TECHNICAL REPORT:

HUMAN REPRODUCTIVE ENDOCRINE EFFECTS OF OCCUPATIONAL SOLVENT EXPOSURE

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28 February 2000

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Grant Numbers: 5 R03 OH03468-01, 5 R03 OH03468-02

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ABSTRACT

Although organic solvents are widely used in many different industries, there is very little data on the effects of exposure to all but a few solvents on male reproductive health. Painters comprise one large occupational group with significant solvent exposure that has not been well-studied in this regard. We therefore chose to study fertility and reproductive endocrine function in male painters. We assessed time-to-pregnancy (fecundability, an indicator of fertility) using a retrospective cohort design and cross-sectionally measured reproductive hormone concentrations in a group of painters compared to a control group of carpenters and to a group of millwrights with intermediate solvent exposure. Detailed occupational, exposure, medical, and time-to-pregnancy histories were obtained by telephone interview using previously validated instruments. Blood samples were obtained, and serum luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone concentrations were determined by immunoassay. Whole blood lead was also measured. Using Cox regression analysis we found that time-to-pregnancy was non-significantly longer in the painters and millwrights than the carpenters (relative probability of pregnancy in the painters and millwrights combined compared to the carpenters of 0.76, 95% CI 0.45-1.27). In the multivariate analysis time-to-pregnancy was significantly affected by age of the father at the time of the pregnancy of interest and whether the couple was trying to become pregnant. Using analysis of covariance, LH, FSH, and testosterone concentrations did not differ by exposure group, but LH varied significantly with body mass index and education, and testosterone varied significantly with body mass index. The non-significantly reduced fecundability in the two solvent-exposed groups suggests the need for further study of fertility in solvent-exposed men.

SIGNIFICANT FINDINGS

1. Fecundability was non-significantly decreased in solvent-exposed painters and millwrights compared to unexposed carpenters.

2. Fecundability decreased significantly as the father's age at the time of the pregnancy of interest increased and increased for couples that were trying to become pregnant.

3. LH, FSH, and testosterone concentrations did not vary by solvent-exposure group.

4. LH and testosterone concentrations were significantly affected by body mass index, a measure of adiposity. Total years of education, an indicator of socioeconomic status, had a significant effect on LH concentration.

USEFULNESS OF FINDINGS

This was only the third study of which we are aware that examined time-to-pregnancy in solvent-exposed men. The results of the two previous studies were contradictory, with one suggesting decreased fecundability in a heterogeneous group of men with occupational solvent exposure, and the other finding no effect on fecundability of toluene exposure in male printing workers. Our finding of 24% lower fecundability in solvent-exposed painters and millwrights

than carpenters, although not statistically significant, supports the need for further study of fertility in solvent-exposed men. A follow-up study would require a study population of about 350 exposed subjects and an equal number of unexposed subjects to detect a 24% difference in fecundability with 80% power. Ideally, such a follow-up study would include semen analyses in addition to hormone measurements.

SCIENTIFIC REPORT

BACKGROUND

Of the many manmade chemicals which have been implicated as possible reproductive toxicants, the mechanism of action has been delineated for very few (Colborn et al. 1993; Soto et al. 1995). Moreover, recommended human occupational and environmental exposure limits were based on studies which were not designed to detect subtle reproductive effects. The ability to identify such subtle, likely reversible, effects is critical to preventing more serious, potentially irreversible outcomes such as infertility and spontaneous abortion. A number of epidemiologic and animal studies have suggested that solvents are male and female reproductive toxicants. Several epidemiologic studies have found elevated odds ratios for spontaneous abortion (SAB) in women with occupational solvent exposure (Pastides et al. 1988; Lindbohm et al. 1990; Lipscomb et al. 1991; Windham et al. 1991), and one study found increased SAB rates in wives of men exposed occupationally to solvents (Taskinen et al. 1989). Three studies have not found significant associations between painting occupations and infertility (IARC 1989). More recently, fecundability (time-to-pregnancy, an indicator of the overall fertility of a couple) has been recognized as a more sensitive measure of effects of exposures on fertility than rates of diagnosis of infertility (Baird et al. 1986). In women reduced fecundability was associated with daily or high exposure to organic solvents in general and in shoe factories, dry-cleaning shops and the metal industry (Sallmén et al. 1995). Time-to-pregnancy also increased in primigravida wives of men occupationally exposed to solvents, but not in multigravida wives (Sallmén et al. 1998), and time-to-pregnancy was unaffected in the wives of printing workers exposed to toluene (Plenge-Bönig and Karmaus 1998).

Other evidence for adverse effects of solvent exposure on reproductive function in women includes observations of increased rates of prolonged or heavy menstrual bleeding, dysmenorrhea and irregular cycles in women working with aromatic solvents in electronics (Ng et al. 1992) and shoe manufacturing (Barlow and Sullivan 1982; Huang 1991).

Several studies have reported on the reproductive function of male workers exposed to the aromatic solvent toluene. Mørck *et al.*, (Mørck et al. 1988) found plasma follicle-stimulating hormone (FSH) and self-report of sexual problems to be positively correlated with toluene exposure index and found that testosterone levels increased after 6 weeks without exposure. Svensson *et al.*, (Svensson et al. 1992b) investigated reproductive hormone levels in men exposed to toluene in 2 rotogravure printing companies where average air concentrations were 11 and 47 ppm, well below the Swedish TLV-TWA of 80 ppm. They found a significant negative correlation between toluene exposure and plasma luteinizing hormone (LH), FSH and testosterone levels, but the significance for FSH was eliminated when age was controlled for in the analysis (Svensson et al. 1992b). Svensson *et al.*, (Svensson et al. 1992a) also found lower

LH, FSH and testosterone levels in 20 men exposed to toluene in another rotogravure company, where median air levels were 36 ppm, compared to unexposed controls. FSH and LH levels rose after vacation in a subset of this group. There was a negative association between blood toluene and plasma prolactin levels, but no association of blood toluene was found with the other reproductive hormones measured (Svensson et al. 1992a).

Experimental animal studies suggest that one mechanism whereby solvents could disrupt reproductive function in males and females is by directly or indirectly altering hypothalamic gonadotropin releasing hormone (GnRH) secretion, which would in turn disrupt pituitary LH and FSH secretion. In female rats toluene exposure has been shown to reduce hypothalamic GnRH content (Stepanov et al. 1990). In male rats toluene and xylene exposure have been shown to alter hypothalamic norepinephrine levels and turnover (Andersson et al. 1980; Andersson et al. 1981; von Euler et al. 1988). Norepinephrine is the primary neurotransmitter involved in regulating GnRH secretion. In year 1 of this grant, we tested the hypothesis that exposure to the aromatic solvent toluene acutely suppresses gonadotropin secretion by suppressing hypothalamic secretion of GnRH in men and women. We used an experimental, controlled exposure approach, the results of which are summarized in the companion summary report. In brief, three hour exposures to 50 ppm toluene did not appreciably alter LH or FSH mean concentrations, pulse frequency, or pulse amplitude in men or in women in the luteal or follicular phases of the menstrual cycle. As it is not practical to extend controlled exposures for longer than 3 hours using our exposure system, we proposed studying solvent exposures of chronic duration in the occupational setting during year 2 of the grant, rather than performing additional controlled exposures.

The goals of the current study were to 1) test the hypothesis that occupational exposure to solvents in the painting profession alters gonadotropin secretion and 2) to test the hypothesis that this exposure also reduces fertility, which may be a consequence of altered gonadotropin secretion.

SPECIFIC AIMS

The Specific Aims for the epidemiological study described herein were essentially the same as for the preceding controlled exposure study (performed in year 1 of the grant period). However, the aims were broadened to include occupational solvent exposure in general, rather than toluene exposure specifically.

Specific Aim 1. To assess whether exposure to levels of solvents under the currently allowable limits alters gonadotropin (luteinizing hormone and follicle stimulating hormone) secretion in humans.

Specific Aim 2. To explore whether the differences in solvent pharmacokinetics and hormonal milieux between men and women affect their gonadotropin responses to solvent exposure.

Specific Aim 3. To explore how gonadal feedback modulates the effects of solvent exposure on gonadotropin secretion in women.

As a result of the very few women recruited into the study, we were unable to address Specific Aims 2 and 3. Therefore, the epidemiological study described herein focused on assessing the effects of occupational solvent exposure on gonadotropin secretion and fertility in men.

PROCEDURES AND METHODOLOGY

Experimental design

Time-to-pregnancy was retrospectively determined and serum LH, FSH, and testosterone were measured cross-sectionally in a group of male painters who were occupationally exposed to solvents and in a control group of carpenters. These subjects were recruited for a larger study of which Dr. Drew Brodkin was the Principal Investigator and which included tests of hepatic and renal function.

Subjects

The subjects were recruited from among members of several locals of the International Brotherhood of Painters and Allied Trades and the United Brotherhood of Carpenters, most of whom were employees at a large Department of Energy facility in eastern Washington, the Hanford Reservation. Based on industrial hygiene monitoring of painters on the Hanford site, toluene is one of the most commonly used solvents there. In addition, xylene, octane, methyl ethyl ketone, n-butyl acetate, iso butyl ketone, coal tar naptha, Stoddard solvent, and other solvents are also used, either individually or as components of paints and coatings.

The initial study protocol proposed recruiting both male and female participants, but due to the small numbers of women members of the union locals, only three eligible women were recruited. Therefore, the study population consisted of 98 men.

Union locals sent study information packets and return-addressed postcards directly to their membership. Union members who were interested in participating in the study returned the postcards and were contacted by the study coordinator. Study eligibility was determined by a structured telephone interview, and written informed consent was obtained by mailing potential subjects the informed consent forms after determining their eligibility and verbally explaining the study to them. Subjects were excluded for known infertility or conditions associated with infertility such as mumps orchitis or varicocele. Subjects were also excluded for known renal and liver disease as well as for conditions associated with renal and liver disease such as heavy alcohol use.

Of the 98 men who participated in the study, 32 were painters and 66 were carpenters. Of the 66 carpenters, 2 did not complete interviews. Moreover, it was discovered during the course of the interviews that a subset of carpenters, the millwrights, had intermediate solvent exposure. Therefore, analyses were performed using 3 exposure groups consisting of 32 painters, 40 carpenters, and 24 millwrights, for a total of 96 subjects.

Subject Interviews

Detailed employment, exposure, medical, and time-to-pregnancy histories were obtained by structured telephone interview by the study coordinator. Each subject also underwent

phlebotomy, provided a urine sample, and had a hepatic ultrasound at Kadlec Medical Center in Richland, WA. One aliquot of blood was centrifuged, serum was separated, and serum was stored at -20 C until the hormone assays were performed. Another aliquot of whole blood was stored at -20 C until assay for lead.

Time-to-pregnancy data were gathered using a previously validated structured interview form for men (Baird et al. 1986). Data were gathered about the dates and outcomes of all pregnancies fathered by the individual. Detailed information was then gathered on the most recent pregnancy, including number of months of uncontracepted intercourse before pregnancy, breastfeeding before that pregnancy, use of contraception before that pregnancy, and smoking of the female partner during that pregnancy.

The structured occupational history asked about job history, including job titles, number of years in each job, average hours per week and months per year in each job, and time spent in painting-related tasks in each job (supervision, prep, painting indoors, painting outdoors, etc). For each method of applying paint (spraying, brushing, rolling, other) the type, if any, of respiratory protection used was also asked.

The structured exposure interview was designed for painters and previously used in studies of neurobehavioral effects of chronic occupational solvent exposure (Daniell et al. 1999). The subjects were asked to estimate the number of years and number of hours per week they worked with each of 69 substances, including various metals and solvents. These data will be used in the future to calculate a solvent exposure index for each subject, providing a continuous exposure variable that will be used to reanalyze the data. The analyses in the present report used job category as an index of exposure as described above.

Additionally, data were gathered on neurological, respiratory, and mucous membrane irritation symptoms, medications, educational level achieved, marital status, age, ethnicity, tobacco use, and alcohol use.

Hormone Assays

Plasma LH and FSH were measured using Wallac DELFIA time-resolved fluoroimmunoassays in the laboratory of Dr. William Bremner (University of Washington, Seattle, WA). These are solid-phase, two-site, sandwich fluoroimmunoassays. They use two monoclonal antibodies (mouse anti-human) directed against two different antigenic determinants of the gonadotropin , one of which is labelled with eutropium. For LH the laboratory interassay coefficients of variation (CV) are 6.2%, 6.9% and 14.2% and the intraassay CVs are 3.5%, 2.8% and 7.2% for the high, medium and low control samples. For FSH the interassay CVs are 3.2%, 4.1% and 20.9% and the intraasay CVs are 2.5%, 2.3% and 12.9% for the high, medium and low control samples, respectively.

Plasma testosterone was measured by radioimmunoassay using reagents from the World Health Organization Match Reagent Program by previously described methods (Matsumoto et al. 1983). Interassay CVs ranged from 14% to 24% for the different control serum pools.

Statistical Analyses

The time-to-pregnancy data were analyzed using a Cox proportional hazards model (Baird et al. 1986; Weinberg et al. 1994; Joffe 1997). Our target sample size of 100 exposed and

100 control pregnancies was estimated to have greater than 90% power to detect a 50% difference in fecundability at a significance level of 0.05 based on the power curves generated by Baird and coworkers (Baird et al. 1986). Our final sample size, with 25 control pregnancies (carpenters who had ever fathered a pregnancy) and 49 exposed pregnancies (painters plus millwrights who had ever fathered a pregnancy) had only about 50% power to detect a 50% difference in fecundability at a significance level of 0.05 (Baird et al. 1986).

Analysis of covariance (ANCOVA) was used to compare reproductive hormone concentrations among the three exposure groups. As LH and FSH concentrations were not normally distributed, log transformations were used. We estimated that our sample size of 42 control (carpenters) and 64 exposed (painters plus millwrights) subjects had greater than 80% power to detect differences in LH, FSH, and testosterone concentrations between the exposed and control groups of the magnitude observed in the studies of Svensson and coworkers (Svensson et al. 1992; Svensson et al. 1992), i.e. 11%, 35%, and 12% differences in LH, FSH and testosterone, respectively.

RESULTS

Demographic Variables

The demographic and other characteristics of the three exposure groups are shown in Table 1. There was a significant difference among the three groups in terms of age ($p<0.05$), with the painters being younger than the millwrights or carpenters. Millwrights had significantly higher whole blood lead concentrations than painters or carpenters ($p<0.05$), but the highest lead level was only 8 μg/dL. There was a tendency for fewer of the painters to have completed high school ($p=0.06$) and for the painters to have fewer years of vocational training ($p=0.09$). However, the three groups did not differ significantly by years of college completed. The groups also did not differ significantly by body composition, number of years in the job category, ethnicity, marital status, whether or not they had had a child, or current or past alcohol use. Table 2 displays variables related to the pregnancy for which time-to-pregnancy data were gathered for the 74 subjects who were able to estimate time-to-pregnancy, out of the 83 subjects who had ever had a child. The three exposure groups again differed significantly by age at the time of interview ($p<0.01$), but not in terms of age at the time of the pregnancy of interest, use of contraception before that pregnancy, whether or not they were trying to become pregnant before that pregnancy, whether their partner was breastfeeding before that pregnancy, or whether their partner smoked during the pregnancy.

Table 1: Demographic and other variables by job category

Variable	Carpenter n = 40	Millwright n = 24	Painter n = 32	p-value
		(mean ± SD)		
Age (years)	46.8 ± 8.3	49.9 ± 9.4	42.7 ± 7.8	0.007
Height (m)	1.8 ± 0.06	1.8 ± 0.06	1.8 ± 0.05	0.910
Weight (kg)	91.2 ± 12.2	93.6 ± 13.6	88.6 ± 14.1	0.368
BMI[1] (kg/m^2)	28.8 ± 4.3	29.5 ± 4.3	28.0 ± 4.0	0.448
Years in job	22.8 ± 7.5	22.4 ± 9.1	19.5 ± 8.5	0.221
Years of college	0.82 ± 1.43	0.67 ± 1.09	0.31 ± 0.82	0.190
Years of vocational training	0.75 1.48	0.71 ± 1.30	0.16 ± 0.57	0.090
Blood lead (ug/dL)	1.1 ± 1.5	2.2 ± 1.8	1.7 ± 1.2	0.029
		(percent)		
Ethnicity				0.326[2]
caucasian	92.5	100.0	90.6	
hispanic	0	0	6.3	
black	0	0	3.1	
other	7.5	0	0	
Education (completed high school or more)	80.0	66.6	75.0	0.064
Alcohol use (≥30 gm EtOH per day)	19.4	26.3	22.2	0.847
Maximum past EtOH per day (≥30 gm/day)	42.1	59.1	43.8	0.408
Marital status				0.291
Single	12.5	4.2	18.8	
Married	80.0	87.5	81.2	
Other	7.5	8.3	0	
Have had a child	80.0	87.5	93.8	0.235

[1] body mass index; [2] comparison of caucasian versus other
Significant differences among groups were determined by one-way analysis of variance for the continuous variables and by Pearson's Chi-square test for the categorical variables.

Table 2: Time-to-pregnancy variables by job category

Variable	Carpenter n = 25	Millwright n = 20	Painter n = 29	p-value
		(mean ± SD)		
Age at interview (years)	48.3 ± 7.4	50.3 ± 9.3	43.4 ± 7.6	0.006
Age at TTP pregnancy	30.7 ± 6.3	28.6 ± 3.9	28.9 ± 4.8	0.269
		(percent of exposure group)		
Contraception*				0.512
Not using contraception	48.4	47.6	43.3	
Irregular contraception	25.8	42.9	30.0	
OCPs last method used*	39.3	31.6	24.0	0.756
Wife breastfeeding*	61.3	52.4	56.7	0.149
Trying or unconcerned*	83.9	71.4	80.0	0.550
Partner smoked during pregnancy				

*These variables refer to the time period before the pregnancy for which time-to-pregnancy was determined (most recent pregnancy).

Effect of Solvent Exposure on Time-to-pregnancy

Cox proportional hazards modeling was performed to analyze the effect of job category as an index of exposure on time-to-pregnancy (TTP). Some individuals were able to provide an actual number of months it took them and their partner to conceive. Others were only able to approximate TTP as "less than 3 months", "3 to 12 months", or "greater than 12 months". Therefore, TTP was included in the model either as a composite variable, combining data from these two groups of individuals, or as a variable containing only data from those individuals who were able to give an actual number. For the combined variable, "less than 3 months" was coded as 3 months, "3 to 12 months" was coded as 8 months, and "greater than 12 months" was censored at 12 months. The results of the analyses using these two exposure variables were essentially the same. Therefore, the combined variable is presented here. Additionally, the model was run using all of the actual values for TTP or censoring them at 12 months. The reason for doing the latter is the problem of "medical intervention bias", an association between the exposure of interest and the probability that an infertile couple will seek treatment for infertility (Weinberg et al. 1994). Censoring all TTPs at 12 months, the time at which a couple is medically defined as infertile, guards against this type of bias (Weinberg et al. 1994). Again, the results were essentially the same whether the data were censored or not. The censored results are presented here.

Table 3 shows the crude relative risks for pregnancy for the potential confounding variables. These included age at interview; age at the time of the pregnancy of interest (estimated by age at birth of the child or other pregnancy outcome); years between collection of the data and the pregnancy; whether the couple was trying to become pregnant, trying not to become pregnant, or not concerned; whether and how consistently the couple was using contraception prior to the pregnancy; whether and how consistently the couple was using oral contraceptives prior to the pregnancy; whether the female partner was breastfeeding prior to the pregnancy; whether the female partner smoked during the pregnancy; number of years in the job; and years of education. Only three of the potential confounders were significantly associated with TTP. Older men tended to have longer TTPs ($p<0.01$ for the regression coefficient for age at pregnancy of interest). Couples who were trying to become pregnant had shorter TTPs than couples who were unconcerned one way or the other, and the latter, in turn, had shorter TTPs than couples who were trying not to become pregnant ($p<0.10$ for couples trying not to become pregnant compared to couples who were trying; Figure 2). Finally, couples who stopped using oral contraception and then abstained from intercourse or used another method for a while had shorter times-to-pregnancy than couples who stopped using oral contraceptives and began having uncontracepted intercourse immediately ($p<0.05$).

In all of the models, TTP tended to be longer in the painters and millwrights than in the carpenters, but this was not statistically significant. This can be seen in Figure 1, which depicts the survival curves for the final model, which included job, age at the time of the pregnancy of interest, and whether the couple was trying to become pregnant. In most of the models tested, as demonstrated in Figure 1a, time-to-pregnancy was longer in the millwrights than in the painters. The difference in fecundability was also not statistically significant when painters and millwrights were combined into a single exposed group for comparison to carpenters (Figure 1b). The relative risk of pregnancy for painters plus millwrights compared to carpenters was 0.76 (95% C.I. = 0.45-1.27). Therefore, solvent-exposed men had a 24% lower probability of pregnancy per month of trying than did the unexposed carpenters. Restricting the analysis only to pregnancies which ended in a live birth or only to couples who were trying to become pregnant or unconcerned about becoming pregnant also did not alter the results of the analysis substantially.

Table 3: Crude relative risks of pregnancy, adjusted for job category; Cox proportional hazards models

Factor	n (%)#	RR*	95% CI
Age	74 (100)	0.98	0.95-1.01
Age at TTP	74 (100)	0.94†	0.90-0.99
Years since TTP	74 (100)	1.00	0.98-1.03
Breastfeeding			
no	29 (39)	1.00	
yes	41 (55)	1.18	0.91-1.53
Contraception			
regular and consistent	14 (19)	1.00	
no contraception	36 (49)	1.14	0.57-2.27
irregular	24 (32)	0.99	0.47-2.05
Oral contraceptives			
last method before TTP	20 (27)	1.00	
never used before TTP	22 (30)	0.95	0.48-1.86
stopped OCP, then abstained	7 (1)	2.55 †	1.02-6.41
not last method	16 (22)	1.21	0.59-2.49
Education			
did not finish high school	10 (14)	1.00	
finished high school	54 (73)	1.08	0.52-2.22
Total years of education	72 (97)	0.99	0.88-1.11
Current alcohol use			
< 30 gm/day	45 (61)	1.00	
≥ 30 gm/day	13 (18)	0.85	0.41-1.73
Maximum past alcohol use			
< 30 gm/day	38 (51)	1.00	
≥ 30 gm/day	33 (45)	0.82	0.49-1.37
Marital status			
single	4 (5)	1.00	
married	68 (92)	0.96	0.34-2.70
divorced	2 (3)	1.51	0.27-8.35
Partner's smoking during pregnancy			
no	47 (64)	1.00	
yes	26 (35)	1.39	0.82-2.37
Trying to conceive during TTP			
trying	25 (38)	1.00	
unconcerned	35 (47)	0.66	0.38-1.16
trying not to	13 (18)	0.48††	0.22-1.05

Percentages may not total to 100 due to missing values and rounding.
* For continuous variables this is the relative risk per unit of the variable (e.g. per year for the age variables). † $p<0.05$; †† $p<0.10$

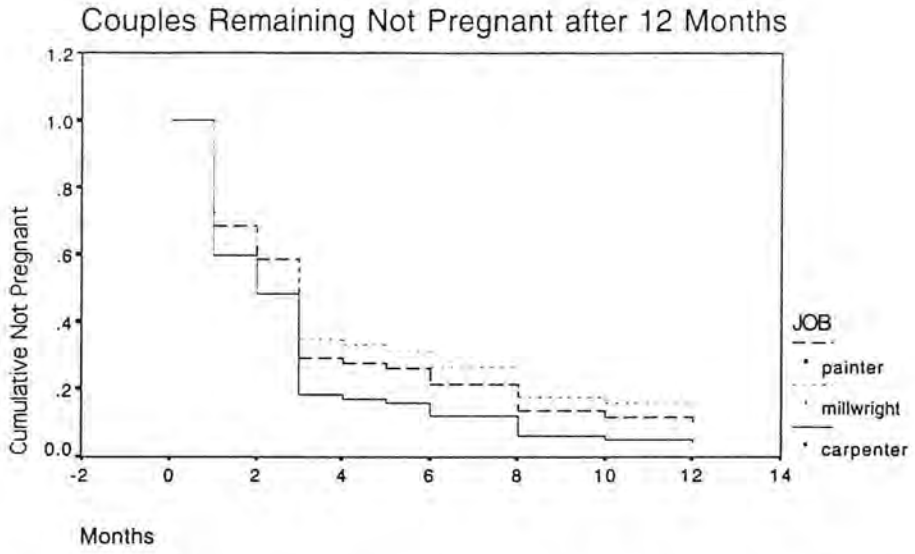

Figure 1a: "Survival" curve from Cox regression analysis of the effect of job category on the number of months to pregnancy (censored at 12 months) adjusted for age at the time of the pregnancy of interest and for whether or not the couple was trying to conceive. The effects of age and trying were significant ($p<0.05$); the effect of job was not ($p=0.34$).

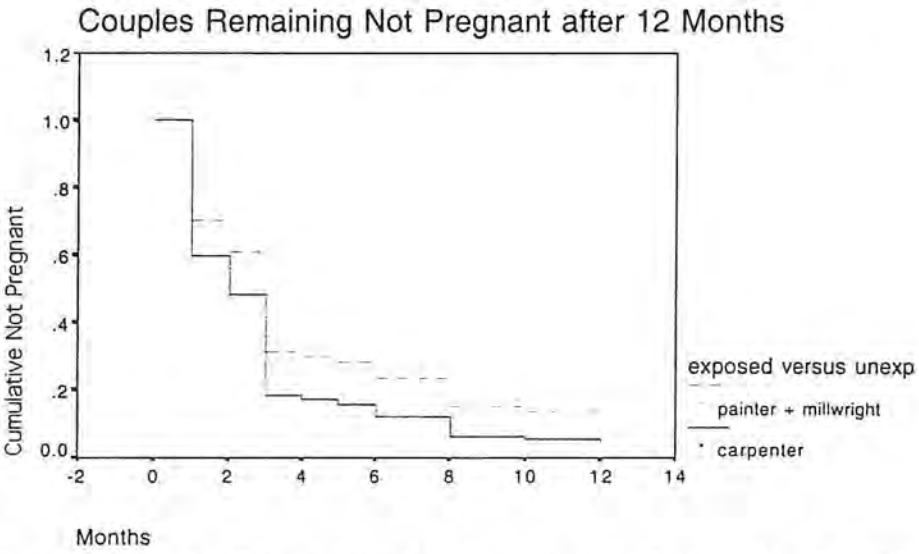

Figure 1b: "Survival" curve from Cox regression analysis of the effect of exposure to solvents (painters and millwrights combined) on months to pregnancy (censored at 12 months) adjusted for age at the time of the pregnancy and for trying to conceive. The effects of age and trying were significant ($p<0.01$, $p<0.05$); the effect of job was not ($p=0.16$).

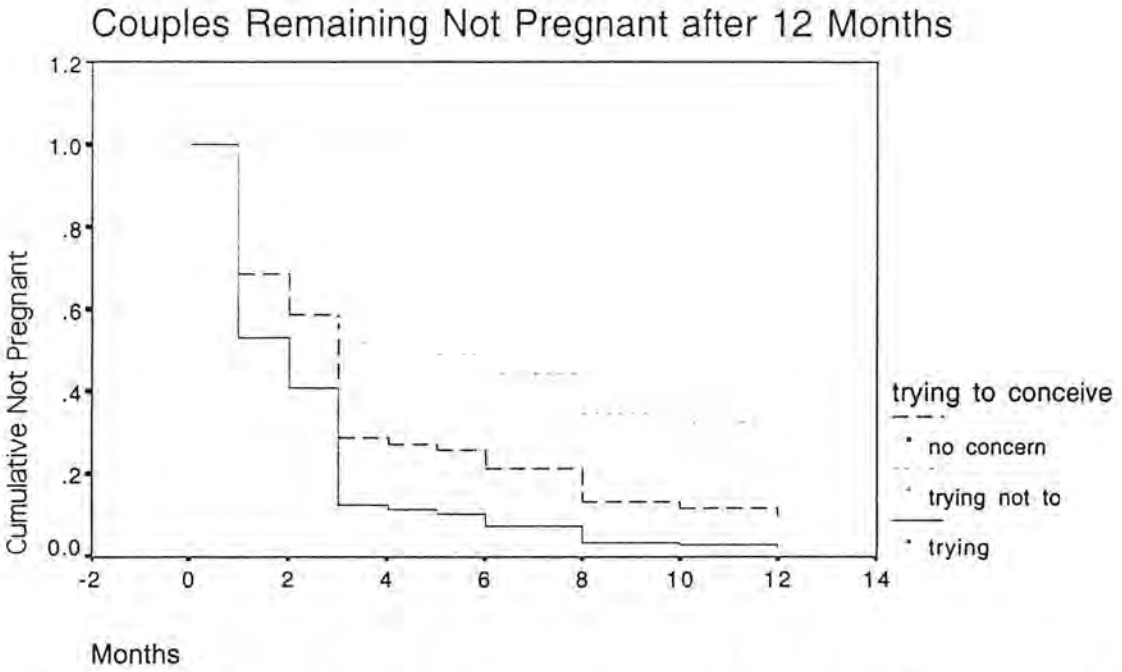

Figure 2: "Survival" curve from Cox Regression Analysis of the effect of whether or not the couple was trying to become pregnant on the number of months to pregnancy (censored at 12 months) adjusted for age at the time of the pregnancy of interest and for job category. The relative risk of pregnancy in those trying not to conceive compared to those who were trying plus those who were unconcerned was 0.44 (95% CI 0.21-0.92).

Effect of Solvent Exposure on Reproductive Hormone Concentrations

Figure 3 depicts the reproductive hormone concentrations by job category. As LH and FSH concentrations were not normally distributed, the logs of their concentrations were used in the analyses. Analysis of covariance (ANCOVA) was used to assess the effects of job category on LH, FSH, and testosterone concentrations. Age, body mass index, years of college, years of vocational training, total years of education, current alcohol use (greater than or less than 30 mg/day), past maximum alcohol use (greater than or less than 30 mg/day), blood lead concentration, ethnicity (caucasian or other), marital status, and number of years in the profession were all evaluated as covariates. Analyses using each of these possible covariates individually revealed that only age, body mass index (kg/m^2), and total number of years of education were associated with at least one of the hormone concentrations at a significance level of 0.1 or lower. Therefore, these three covariates were included in the final model. In the final model there was no significant effect of job category on LH, FSH, or testosterone concentration. The log of LH concentration decreased significantly with increasing body mass index and increasing total years of education ($p<0.05$), and testosterone concentration also significantly decreased with increasing body mass index ($p<0.01$). FSH also tended to decrease with increasing BMI, although not significantly. Both LH and FSH increased with increasing age, but not significantly ($p=0.19$, $p=0.09$).

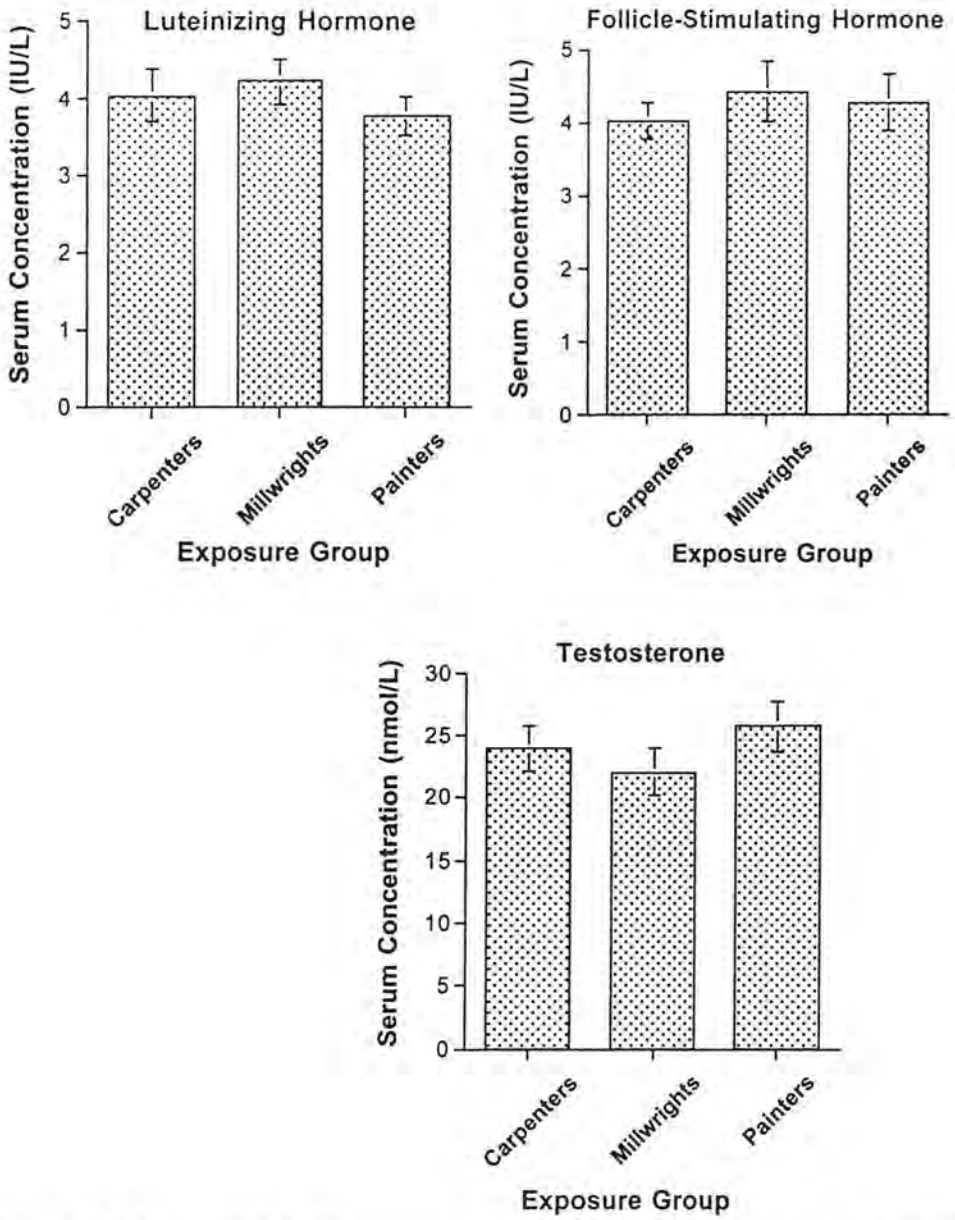

Figure 3: Mean ± SEM of hormone concentrations by job category. ANCOVA with age, body mass index, and number of years of education as covariates revealed no significant effects of job category on logLH, logFSH, or testosterone concentrations.

DISCUSSION

This study investigated fecundability and reproductive hormone concentrations in three groups of men with different levels of occupational solvent exposure. Our results suggest that fecundability may be reduced in solvent-exposed millwrights and painters compared to unexposed carpenters, but this difference was not statistically significant. Based on power curves generated by Baird and coworkers (Baird et al, 1986), we estimate that a sample size of approximately 350 exposed subjects and 350 unexposed subjects would be required for a

definitive study to have 80% power at a 0.05 level of significance to detect a 25% lower fecundability in the exposed group. No differences in LH, FSH, or testosterone concentrations were observed among the three occupational groups. This suggests that the difference in fecundability, if it is substantiated, is not due to a primary effect of solvent exposure on pituitary gonadotropin secretion, as we had hypothesized.

Fecundability has not previously been studied in painters and millwrights, however, there have been several studies which looked for associations between employment as a painter and infertility. Standardized fertility ratios were not found to be decreased in painters in England and Wales between 1980 and 1982 (IARC 1989). Similarly, a Danish case-control study did not find significant associations between painting and a diagnosis of idiopathic infertility in men (IARC 1989). In contrast, another Danish study did find that 18% of painters compared to only 10% of construction workers reported failure to conceive after more than two years of trying (IARC 1989). An association between occupational solvent exposure in a heterogeneous group of men from many occupations and reduced fecundability was found for primigravida wives of solvent exposed men, but not for multigravida wives (Sallmén et al. 1998). As our study only had time-to-pregnancy data for 14 first pregnancies, we were unable to perform a valid statistical analysis, however, time-to-pregnancy tended to be longer in the primigravida wives of the painters and millwrights than carpenters (data not shown). A study of printing workers with exposure to the aromatic solvent toluene failed to find an effect on fecundability in male workers (Plenge-Bönig and Karmaus 1998).

Other studies have looked at semen quality in men exposed occupationally to various solvents. Painters and other workers exposed to glycol ether solvents had abnormalities in some semen parameters compared to control workers (Welch et al. 1988; Ratcliffe et al. 1989). Occupational exposure to styrene was associated with increased abnormal sperm morphology, *decreased* percentages of dead sperm, and *increased* percentages of live sperm, however, the control group in that study was men attending an infertility clinic (Jelnes 1988). Abnormalities in reproductive hormone concentrations have also been inconsistently associated with occupational solvent exposure in men. Reduced LH, FSH, and testosterone concentrations were associated with recent toluene exposure in the printing industry in two studies (Svensson et al. 1992; Svensson et al. 1992), but not with chronic toluene exposure in a third study (Mørck et al. 1988).

This study has a number of strengths and limitations. One strength of this study is that we examined the effects of solvent exposure on fecundability and reproductive hormone concentrations in two distinct occupational groups with different patterns of solvent use, rather than grouping together solvent-exposed workers of many occupations. Information about specific occupations can be useful in beginning to sort out which solvents or patterns of solvent use have the greatest effects on reproductive health. An additional strength is that our exposure groups were homogeneous in terms of primary work site, removing job location as a source of confounding. Limitations of this study include the low power to detect effects on fecundability due to the smaller than planned sample size, the absence of exposure measurements, the use of single measurements of hormone concentration for LH and FSH, which have significant minute-to-minute variability, and the lack of semen analyses. Regarding exposure assessment, we did obtain detailed historical exposure information, which we plan to use to calculate exposure

indices for each subject. We were also able to view the results of industrial hygiene monitoring for solvents which had been performed for painters working at the same site during the years preceding our study. For the most part, all of these personal breathing zone samples were well below the permissible exposure limit for each solvent. Finally, it is difficult to do exposure monitoring in a truly representative way for a study of this kind because of the heterogeneous patterns of solvent use in the occupations we studied. Regarding the reproductive hormone measurements, our study had sufficient power to detect differences of the magnitudes previously observed with toluene exposure in the printing industry even though we only obtained single measurements on hormones with episodic secretion. Future studies should address these limitations by investigating fecundability using a sufficiently large sample size to detect a 25% lower fecundability with 80% power and should also include semen analyses in order to begin to sort out the mechanism by which solvent exposure in these occupations affects fertility.

In addition to the effect of job category on fecundability and reproductive hormone concentrations, we also examined the effects of a number of possible confounding variables. We observed significant effects of age at the time of the pregnancy of interest and of whether or not the couple was trying to become pregnant. Neither of these is surprising. Fertility is well-known to decline with age (Vermeulen 1993), and one would therefore expect time-to-pregnancy to increase with increasing age, as we found. Couples who were trying to become pregnant had shorter times-to-pregnancy. This may have been because they had lower uses of contraception than couples who were not trying to become pregnant ($p<0.001$ by Pearson's Chi Square), however, we did not observe a significant effect of contraceptive use on TTP. Planning bias is a concern in time-to-pregnancy studies when exposed couples use contraception with different frequency and consistency than do unexposed couples, however, we observed no differences among the exposure groups in terms of whether or not the couples were trying to conceive or in terms of contraception use ($p=0.67$, $p=0.51$ by Pearson's Chi Square).

In the ANCOVAs for LH and testosterone, body mass index was a significant covariate; FSH also tended to decrease with increasing body mass index. The association of increasing adiposity with decreasing LH, FSH, and testosterone concentrations has been reported previously in obese men compared to men of normal weight (Vermeulen et al. 1993; Blank et al. 1994). Total years of education was a significant covariate for LH only. We are unaware of any published studies demonstrating a negative association between education or socioeconomic status and LH or FSH concentrations. However, low socioeconomic status has been associated with delayed puberty, and a stress-mediated suppression of the hypothalamic GnRH pulse generator has been postulated as a mechanism (Vermeulen 1993). LH and FSH increased non-significantly with increasing age. This increase in gonadotropin secretion with age has also been previously described (Deslypere and Vermeulen 1984).

CONCLUSIONS

We observed non-significantly lower fecundability (longer time-to-pregnancy) in solvent-exposed painters and millwrights compared to unexposed carpenters. We detected no effects of occupational solvent exposure on plasma LH, FSH, or testosterone concentrations, suggesting that any effect on fecundability is not mediated by effects of solvent exposure on pituitary hormone secretion. The results of this preliminary study suggest the need for further studies of

fertility in larger groups of solvent-exposed men. In accordance with previous work, we found that time-to-pregnancy was longer for men who were older during the preconceptional period and for couples who were actively trying to avoid pregnancy. We also found, as has been reported previously, that serum LH and testosterone concentrations varied inversely with body mass indices.

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PUBLICATIONS

It is anticipated that the work described in this Technical Report will be submitted for publication. The working title is "Effects of Solvent Exposure in Painters and Millwrights on Fecundability and Reproductive Hormone Concentrations."

EQUIPMENT INVENTORY

No equipment was purchased under this grant.

FINAL INVENTION STATEMENT

No inventions were conceived under this grant.