RELATIONSHIPS BETWEEN EXPOSURE TO DIOXIN-LIKE CHEMICALS, TESTOSTERONE LEVELS, AND SEX OF THE CHILDREN OF PESTICIDE APPLICATORS

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Introduction

A cohort of pesticide applicators from the Red River Valley of Northwest Minnesota is the subject of a continuing epidemiological study to evaluate health effects of exposure to pesticides. These workers have a long-term history of applying insecticides, herbicides, and fungicides. The total cohort contains 1067/ 1397 men (state certified pesticide applicators) who were contacted for this study. Approximately 80% were married or had a significant other and eight hundred thirty five (835) women who were the spouse of the pesticide applicator (farmer) have also volunteered for the survey. A sub-cohort of 144 men volunteered for a study to investigate the relationship of blood testosterone levels to the sex outcome of their children and any association with exposure to pesticides. Previous work has suggested that there may exist a relationship between the sex outcome of offspring and parental hormonal status at the time of conception (1, 2). Follow-up of the Seveso Cohort in Italy that was exposed to high levels of dioxins has also reported a linkage with altered sex ratios in the children of highly exposed adults (3,4,5). These observations caused us to investigate the relationships between serum testosterone levels of men, the sex of their children, and the extent of exposure to dioxin-like chemicals that may contaminate herbicides.

We have begun characterizing the exposure scenario in the Red River Valley Cohort. Twelve commercial chlorophenoxy mixtures that had been used during herbicide applications were analyzed by DIPS-CALUX, a reporter gene bioassay that responds to dioxin-like chemicals with production of luciferase. Ten of the twelve commercial preparations of chlorophenoxy

herbicides were highly contaminated with dioxin-like chemicals. In addition, analysis of blood levels of dioxin-like chemicals in these pesticide workers with the DIPS-CALUX bioassay demonstrated significantly elevated concentrations of dioxin-like chemicals.

Materials and Methods

Cohort inclusion criteria and survey

One hundred and forty four (male) subjects agreed to take part in this study. Criteria for inclusion were no chronic disease or medication use, use of fungicides at least 5 days/year or use of herbicides 6-10 days or more per year but no use of fungicides. Of the 144 subjects in the study, 96 had fathered a child. Phone surveys were conducted with the pesticide applicator and their spouses and information gathered on pesticide use, health, and reproduction, including birth date and sex of offspring fathered by the subject.

Blood Specimen Collection and Data Analysis

Blood specimens were collected in July and October of 1998 at local clinics in the Red River Valley between 7:00 AM and 11:00 AM and subjects were instructed to fast prior to blood draw visits. Blood was centrifuged and serum shipped to the University of Minnesota. Total testosterone was measured using a solid-phase radioimmunoassay (catalog number TKTTI; Diagnostic Products Corporation, Los Angeles, CA). The mean values of the July and October blood draw were calculated for each individual. Applicators were assigned to quartiles based upon their mean total serum testosterone concentrations. Statistical analysis of the relationship of serum testosterone and sex ratio (male/female live births) was analyzed based on quartiles with a two-tailed Fisher's exact test.

DIPS-CALUX bioassay of dioxin-like contaminants in Herbicide Samples and Serum

Archived samples of chlorphenoxy herbicides that were widely used in the Red River Valley of Minnesota were sent from the University of Minnesota to XDS laboratory for analysis. A sample of 0.5 g of each of these herbicides was dissolved in hexane and processed through our patent pending procedure to specifically isolate halogenated dioxin/furans and halogenated biphenyls (6). Serum samples were sent from the University of Minnesota and similarly processed for isolation of halogenated dioxin/furan isolation and halogenated biphenyl isolation. The dioxin-like TEQ activity was estimated following application of these extracts to our genetically engineered cell line that contains the firefly luciferase gene under trans-activational control of the aryl hydrocarbon receptor (7). Dioxin-like TEQ activity was estimated from a standard curve of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin versus induction of luciferase activity.

Results and Discussion

Testosterone Quartiles and Sex Ratio Analysis

Shown in Table 1 are the results of the Testosterone Quartiles and Sex Ratio Analysis for pesticide applicators under study in the Red River Valley.

Quartile	Total Testosterone Range (ng/ml)*	Age Mean ±» SE	No. of Fathers Children	No. of Male Children	No. of Female	Sex Ratio
1st	≤ 3.73	46.0 ±1.7	24	29	38	0.76
2nd	3.74-4.37	45.0 ±1.6	24	30	42	0.71
3rd	4.38-5.63	49.4 ±2.2	24	33	34	0.97
4th	>5.64	44.0 ±2.3	24	43	27	1.59
Total of all Quartiles			96	135	141	0.96
NHS**				2,026,854	1,932.563	1.049

*normal clinical range 3.00-12.00 ng/dl; coefficient of variation = 6.7%
** National Health Statistics for 1999 (National Center for Health Statistics, 2002)

Significant differences exist (two-tailed Fisher's exact test) in the sex ratios observed between the 1st and 4th quartiles (P=0.04), 2nd and 4th (P=0.02), and combined 1st + 2nd compared to combined 3rd + 4th (P=0.04). These results indicate that the sex ratio of children borne to pesticide applicators is related to the applicators' total serum testosterone levels.

Notably, there is a significant difference in the percentage of fungicide-exposed subjects across quartiles. During the 1998 season, 47.9 % of subjects in the combined 1st + 2nd quartiles used fungicides vs. 27.1 % in the combined 3rd + 4th (p=0.035). Historically, 72.9 % of subjects in the 1st + 2nd quartiles had used fungicides during their lives vs. 45.8% in the 3rd +4th (p=0.007). The most commonly used fungicides in this region of Minnesota are of the organotin class. It is not known whether the association between lower testosterone levels and lower sex ratios reflect a cause and effect, or if they may be a parallel response to a common toxicant mechanism.

Analysis of quantitative pesticide use data revealed a general trend of greater pesticide use by subjects in the 1st and 2nd quartiles compared to the 3rd and 4th. Information recorded by subjects in the written survey indicated that subjects in the 1st and 2nd quartiles treated more acres per year (sum of all crop acres treated) on average with herbicides, insecticides and fungicides than those in the 3rd and 4th quartiles.

Exposure to dioxins and dioxin-like chemicals has been found to both lower testosterone and shift the sex ratio of children born to exposed male subjects (3,4,5). W.H. James (2) has suggested that exposure to dioxin, the pesticide dibromochloropropane (DBCP), borates, vinclozolin, and non-ionizing radiation can produce excesses of female offspring. James' hypotheses are potentially relevant and require further evaluation as chlorophenoxy herbicides are among the most commonly used pesticides in the Red River Valley region and can be contaminated by dioxin. It is known that subjects in the 1st and 2nd quartiles used more herbicides on average than those in the upper ones, so the potential exists for greater lifetime dioxin exposure in lower quartile subjects.

DIPS-CALUX bioassay of dioxin-like contaminants in Herbicide Samples and Serum

Twelve samples of chlorphenloxy herbicides used in spraying operations were sent to Xenobiotic Detection Systems for DIPS-CALUX bioassay analysis of TEQ contamination and the results are presented in Table 2.

Table 2. Contamination of Commercial Chlorphenoherbicides with Dioxin-Like TEQ Activity

Client ID #	TCDD TEQ pg/ml sample
1	26.2 ± 0.5
2	ND
3	6.0 ± 2.2
4	3392 ± 257
5	13.7 ± 2.6
6	1637 ± 212
7	800 ± 180
8	1668 ± 419
9	19.7 ± 6.4
10	51.4 ±5.4
11	1882 ± 311
12	ND

Five of the commercial herbicides demonstrated a high degree of contamination (parts per billion level of dioxin-like TEQ contamination). Five of the herbicide mixtures demonstrated trace contamination with dioxin-like chemicals (5-100 parts per trillion of dioxin-like TEQ contamination). Two of the herbicide mixtures demonstrated less than 1 part per trillion contamination with dioxin-like chemicals. These data suggest that some herbicide applicators could have been exposed to high levels of dioxin-like chemicals.

The data on serum samples suggest that indeed some of these applicators have significant exposure to dioxins-like chemicals. Determination of dioxin-like TEQ activity in blood of 4 of these pesticide applicators is shown in table 3.

Table 3. DIPS-CALUX Assay: Dioxin-like TEQ (= toxic equivalents) Activity in Serum Samples from Herbicide Applicators

ID #	TEQ Pg TCDD TEQ/g lipid	Years Herbicide Application	Avg Days/yr	Avg. Acres Treated	Chlorinated Herbicides
133	83.2	43	30	6477	A, B, C, D, F, H, J
351	57.6	19	30	4820	B, C, D, E, H, J
3205	204.9	10	60	21,609	A, C, F, J
3215*	90.44	35	7	2590	G, H, I

A – 2,4-D (chlorophenoxy)
B – Bronate (MCPA + Bromoxynil)
C – Curtail (3,6-dichloropicolinic acid + 2,4-D)
D – Cheyenne (MCPA + Fenoxaprop-P-ethyl + Tribenuron-methyl + Thifensulfuron-methyl)
E – Assure II (quizaflop-P-ethyl)
F – Tiller (2,4-D + MCPA + fenoxaprop-P-ethyl)
G – Carbyne (barban)
H – Hoelon (diclofop-methyl)
I – Dowpon (2,2-dichloropropionic acid)
J – Stinger (3,6-dichloropicolinic acid)

Conclusion

The data presented provide strong suggestive data that there may be a relationship between testosterone levels, sex of the children of these workers and the levels of exposure to dioxin-like chemicals. We are currently searching for funding to analyze the remaining blood samples within the cohort for dioxin-like activity in the blood of these workers. This would provide more data to help establish if there is a causal relationship to exposure and alterations in reproductive function. This cohort also may provide useful dosimetry to help determine the levels of dioxin in blood that may contribute to altered reproductive functions and alteration of hormonal homeostasis.

References

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