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Total Serum Testosterone and Gonadotropins in Workers Exposed to Dioxin

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Human reproductive endocrine data may be an important source of epidemiologic information in regard to the toxic potential of 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin). The association of serum dioxin with total serum testosterone, luteinizing hormone, and follicle-stimulating hormone was examined in 248 chemical production workers from New Jersey and Missouri plants and 231 nonexposed neighborhood referents who participated in a medical evaluation in 1987. In linear regression analyses, current serum dioxin was positively and significantly related to luteinizing hormone and follicle-stimulating hormone and inversely related to total testosterone after adjustment for potential confounders ($p < 0.05$). These trends were also apparent in logistic regression analyses, in which the authors examined the odds ratios of high luteinizing hormone (>28 IU/liter), high follicle-stimulating hormone (>31 IU/liter), and low testosterone (<10.4 nmol/liter) by serum dioxin quartiles. There was a greater prevalence of high luteinizing hormone among workers in the second (odds ratio (OR) = 1.9, 95% confidence interval (CI) 0.7–5.5), third (OR = 2.5, 95% CI 0.9–7.3), and fourth (OR = 1.9, 95% CI 0.7–5.0) quartiles of serum dioxin compared with referents. For follicle-stimulating hormone, the authors observed a greater prevalence of high follicle-stimulating hormone among workers in the fourth quartile (OR = 2.0, 95% CI 0.7–5.6) compared with referents. Similarly, the prevalence of low testosterone was two to four times greater among workers in the second (OR = 3.9, 95% CI 1.3–11.3), third (OR = 2.7, 95% CI 0.9–8.2), and fourth quartiles (OR = 2.1, 95% CI 0.8–5.8) than among referents. The trends observed in these data offer human evidence of alterations in male reproductive hormone levels associated with dioxin exposure. The results support the animal literature in which dioxin-related effects have been observed on the hypothalamic-pituitary-Leydig-cell axis and on testosterone synthesis. *Am J Epidemiol* 1994;139:272–81.

dioxins; FSH; LH; testosterone

Human risk assessment for 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin) exposure has been a topic of debate, owing in part to the large animal intraspecies differences in its toxicity and median lethal dose (LD_{50}), ranging from 0.6 μg/kg in the guinea pig to 5,000 μg/kg in the hamster (1–3). Many of the physiologic effects of dioxin in animals resemble those evoked by hyper- and hypohormonal states (4–7). Theoretically, dioxin's ability to affect endocrine organs and to modulate hormone receptors (8–11), particularly estrogen receptors, has important implications for understanding the toxicity of dioxin (12).

Human reproductive endocrine data may be an important source of epidemiologic information in regard to the toxic potential of dioxin. In laboratory animals, lethal levels of dioxin result in testicular hypoplasia and

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Abbreviations: CI, confidence interval; OR, odds ratio; SE, standard error.

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impaired spermatogenesis (13–16). At sublethal but overtly toxic doses, these effects have been observed in monkeys (17). Sublethal levels of dioxin also decrease seminal vesicle and ventral prostate weights and total plasma testosterone in rats (18).

In humans, accidental or high occupational exposure during manufacturing processes has been associated with complaints of impotency and loss of libido (19). In the US Air Force Health Study of Ranch Hand veterans who participated in the aerial spraying of Agent Orange in Vietnam, serum dioxin was significantly related to decreased testicular size, but trends observed for decreased serum testosterone levels were weak and nonsignificant (20). Additionally, serum dioxin appeared to be unrelated to sperm count, miscarriage, birth weight, and birth defects (21).

In the current report, we examine whether serum levels of dioxin are associated with serum testosterone and gonadotropin levels among men previously occupationally exposed to dioxin-contaminated chemicals and nonexposed neighborhood referents who participated in a cross-sectional medical examination.

MATERIALS AND METHODS

The study population of exposed workers was recruited from current and former employees of two plants of the 12 included in the National Institute for Occupational Safety and Health (NIOSH) cohort of workers exposed to dioxin (22). The two plants are located in Verona, Missouri, and Newark, New Jersey. Dioxin exposure resulted from the manufacturing of 2,4,5-trichlorophenol and its derivatives. Exposure to dioxin occurred between 1951 and 1969 at the New Jersey plant and between 1968 and 1972 at the Missouri plant. A cross-sectional medical study of workers from the two plants was conducted in 1987 (23, 24), 15–37 years since the last occurrence of occupational exposure to dioxin.

A total of 586 workers were identified. Of the 586 workers, 143 (24 percent) were deceased and 43 (7.3 percent) were not located, leaving 400 workers (68 percent) eligible for study participation. A total of 357 of the eligible workers (89 percent) completed an interview, of whom 281 (70 percent of the eligible or 48 percent of the original cohort) participated in the medical examination.

Neighborhood referents, with no prior employment history in facilities that produced phenoxy herbicides, were recruited by means of a random sampling procedure described elsewhere (25). Potential age- (within 5 years), race-, and sex-matched referents were identified by a door-to-door screening of each worker's census tract neighborhood. A total of 325 referents consented to an interview and 260 participated in the medical examination. A matched referent was obtained for 91 percent of the interviewed workers and for 76 percent of the workers who participated in the medical examination. We excluded all women (14 exposed, 17 referents) from the analyses. A total of 23 men were also excluded (11 exposed, 12 referents), because of conditions known to influence gonadotropin and/or testosterone levels: history of prostate cancer, thyroid or other hormone usage, and liver cirrhosis.

Trained interviewers administered a questionnaire on occupational histories. Different interviewers, blinded to occupational histories, administered questionnaires on medical, demographic, and life-style characteristics. The medical examination visit included the drawing of blood for determination of serum dioxin and hormone concentrations.

Determination of serum dioxin

Each participant in the medical study donated 10–50 ml of serum for determination of serum dioxin levels. The methods of collection and preparation, the analytic techniques, and quality control standards of the laboratory analyses have been presented elsewhere (24–27). Serum dioxin concentration was determined for all but eight of

the examined dioxin-exposed workers. One worker was excluded because of the poor patency of his veins, and seven other workers were excluded because the collected serum samples did not meet laboratory quality control standards. Serum dioxin levels were measured in a random sample of the examined referents (n = 99 or 38 percent). All serum samples of the nonexposed referents fell below 20 picograms/gram (pg/g) of lipid (lipid-adjusted). For the 161 referents whose serum level was not measured, the median level for the referents (6.08 pg/g) was assigned and used in the analyses. For 14 samples with serum levels below the limit of detection, the level of serum dioxin was calculated by dividing the limit of detection of each sample by the square root of 2, as described by Hornung and Reed (28).

Because current serum dioxin levels were measured 15–37 years after exposure ceased, each worker's past serum dioxin levels (at the time occupational exposure ceased) were estimated using a standard half-life decay equation (29). The equation takes into account the number of years since each worker was occupationally exposed to dioxin (30), and assumes a dioxin half-life of 7.1 years (31), a steady state dioxin background level of 6.08 pg/g (the median level for referents), and that no occupational dioxin exposure occurred after the termination of work. The half-life extrapolated values provide an indication of the extent of previous exposure among the workers.

Hormone assays

A single measurement of total serum testosterone, follicle-stimulating hormone, and luteinizing hormone was obtained from a morning blood draw. Serum concentrations for these hormones were determined using standard double antibody radioimmunoassay procedures. The laboratory standards for normal value ranges were 3–31 IU/liter for follicle-stimulating hormone, 5–28 IU/liter for luteinizing hormone, and 9.4–34.7 nmol/liter for total testosterone. The acceptable limit for the within- and between-assay variation for follicle-stimulating hormone, luteinizing hormone, and testosterone was set at 10 percent. No samples exceeded this limit.

Statistical analyses

We used multiple linear regression analysis to separately examine the association of serum dioxin with testosterone, follicle-stimulating hormone, and luteinizing hormone while controlling for potential confounders (32). In these analyses, serum dioxin, testosterone, follicle-stimulating hormone, and luteinizing hormone were handled as lognormal continuous variables.

In addition to linear regression analyses, we examined the distribution of high follicle-stimulating hormone, high luteinizing hormone, and low testosterone by quartiles of serum dioxin concentrations in multiple logistic regression analyses. The group of workers in the lowest exposure group had serum dioxin levels below 20 pg/g with a median of 10 pg/g, not unlike the comparison group of referents, who had dioxin levels below 20 pg/g with a median of 6.08 pg/g. For the remaining three groups of workers, serum dioxin levels were 20–75 pg/g, 76–240 pg/g, and 241–3,400 pg/g. The quartiles of half-life extrapolated serum dioxin levels were: <140 pg/g, 140–495 pg/g, 496–1,860 pg/g, and 1,860–30,000 pg/g. In these analyses, serum dioxin was handled as a continuous variable to examine trends and as a categorical variable for the presentation of adjusted odds ratios and 95 percent confidence intervals. The definitions of high follicle-stimulating hormone (>31 IU/liter) and high luteinizing hormone (>28 IU/liter) were based on the cutoff points associated with the upper end of normal values provided by the laboratory. These cutoff points coincided with the top eighth percentile of the luteinizing hormone and follicle-stimulating hormone distributions. To be consistent with the luteinizing hormone and follicle-stimulating hormone analyses, the definition of low testosterone (<10.4 nmol/liter) was also based on the cutoff point associated with the lowest eighth percentile of the testosterone distribution. For testoster-

one, however, the eighth percentile cutoff point was slightly higher than the lower end of the normal range (9.4 nmol/liter) provided by the laboratory.

The potential confounders examined included age (in years), body mass index (kg/m^2), diabetes mellitus (yes vs. no), current alcohol consumption (yes vs. no), race (white vs. other), and smoking status (smoker vs. nonsmoker). We included a variable in the multivariate model when the variable was significant at the $p < 0.10$ level or when the inclusion of the variable modified the regression coefficient of serum dioxin by more than 5 percent. To ensure comparability of the presented data, we retained a variable that was significant or a confounder in one analysis in all analyses of that particular hormone.

Because the use of matched analyses would have resulted in the exclusion of 28 percent of the examined workers and 23 percent of the examined referents, we used an unmatched design in our analyses. Unmatched analyses were also preferable as they allowed us to examine dose-response relations that would not have been possible in matched analyses because of the small numbers of matched pairs for any given exposure category.

RESULTS

The age of the study population ranged from 31 to 81 years, with a mean age of 55 years at the time of the medical examination. The distribution of smoking, alcohol consumption, race, age, and body mass index by category of serum dioxin is presented in table 1. The dioxin-exposed workers had been exposed for an average of 2.7 years (ranging between 1 and 18.5 years of exposure) between 1951 and 1969. Workers in the highest quartile of serum dioxin were significantly older (mean age, 61 years) than referents (mean age, 56 years, $p < 0.01$), and other exposed workers (mean ages, <54 years) (F test, $p < 0.01$). The prevalence of current smoking tended to decrease with increasing serum dioxin category, but the observed differences were not significant (chi-square $p = 0.14$). There were no trends observed for differences in race, alcohol consumption, or body mass index by category of serum dioxin. In all multiple regression analyses of follicle-stimulating hormone, we adjusted for age, current alcohol consumption, smoking, and diabetes mellitus. In all regression analyses of luteinizing hormone and total testosterone, we adjusted for age, current alcohol consumption, smoking, diabetes, and body mass index.

In linear regression analyses, current serum dioxin was positively related to follicle-stimulating hormone (serum exposure coefficient = 0.04, standard error (SE) = 0.02) and luteinizing hormone (serum exposure coefficient = 0.03, SE = 0.01), and inversely related to total testosterone (serum exposure coefficient = −0.02, SE = 0.01) ($p < 0.05$). Although statistically significant, the magnitude of the unit increase in gonadotropins or decrease in testosterone

TABLE 1. Distribution of demographic characteristics by serum 2,3,7,8-TCDD* category, New Jersey and Missouri dioxin-exposed production workers, 1987

Serum 2,3,7,8-TCDD category (pg/g)	No. of participants	Mean age (years)†	Mean body mass index (kg/m^2)†	% white	% smokers	% current alcohol drinkers
Referents						
< 20	231	56.0 ± 0.7	27.1 ± 0.3	88.7	32.8	74.9
Workers						
< 20	65	52.8 ± 1.3	27.7 ± 0.5	90.8	40.6	64.6
20–75	60	53.2 ± 1.3	27.2 ± 0.6	81.7	38.3	65.0
76–240	62	53.6 ± 1.3	28.2 ± 0.6	87.1	22.0	77.4
241–3,400	61	61.0 ± 1.3‡	27.6 ± 0.6	95.1	20.3	73.8

* 2,3,7,8-TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin.
† ± standard deviation.
‡ $p < 0.01$, F test.

for every unit increase in serum dioxin was small. To illustrate, a serum dioxin level of 2,980 pg/g predicted a mean luteinizing hormone that was 3.26 IU/liter greater, a mean follicle-stimulating hormone that was 2.5 IU/liter greater, and a mean testosterone that was 2.29 nmol/liter less than the predicted means associated with the background level of 6 pg/g (figure 1). To calculate the points depicted, we fixed diabetes and current cigarette smoking to no, current alcohol use to yes, body mass index to 27, and age to 55 years.

In the multiple logistic regression analyses, there was a greater prevalence of high luteinizing hormone among workers in the second (10.0 percent, odds ratio (OR) = 1.9, 95 percent confidence interval (CI) 0.7–5.5), third (9.7 percent, OR = 2.5, 95 percent CI 0.9–7.3), and fourth (13.1 percent, OR = 1.9, 95 percent CI 0.7–5.0) quartiles of serum dioxin compared with referents (6.5 percent). Although each group difference had wide confidence intervals that included one, the positive trend for high luteinizing hormone was significant (serum exposure coefficient = 0.21, SE = 0.01, p = 0.03) (table 2). For follicle-stimulating hormone, there was a greater prevalence of high follicle-stimulating hormone among workers in the fourth quartile (11.5 percent; OR = 2.0, 95 percent CI 0.7–5.6) compared with referents (6.1 percent). Each group difference had wide confidence intervals that included one and the overall positive trend was not significant (serum exposure coefficient = 0.17, SE = 0.10, p = 0.10) (table 3). The prevalence of low testosterone was 2–3 times as high among the second (11.7 percent vs. 4.8 percent, OR = 3.9), third (9.7 percent vs. 4.8 percent, OR = 2.7), and fourth (16.4 percent vs. 4.8 percent, OR = 2.1) quartiles of serum dioxin than among the referent group (table 4). Except for the second quartile, these group differences had wide confidence intervals. The overall trend was not significant (serum exposure coefficient = 0.15, SE = 0.10, p = 0.10).

Similar or slightly stronger trends were observed in the analyses that used half-life

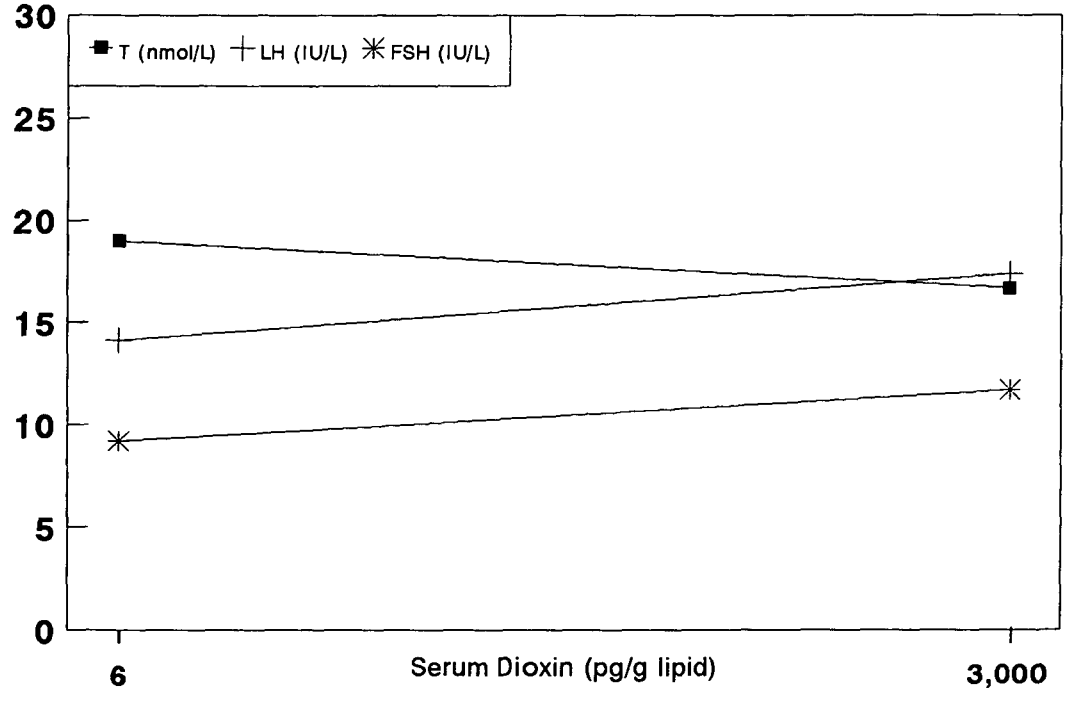

FIGURE 1. Predicted mean total testosterone (T), luteinizing hormone (LH), and follicle-stimulating hormone (FSH), by serum dioxin level (pg/g lipid), in New Jersey and Missouri dioxin-exposed production workers, 1987. (Predicted means are based on adjusted linear regression exposure coefficients.)

TABLE 2. Adjusted mean luteinizing hormone and adjusted odds ratios and 95% confidence intervals for high serum luteinizing hormone by serum 2,3,7,8-TCDD* category, New Jersey and Missouri dioxin-exposed production workers, 1987

Serum 2,3,7,8-TCDD category (pg/g)	No.	Adjusted† mean	SE‡	% High§,‖	Adjusted† odds ratio	95% confidence interval
Referents						
<20	231	16.5	1.05	6.5	1.0	
Workers	248	17.0	1.04	9.3	1.6	0.8–3.3
<20	65	15.3	1.06	4.6	0.8	0.2–3.0
20–75	60	17.3	1.07	10.0	1.9	0.7–5.5
76–240	62	17.1	1.07	9.7	2.5	0.9–7.3
241–3,400	61	19.1	1.07	13.1	1.9	0.7–5.0

* 2,3,7,8-TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin.
† Adjusted for age, body mass index, alcohol, smoking, and diabetes mellitus.
‡ SE, geometric standard error.
§ >28 IU/liter.
‖ Test for trend, p value = 0.03.

TABLE 3. Adjusted mean follicle-stimulating hormone and adjusted odds ratios and 95% confidence intervals for high serum follicle-stimulating hormone by serum 2,3,7,8-TCDD* category, New Jersey and Missouri dioxin-exposed production workers, 1987

Serum 2,3,7,8-TCDD category (pg/g)	No.	Adjusted† mean	SE‡	% High§,‖	Adjusted† odds ratio	95% confidence interval
Referents						
<20	231	10.9	1.07	6.1	1.0	
Workers	248	12.2	1.07	8.1	1.5	0.7–3.3
<20	65	11.2	1.09	6.2	1.1	0.3–3.9
20–75	60	13.0	1.09	8.3	1.7	0.5–5.4
76–240	62	12.0	1.10	6.5	1.7	0.5–5.6
241–3,400	61	12.8	1.10	11.5	2.0	0.7–5.6

* 2,3,7,8-TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin.
† Adjusted for age, alcohol, smoking, and diabetes mellitus.
‡ SE, geometric standard error.
§ >31 IU/liter.
‖ Test for trend, p value = 0.10.

extrapolated serum dioxin, to represent the serum dioxin levels at the time occupational exposure ceased (table 5).

DISCUSSION

In summary, current and half-life serum dioxin levels were positively and significantly related to luteinizing hormone and follicle-stimulating hormone and inversely related to testosterone in linear regression analyses adjusting for confounders. In the logistic regression analyses, there was a greater prevalence of high luteinizing hormone among workers in the second (OR = 1.9), third (OR = 2.5), and fourth (OR = 1.9) quartiles of serum dioxin compared with referents, and, although the confidence intervals were wide for each odds ratio, the overall test for trend was significant. Also, there was a greater prevalence of high follicle-stimulating hormone among workers in the fourth quartile (OR = 2.0), and a greater prevalence of low testosterone among workers in the second (OR = 3.9), third (OR = 2.7), and fourth quartiles (OR = 2.1) of serum dioxin compared with referents. Although the odds ratios had wide confidence intervals and the tests for trend were not significant, the direction of the associations observed in the multiple logistic regression analyses were consistent with the results obtained from the linear regression analyses.

As illustrated in figure 1, the magnitude of the predicted differences in mean gonado-

TABLE 4. Adjusted mean total testosterone (nmol/liter) and adjusted odds ratios and 95% confidence intervals for low total testosterone by serum 2,3,7,8-TCDD* category, New Jersey and Missouri dioxin-exposed production workers, 1987

Serum 2,3,7,8-TCDD category (pg/g)	No	Adjusted† mean	SE‡	% Low§,‖	Adjusted† odds ratio	95% confidence interval
Referents						
<20	231	18.2	1.04	4.8	1.0	
Workers	248	17.3	1.03	10.1	2.1	1.0–4.6
<20	65	18.7	1.05	3.1	0.9	0.2–4.5
20–75	60	16.8	1.05	11.7	3.9	1.3–11.3
76–240	62	17.2	1.05	9.7	2.7	0.9–8.2
241–3,400	61	16.4	1.05	16.4	2.1	0.8–5.8

* 2,3,7,8-TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin.
† Adjusted for age, body mass index, alcohol, smoking, and diabetes mellitus
‡ SE, geometric standard error.
§ <10.4 nmol/liter.
‖ Test for trend, p value = 0.10.

TABLE 5. Adjusted odds ratios (OR) and 95% confidence intervals (CI) for high follicle-stimulating hormone (FSH), high luteinizing hormone (LH), and low total serum testosterone by half-life extrapolated serum 2,3,7,8-TCDD* category, New Jersey and Missouri dioxin-exposed production workers, 1987

Half-life extrapolated 2,3,7,8-TCDD (pg/g)	No.	FSH†			LH†,‡			Testosterone†,‡		
		% High§,‖	OR	95% CI	% High§,‖	OR	95% CI	% Low§,‖	OR	95% CI
Referents										
<20	231	6.1	1.0		6.5	1.0		4.8	1.0	
Workers	248	8.1	1.5	0.7–3.3	9.3	1.6	0.8–3.3	10.1	2.1	1.0–4.6
<140	62	3.2	0.7	0.1–3.5	1.6	0.3	0.0–2.3	3.2	1.2	0.2–5.8
140–495	62	10.0	1 9	0.6–5.6	8.1	1.5	0.5–4.5	6.5	1.8	0.5–6.1
496–1,860	62	6.5	1.4	0.4–1.6	11.3	2.4	0.9–6.7	11.3	3.5	1.2–10.0
1,861–30,000	62	12.9	2.1	0.8–2.1	16.1	2.4	0.9–6.1	19.4	2.2	0.8–5.7

* 2,3,7,8-TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin.
† Adjusted for age, alcohol, smoking, and diabetes mellitus.
‡ Adjusted for body mass index.
§ High FSH (>31 IU/liter); high LH (>28 IU/liter); low testosterone (<10.4 nmol/liter).
‖ FSH test for trend, p = 0.12; LH test for trend, p = 0.04; testosterone test for trend, p = 0.12.

tropin and testosterone concentrations across the spectrum of serum dioxin exposure was small. However, the logistic regression analyses suggest that these minimal changes for the group as a whole indicate that a greater proportion of men fell above the eighth percentile for gonadotropins and below the eighth percentile for testosterone.

Because of the high variability in gonadotropins and testosterone levels in men, the reproductive significance of the observed differences in gonadotropins and testosterone by dioxin levels cannot be directly addressed by our cross-sectional one-sample data. Testosterone is necessary for the maintenance of libido and potency and for the initiation and maintenance of spermatogenesis, and high luteinizing hormone and follicle-stimulating hormone are correlates of low sperm concentrations (33). However, because the presence of both low testosterone and high luteinizing hormone was not observed in the same individuals (an indication of primary gonadal failure), we interpret these dioxin-related findings as being suggestive of more subtle alterations in gonadotropins and testosterone than that of primary gonadal failure. In the animal literature, dioxin-related effects have been attributed to dioxin's ability to influence the hypothalamic-pituitary axis and testosterone synthesis.

In rats, dioxin decreases testosterone levels (34, 35). There is no evidence that the decrease in testosterone is a result of an enhancement in testosterone catabolism or

excretion, but there is evidence that dioxin decreases testosterone synthesis (36). Two mechanisms by which dioxin may reduce testosterone synthesis have been postulated: a dioxin-induced decrease in testicular cytochrome P450scc activity and/or an impairment in the ability of luteinizing hormone receptor binding to initiate the mobilization of cholesterol to cytochrome P450scc (37). Both postulates are plausible given the documented dioxin effect on the P450 system (38) and dioxin's ability to modulate hormone receptors (8–12). The dioxin-related decrease in total plasma testosterone in rats parallels a reduction in testicular microsomal cytochrome P-450 and its dependent enzymes, 17-hydroxylase and 17,20-lyase (38), enzymes in the biosynthetic pathway between pregnenolone and testosterone. In addition, there is evidence that dioxin may decrease testosterone levels by decreasing the production of pregnenolone from cholesterol (37). In laboratory rats, the dioxin-related decrease in testosterone is not accompanied by a compensatory increase in plasma luteinizing hormone (37, 39). In addition, dioxin has been shown to reduce the responsiveness of the pituitary gland to testosterone (40) and of the Leydig cells to luteinizing hormone stimulation (41).

Our findings in the occupationally exposed workers are plausible given the pharmacologic and toxicologic properties of dioxin. The observed associations in these data may reflect multiple pathways of dioxin activity, including a potential effect on testosterone synthesis and impaired pituitary and Leydig cell responsiveness. The common mechanism responsible for these multiple effects may lie in dioxin's ability to influence hormone receptors. The aromatic hydrocarbon (Ah) receptor, to which dioxin binds to trigger its toxic effects, resembles steroid hormone receptors in both structure and mode of action (42, 43). Studies suggest that dioxin modulates hormone receptors, including estrogens (8, 9), prolactin, and its own Ah receptors (44, 45). The effect of dioxin on testosterone receptors, however, has not been examined.

The US Air Force Ranch Hand Study provides the only other human data available on serum dioxin and testosterone (21). Twenty-six percent of the Ranch Hand Veterans had current dioxin levels at the time of measurement that were greater than 33.3 pg/g and half-life extrapolated serum dioxin levels that were greater than 390 pg/g (21). In contrast, 63 percent of our exposed men had serum dioxin levels that were greater than these concentrations. Ranch Hand Veterans with current serum dioxin levels exceeding 33.3 pg/g were reported to have a nearly fourfold excess in unspecified testicular abnormalities relative to individuals with background serum dioxin levels after adjusting for potential confounders (relative risk = 3.8, 95 percent CI 1.7–8.6). The same exposure group had a lower mean total serum testosterone level than the nonexposed comparison group, but the difference was considered clinically and statistically nonsignificant after controlling for potential confounders including percent body fat. No association between serum dioxin and follicle-stimulating hormone was observed in the Ranch Hand population, and luteinizing hormone was not examined.

Our ability to draw inferences from the available data on our study population is limited by the cross-sectional nature of the data, and the pulsatile nature of gonadotropins and testosterone measurements. Observed differences based on one measurement are likely to be more conservative than those based on multiple measurements. For example, the lack of a monotonic dose-response relation by quartiles of serum dioxin could reflect the widely fluctuating nature of the parameters examined. One may argue, on the other hand, that the pulsatile nature of the data may result in spurious differences, but we find it difficult to explain why spurious differences would be more likely to occur among the higher exposure categories. Ideally, endocrine challenge tests, with multiple gonadotropin and testosterone measurements, would provide a better assessment of possible dioxin-related effects. The high attrition rate, with 31 per-

cent of the original cohort deceased or lost to follow-up and only 48 percent of the original cohort participating in the medical examination, poses a methodological problem in drawing inferences from these data. In addition, the possibility of reverse causality, where gonadotropin and testosterone levels may influence dioxin measures, cannot be ignored. Nonetheless, the available data do provide human evidence indicating subtle alterations in male reproductive hormone levels associated with dioxin exposure.

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