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COMPARISON OF THE CALUX BIOASSAY AND ID-HRGC/HRMS AS MEASURES OF DIOXIN TOXIC EQUIVALENTS IN SERUM

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Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs) including 2,3,7,8, tetrachlorodibenzo-*p*-dioxin (TCDD), dibenzofurans (PCDFs), and biphenyls (PCBs) constitute a group of polyhalogenated aromatic hydrocarbons that are persistent and widespread environmental contaminants.[1] Since TCDD and other dioxin-like compounds exist as complex mixtures of various congeners throughout the environment, calculating total TCDD toxic equivalent (TEQ) concentrations has become widely accepted as the most relevant exposure measure in studies of health effects of dioxins and dioxin-like compounds. [2, 3] In particular, in background-exposed populations, these other congeners may contribute a large portion of TEQ relative to TCDD. To date, analytical methods to quantify exposure to these chemicals in biologic samples have included very sensitive and specific techniques such as high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS). However, these methods are time-consuming and expensive, require large sample volumes and extensive sample clean-up.

An AhR-dependent recombinant bioassay was developed that reportedly can measure the total TEQ concentration of a complex mixture in human serum, without extensive cleanup and chemical analysis procedures. It requires a relatively smaller volume of serum (<5ml) and less time to complete. The chemical-activated luciferase gene expression (CALUX) bioassay measures the ability of a chemical mixture to activate AhR-dependent gene expression in cells in culture.[4-6] It is based on AhR-mediated firefly luciferase expression in genetically modified cell lines. To date, limited validation studies of the CALUX results against HRGC/HRMS measurements have been completed.[7-9] Despite this, the CALUX bioassay has already found application in studies of health outcomes.[10-13]

The purpose of this study is to examine the validity of the CALUX bioassay to measure TEQ in a background-exposed population. We compare the CALUX-TEQ with the "gold standard" WHO-TEQ, derived from Isotope Dilution-HRGC/HRMS measurements in a sample of Italian women.

Methods and Materials

Study Population Study participants included women who participated in an endometriosis case-control study at Desio Hospital, Italy, about 25 kilometers north of Milan.[14] To be eligible, a woman had to be 20 to 50 years old, and scheduled to undergo laparoscopy for pelvic pain, infertility, tubal ligation, or adnexal/uterine mass at the Hospital of Desio, between July 1998 and December 1999. As part of the study, women were interviewed by trained nurse-interviewers and asked about sociodemographic information, personal habits, and reproductive history. A total of 78 women agreed to provide a 70-mL blood sample.

HRGC/HRMS Chemical Analysis Informed consent was obtained and serum samples were aliquoted and stored at -20°C at Hospital of Desio. For each woman, a 15-mL serum sample was sent to the U.S. Centers for Disease Control and Prevention (CDC) for analysis of PCDDs, PCDFs, and PCBs by high-resolution mass spectrometry methods.[15] Serum samples were analyzed for the 17 PCDD and PCDF toxic congeners and coplanar PCBs (PCB 77, 81, 126, 169). In addition, 36 PCBs were measured including the mono-*ortho* PCBs (PCB 105, 118, 156, 157, 167). Serum total lipids were calculated using an enzymatic 'summation' method.[16] Values were reported on a lipid-weight basis in parts per trillion (ppt) or parts per billion (ppb). To aid in the statistical analysis of data from truncated frequency distributions, quantifiable results less than the respective method detection limits were reported when observed for individual analytes. For individual analyte data with non-detectable values, a value equal to one-half the detection limit was assigned.[17]

International toxicity equivalents were calculated separately for PCDDs (PCDD-TEQ), PCDFs (PCDF-TEQ), coplanar PCBs (cPCB-TEQ), mono-*ortho* PCBs (oPCB-TEQ) and Total TEQ, using the TEF scheme of the WHO.[2] Non-detectable data were excluded, however, from TEQ calculations.

CALUX bioassay Based on the distribution of Total TEQ data derived from the HRGC/HRMS data for the 78 women, we selected a total of 32 serum samples (4mL) to be sent in 2 shipments to Xenobiotic Detection System, Inc (Durham, North Carolina) for CALUX bioassay analysis. We randomly selected eight samples per quartile of HRGC/HRMS-derived Total TEQ. Those responsible for the CALUX bioassay were blind to the HRGC/HRMS chemical analysis results.

Statistical Analysis Statistical analysis was performed using STATA 7.0.[18] The distributions of individual PCDD, PCDF, and PCB congener data, as well as TEQ data, were initially examined graphically and with standard descriptive statistics. Analysis of variance was used to examine the relation of TEQ levels with covariates. Because these data were not normally distributed, the relationship between PCDD-TEQ, PCDF-TEQ, PCB-TEQ, Total TEQ, and CALUX-TEQ was examined by nonparametric correlation analysis.

Results and Discussion

The total TEQ for this study population of women in Northern Italy averaged 25.3 ppt, lipid-adjusted (range: 0 - 88.3), comparable to other parts of Europe. TCDD was detected in 19 percent of the 73 samples with a median of 1.6 ppt, lipid-adjusted (range: 0.7 - 18.9). The

CALUX TEQ for the 16 women completed thus far averaged 11.0 ppt, lipid-adjusted (range: 0 - 61.8). We will present the results of correlation analysis between CALUX-TEQ and measures of TEQ, derived from HRGC/HRMS data in this sample of Italian women with background exposure to dioxin and dioxin-like compounds.

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