known to cause altered semen quality in animal studies. This study examined individuals from a major PCB exposure episode occurred in Taiwan, referred to as "Yucheng" (oil-disease) exposure. Over 2,000 Taiwanese during 1978-79 ingested rice oil accidentally contaminated with PCBs and its pyrolytic product, mainly polychlorinated dibenzofurans (PCDFs). In 1993, a closely matched control group was identified for Yucheng individuals for long-term follow-up. To determine whether exposure to PCBs/PCDFs can alter reproductive function in the sexually mature men, exposed men 30 years or older as well as their controls were contacted for medical examination in 2000. Informed consent was obtained. Interview, physical examination, and semen collection by masturbation were done. After liquefaction for 15 min at 37 °C, 20 microliter of semen was used for morphology analysis and sperm motion. For morphological evaluation, a total of 200 cells were scored according to World Health Organization (WHO) guidelines at 400x magnification. The samples were held in a Makler chamber (Sefi Medical Instruments, Israel) for sperm motility measurements. The percentage of motile sperm was counted at 100x with the aid of a grid on an eye piece graticule. Computer-assisted sperm analysis (CASA) was obtained for motility parameters using the Hamilton Thorn Research motility analyzer (version 10 HTM-IVOS Specification, Beverly, MA, USA): curvilinear velocity (VCL, $\mu\text{m/s}$), average path velocity (VAP, $\mu\text{m/s}$), straight line velocity (VSL, $\mu\text{m/s}$), straightness (STR, VSL/VAP), linearity (LIN, VSL/VCL), amplitude of lateral head

displacement (ALH, μm), and beat cross frequency (BCF, Hz). Human sperm-hamster oocyte penetration was assessed using the WHO methods later, after frozen in liquid nitrogen and re-thaw. All interview, examination, and laboratory tests were done in a blinded manner. A total of 27 men prenatally exposed to PCBs/PCDFs, and 27 unexposed matched controls volunteered to participate in the study. The age (42 vs 45 years), body weight, as well as the percent of smokers were not different in the exposed and control men. Age of first ejaculation or nocturnal emission, frequency of erection, and period of abstinence from ejaculation were not different between exposed and control men. Semen volume, sperm concentration, percent of normal morphology, percent of motile sperm were not different between exposed and control men. However, 4 (14.8%) of the 27 exposed men were found to have oligospermia (sperm count of less than 20 million/ml) as compared to 0 of the controls. Among motile sperm, exposed and control men had similar velocity and beat cross frequency as measured by CASA. However, reduced hamster oocyte penetration was found in sperm of PCB/PCDF-exposed men (20.7%, vs. 34.9% in controls, $p=0.032$), suggesting reduced fecundability caused by exposure to PCBs/PCDFs.

Potential for exposure to dioxins and furans when recycling sewage biosolids on agricultural land

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Objectives: Increasingly, biosolids (sewage treatment plant sludge) are being proposed as a valuable source of nutrients for agriculture, forestry, land reclamation and horticultural activities. Because most human exposure to polychlorinated dibenzo-*p*-dioxins and dibenzofurans (dioxins and furans) comes from food, it is essential to consider the potential for increased exposures when agricultural products are grown on land treated with biosolids. A systematic review of the published scientific literature was conducted to examine the movement of dioxins and furans to food, either directly via plant foods grown in soil amended with biosolids, or indirectly through the milk or meat of animals grazing on or fed from fields amended with biosolids. **Methods:** The literature was identified using a search of major medical, toxicological, agricultural, and environmental science bibliographic databases, and was supplemented with literature gathered by a Canadian regulatory agency. Additional papers were identified in a review of the references cited in the articles already collected. **Results:** Dioxin and furan levels in municipal sewage sludge ranged from 0.0005 to 8300 pg TEQ/g ($\mu = 81.4$ pg TEQ/g), with a declining trend over time. Background levels in soil ranged from 0.003 to 186 pg TEQ/g ($\mu = 3.7$ pg TEQ/g). In sludge-amended soil samples, dioxin and furan levels ranged from 1.4 to 15 pg TEQ/g, ($\mu = 5.8$ pg TEQ/g); those studies that measured levels before and after biosolids treatment showed a 1.4- to 17-fold increase in soil concentration after biosolids application. Weak positive relationships between soil contamination levels and resulting concentrations in plants were found for unpeeled root crops, leafy vegetables, tree fruits, hay and herbs, but not for peeled root crops, peas and beans, or grass. Stronger relationships were observed for plants of the cucumber family. In all cases, very large increases in soil concentration were required to achieve a measurable increase in plant contamination. A stronger positive relationship than that seen for crops was observed between dioxins and furans in feed and the levels found in cattle tissue. Although dioxins and furans are known to be excreted in milk, no association was found between feed contamination and the levels measured in milk. **Conclusions:** The application of biosolids to agricultural land used for certain crops (leafy vegetables, tree fruits, harvested forage crops) is not expected to present a serious risk to human health, but the use of biosolids on land used to grow crops of the cucumber family or to graze animals may result in increased human exposure to dioxins and furans.

11123 - THE HEALTH EFFECTS OF WORKERS EXPOSED TO FLY ASH AT A MUNICIPAL INCINERATOR IN JAPAN.

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It is well known that the fly ash of municipal solid waste incinerator (MSWI) contains heavy metals, dioxins, polyaromatic hydrocarbons, and other organic materials. The workers at MSWIs have a certain possibility to absorb such materials chronically via exposure to fly ash. The purpose of this paper is to investigate the health effects of chronic exposure to fly ash on MSWI workers. The subjects were 81 male workers (mean age 42.7) from four MSWIs in the same city. Written informed consents were given from all subjects. Questionnaires of health related habits, eating habit, and subjective symptoms were filled before the day of examination. Blood and urine samples were corrected in the morning of each study day. Occupational health doctors interviewed each subject about his job history, evaluated the exposure to fly ash, and calculated its total duration. The subjects were classified into four groups; those were Long duration of exposure to fly ash, Short duration of exposure to fly ash, Limited exposure to fly ash, and Control. Blood chemistry, oxidative stress markers, immunological markers, sex related hormones, and neurobehavioral functions were measured and compared among the groups. There were no significant difference in health related habits, eating habit, incidences of subjective symptoms, and measured variables between 4 groups except an oxidative stress marker and neurobehavioral functions. The oxidative stress marker, which is urinary 8OhdG, and 4 T-scores of POMS (Profile of Mood State), which are Tension-anxiety, Depression-dejection, Fatigue-inertia, and Confusion-bewilderment, increased according to the elongation of the exposure duration. Grip strength of non-dominant side and the ability of short-term memory using a portion of the Symbol Digit Modalities Test increased in Long-exposure-duration group. Although we could not conclude that a certain contaminant of fly ash was responsible to these effects, there may be neuropsychological effects on workers exposed to fly ash.

	T-scores of POMS				
	N	10y<	10y>	Limited	Control
		30	22	19	10
Tension-anxiety		51.6±11.5	48.2± 6.9	42.8± 7.1	44.6± 4.2
Depression-dejection		55.8±11.8	50.5± 9.5	46.7± 7.2	48.3± 3.5
Anger-hostility		53.3±12.2	50.7±11.3	47.6± 8.2	48.8± 5.5
Vigor		47.1± 8.9	45.1± 9.9	48.1±10.0	54.4± 6.1
Fatigue-inertia		51.5±12.6	48.2± 9.2	42.9± 7.4	44.5± 5.0
Confusion-bewilderment		55.2±11.7	52.1± 9.1	44.5± 8.1	47.7± 5.7