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1. SUMMARY

A National Institute for Occupational Safety and Health (NIOSH) Health Hazard Evaluation (HHE) was conducted in 1991 in response to complaints of impotence and decreased libido among male employees who manufactured the stilbene derivative 4,4'-diaminostilbene-2,2'disulfonic acid (DAS; CAS 81-11-8), an intermediate in the manufacture of fluorescent whitening agents. The chemical structure of DAS is similar to that of the synthetic estrogen diethylstilbestrol (DES). This document contains two reports that present the findings of this HHE.

The first report describes the analysis of reproductive hormone levels. Levels of six hormones in 30 male workers who manufactured DAS (current DAS workers) and 20 former DAS workers who had participated in a previous NIOSH study at the same plant were compared to hormone levels of 35 workers who manufactured plastics additives in a different manufacturing area of the same facility. Current and former DAS workers had lower average total testosterone (TT) levels compared to additives workers (458 and 442 respectively vs. 556 ng/dL; p=0.05 and 0.04). Current and former DAS workers were 3.6 (95% Confidence Interval (CI), 0.5 - 24.4)* and 2.2 (95% CI, 0.3 - 18.0) times more likely than additives workers to have TT values which fell into the lowest 25% of study values (< 386 ng/dL). For both current and former DAS workers, a relationship was found between increasing years of DAS manufacturing work and decreasing TT values. Although average hormone values for each group are within clinically normal limits and sample sizes are small, these hormone data suggest that occupational DAS exposure may be associated with alterations in male reproductive hormone levels.

The second report describes results of the analysis of perceived libido and potency in the same groups of DAS, former DAS, and additives workers. Self-reported sexual function of 30 male workers who manufacture DAS and 20 former DAS workers was compared to that of 35 workers who manufactured plastics additives in a different manufacturing area at the same facility. Questionnaire items were examined by the statistical method of factor analysis, which summarized the questionnaire answers as five components (factors) of sexual function: sexual activity/performance (two factors), interest, satisfaction, and physiologic competence. Current DAS workers were more likely than additives workers to have a value in the lowest 25% of study values for these factors: interest (Odds Ratio (OR)*=1.9, 95% Confidence Interval 0.5-7.2), physiologic competence (OR=1.9, 95% CI 0.6-6.4), and activity/ performance factor II (OR=5.8, 95% CI 1.3-27.3). Former DAS workers reported more problems with activity/performance factors I and II compared to unexposed workers (Factor I OR=2.2, 95% CI 0.5-10.1 and Factor II OR=6.7, 95% CI 1.2-35.9). Although the small study size limits the precision of these results, the pattern of survey results suggests a possible effect on sexual function from working in the DAS manufacturing area.

No environmental standards exist for DAS, and no personal or area exposure measurements were made during this study. However, total serum testosterone values were significantly lower for current and former DAS workers as compared to a group of additives workers from the same company, and a relationship was found between increasing years of DAS manufacturing work and decreasing testosterone values. Further, current and former DAS workers reported more symptoms of sexual dysfunction compared to additives workers. These differences were statistically significant, and were not explained by any other differences between the DAS workers and additives workers. Because of the long DAS work history of many current DAS workers, we could not address the question of whether the current DAS process is independently associated with adverse reproductive health effects, and it is possible that exposures in the current process are not related to the observed effects. Although the exact relationship between DAS manufacturing and male reproductive health is not known, NIOSH considers that the DAS manufacturing process may contain one or more potential male reproductive toxicants and recommends that exposure to chemicals in the DAS manufacturing process be reduced to the lowest feasible concentration. Ciba-Geigy has instituted changes in the DAS process to reduce exposure. However, because health effects were noted in both current and former DAS workers, NIOSH recommends that a review of current control practices be made to identify any further interventions that may reduce occupational exposure.

KEYWORDS: SIC 2865 (cyclic organic crudes and intermediates, and organic dyes and pigments), Stilbene manufacture, amsonic acid; CAS 81-11-8; testosterone; male reproductive hormones; impotence

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* The **Odds Ratio (OR)** is the odds of a health effect being present in one group compared to the odds of the same health effect being present in a comparison group. [Example: An OR of 2 for high blood pressure in a study of men compared to women means that the men are twice as likely as the women to have high blood pressure.] The **95% Confidence Interval (CI)** is the range of possible numbers that the true OR is very likely to fall within. We are 95% sure that the true value of our OR is within the 95% CI.

INTRODUCTION

This investigation was conducted in response to a request from the Oil, Chemical, and Atomic Worker's International Union (OCAW) and OCAW Local 3-562 that NIOSH conduct a health hazard evaluation at the Ciba-Geigy plant in McIntosh, Alabama. The request, which was received on August 8, 1990, indicated employee concern that symptoms such as impotence and decreased libido may be related to their occupational exposure to DAS. A previous study of workers at the same facility, which is discussed later in this document, found low total testosterone levels but was unable to conclusively establish work-relatedness because of the absence of a control group.

In response to the request, NIOSH investigators conducted field surveys on November 28, 1990, August 20, 1991, and December 1-12, 1991. During this study information was collected on reproductive hormone levels and self-reported libido and potency. The results from the analysis of perceived libido and potency were forwarded to the requesters and the company on April 14, 1993. The hormone data were not available at that time. Employees were notified of their specific hormone and medical examination results in April 1993.

This document presents the overall results of both the hormone and libido/potency studies. Two reports are included. The first report describes the analysis of the reproductive hormone levels while the second report describes the results of the analysis of self-reported libido and potency. 3.

REPORT I

Evaluation of Reproductive Function Among Men Occupationally Exposed
to a Stilbene Derivative: I. Hormonal and Physical Status

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ABSTRACT

This is the first of two reports describing a NIOSH Health Hazard Evaluation conducted in 1991 in response to complaints of impotence and decreased libido among male employees who manufactured the stilbene derivative 4,4'-diaminostilbene-2,2'disulfonic acid (DAS; CAS 81-11-8), an intermediate in the manufacture of fluorescent whitening agents. DAS is structurally similar to the synthetic estrogen diethylstilbestrol (DES). This first report describes the analysis of reproductive hormone levels. The second report describes results of the analysis of perceived libido and impotence. Levels of six hormones in 30 male workers who manufactured DAS (current DAS workers) and 20 former DAS workers who had participated in a 1981-1983 study were compared to hormone levels of 35

workers who manufactured plastics additives in a different manufacturing area. Current and former DAS workers had lower mean total testosterone (TT) levels compared to additives workers (458 and 442 respectively vs. 556 ng/dL; p=0.05 and 0.04). In logistic regression analysis, current and former DAS workers were 3.6 (95% CI, 0.5-24.4) and 2.2 (95% CI, 0.3-18.0) times more likely than additives workers to have lowest quartile TT levels (< 386 ng/dL) after adjustment for age and body mass index. Although group hormone mean values are within clinically normal ranges and sample sizes are limited, these data suggest that occupational DAS exposure may be associated with alterations in male reproductive hormone levels.

Key Words: testosterone, workplace exposures, impotence, male reproductive hormones, stilbene manufacture

INTRODUCTION

In 1981, the Oil, Chemical, and Atomic Workers' International Union (OCAW) requested that the National Institute for Occupational Safety and Health conduct a Health Hazard Evaluation (HHE) in response to worker complaints of impotence and decreased libido among employees in a large chemical plant that manufactured the stilbene derivative 4,4'-diaminostilbene-2,2'disulfonic acid (DAS; CAS 81-11-8). DAS is a key intermediate in the manufacture of fluorescent whitening agents which are added to paper, fabrics, and laundry detergents to enhance colors and whiteness by absorbing ultraviolet light and re-emitting the energy as visible light. Although fluorescent whitening agents themselves are not thought to have estrogenic effects, the intermediate DAS is speculated to have estrogenic properties based on structural similarity with estradiol and with the potent synthetic estrogen diethylstilbestrol (DES) (Figure 1). Adverse male reproductive effects from exposure to estrogenic substances such as oral contraceptives and DES have been reported previously (Zaebst et al., 1980; Harrington et al., 1978; Willems, 1981; Shmunes and Burton, 1981). These effects include lowered testosterone levels, decreased libido, impotence, and testicular damage, gynecomastia resultant from estrogen imbalance, and lowered or normal gonadotrophin levels. Clinical reports suggest that these effects may be prolonged (Tomic, 1983; Wortsman et al., 1989). Weak uterotropic effects of DAS, as measured by increases in uterine weight in female rats, have been observed in one report (Smith et al., 1992) but not in other assays (Hostetler et al., 1993).

In 1981-1983, Quinn et al. (1990) conducted an investigation of symptoms and testosterone levels in 43 DAS workers. The workers reported symptoms, including impotence and decreased libido, which were consistent with previous reports of adverse male reproductive effects resulting from occupational and clinical estrogen exposure at other facilities. This study found low total serum testosterone levels (<350 ng/dl) in 37% of the 39 men tested (Quinn et al., 1990). The authors determined that shift work and circadian variability did not fully explain these results. Fourteen percent of the men reported that they experienced impotence (defined as failure rate of 25% or greater to achieve erection during sexual activity) in the preceding six months, and 36% reported experiencing decreased libido (defined as decreased desire for sexual activity leading to a 25% or greater decline in frequency) during the 2 years prior to the study. The investigators suggested but were unable to conclude, because of absence of a control group, that DAS work was associated with low testosterone levels and decreased libido and potency.

In 1990, OCAW requested that NIOSH return to the plant to conduct a follow-up HHE due to continued reports of symptoms of sexual and reproductive dysfunction among its members who manufacture DAS. The objective of the current study was to determine whether male workers who presently manufactured DAS experienced more reproductive health problems when compared to similar workers from the same facility not currently exposed to DAS. A second objective was to determine if employees who manufactured DAS at the time of the first investigation (1981-1983) had more reproductive health problems compared to workers not currently exposed to DAS. This first report describes the analysis of reproductive hormone levels. The accompanying report describes the results of the analysis of self-reported libido and potency.

MATERIALS AND METHODS

1. Study population

We identified a target study population of 129 male workers. Thirty-three were currently working in the DAS manufacturing area ("DAS workers"); 70 were currently DAS-unexposed workers from a separate manufacturing area which produced plastics additives ("Additives workers"), and 26 were former DAS workers who worked in the DAS area at the time of the first investigation (May 1981-December 1983) but were no longer working in the DAS area. Both salaried (technical and administrative) and hourly (chemical operator) employees were invited to participate. Chemical operators controlled and operated all aspects of the chemical processes within the manufacturing area, while personnel in technical and administrative positions varied in their job responsibilities and the proportion of their time spent in direct contact with chemical manufacturing processes. Generally, salaried employees followed a day shift, five-day work week, while the hourly employees in the DAS and additives areas worked according to a 12-hour rotating shift pattern of three days on (7:00 a.m.-7:00 p.m.), three days off, three nights on (7:00 p.m.-7:00 a.m.), and three nights off.

2. Serum hormone determinations and physical examination

A 26 mL blood sample was collected from each individual for hormonal analysis. Because serum testosterone levels have a distinct circadian rhythm and maximum values occur during early morning hours (Bremner et al., 1983), the collection of blood samples was shift-standardized. Blood drawings for hourly employees on a 12-hour rotating shift were conducted at the beginning of the employee's third day of work on the day shift (approximately 7:00 a.m.). Blood drawings for salaried employees on an 8-hour, five day shift were conducted on the third, fourth, or fifth day of the work week at the beginning of the work day (approximately 7:00 a.m.). The blood was allowed to clot and the serum separated and maintained on dry ice at -70^{o} C. Hormone assays for total testosterone (TT), free testosterone (FT), follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin (P), and estradiol (E) were conducted using radioimmunoassays according to standard published procedures (Rodbard et al., 1974; Odell et al., 1967; Odell et al., 1966; Sinha et al., 1973; Bartke et al., 1973; Abraham, 1973; McCann and Kirkish, 1985). Laboratory analyses were conducted according to current FDA Good Laboratory Practices. Each assay was run in duplicate and averaged values used in the analysis. Duplicate immunoassay control serum standards (Lyphochek®, Bio-Rad Laboratories, Anaheim, California) were analyzed as blind samples along with the study samples, and coefficients of variation (CVs) computed for pairs of each standard analyzed. The highest CV was 28% for prolactin in the level two standard. All other CVs were less than 10%. The data were judged acceptable after review of the laboratory's quality assurance information.

A limited reproductive physical examination, consisting of an evaluation of secondary sexual characteristics and external genitalia including testicular volume (Takihara et al., 1983), was administered to all study participants except one worker who refused the examination. The examination was conducted by a NIOSH physician who was blind to the worker's exposure status.

3. Survey instruments

A medical history questionnaire was administered in person to obtain information on personal demographic data, medical history, smoking and drinking habits, work history, and exposures to chemical and physical agents. Questions to assess potency and libido were excerpted from the 21-item Brief Sexual Function Questionnaire of Reynolds et al. (1988). The medical history questionnaire also assessed the presence of other factors that may be associated with reproductive function, such as diabetes mellitus, thyroid disease, obesity, use of certain medications, and neurologic disease. A brief nonparticipant questionnaire, which collected information on selected demographic characteristics, was administered to 22 of the 36 men not willing to participate in the study.

4. DAS process description

Two variations of the DAS production method (Figure 2) had been in use at the study facility. The older process had been in use during the initial 1981-1983 study (Quinn et al., 1990; Hammond et al., 1987). The new process was the primary production process at the time of our study, and was being phased in to replace the older process. In the older process, paranitrotoluene (PNT) was reacted with oleum (SO_3) to form paranitrotoluene sulfonic acid (PNTSA). PNTSA was then converted to dinitrostilbene (DNS) by a reaction involving sodium hydroxide (NaOH) and hypochlorite bleach. The DNS was then converted to a DAS slurry with an iron-catalyzed hydrogenation reaction. When foaming, splashing, and "boiling over" occurred at this reactor, an antifoaming agent, 2-ethyl-1-hexanol, was manually added to the reactor by chemical operators to stop the foaming. This reactor also required regular cleaning, which required that workers enter the reactor and "chip out" chemical residues. The DAS slurry was filtered and crystallized for further processing in aqueous suspension or bagged as wet cake free acid.

The new DAS production process (Figure 2) used more modern engineering that would be expected to reduce worker exposures, but both the new and old processes had potential for dermal and possibly oral exposure to production chemicals. In the new process, PNT was converted to PNTSA in a closed system. The conversion of PNTSA to DNS also took place in a closed system, which utilized a catalyst with a volatile solvent under alkaline conditions. The iron catalyst hydrogenation, used in the older process to form DAS from DNS, was replaced in the new process by an enclosed hydrogenation reaction with catalyst filtration to form DAS. The final product was used in aqueous suspension or bagged as wet cake free acid. Exposure to DAS and other production chemicals was still a possibility in belt filtration areas, the bagging area, and leaking areas in the enclosed systems such as valves, pipes, and filters. Although the two processes differed in their engineering, chemical reaction mechanisms, and extent of operator contact, the raw materials, intermediates, and end products were very similar.

5. Exposure assessment

Air sampling and biomonitoring for exposure to DAS were not conducted because there are no NIOSH-approved methods or other fully validated methods for analyzing air samples or biological specimens for DAS. The evaluation of current exposure potential for specific worker categories was based on observation of job activities and work practices in the DAS manufacturing area and other work areas, and review of environmental sampling records, material safety data sheets, and other

relevant company records. Evaluation of DAS manufacturing exposures during the 1981-1983 study is described by Quinn et al. (1990) and Hammond et al. (1987).

In both old and new processes, the Operator job title in DAS production required both computer monitoring of production processes and performance of operation, sampling, and some housekeeping tasks in the production area. Based on observation of work activities, salaried worker exposure appeared to be more variable and of lesser magnitude than that of the DAS chemical operators. The comparison group of workers for this study was identified from the additives manufacturing area. They were determined to be currently unexposed to DAS on the basis of routine industrial hygiene data provided by the company and information observed during a walkthrough survey. The additives area produces several different formulations of antioxidants and UV protectants which are used in the production of plastics. Processes were generally well enclosed and well maintained, and no substantial exposures to DAS or known reproductive toxicants were present.

6. Data analysis

a. Exposure variables. A current DAS worker was defined as a worker in the DAS production area who had worked there for a minimum of 30 days immediately previous to our study. Former DAS workers were workers currently employed in other areas of the plant who had worked 30 days or more in the DAS production area between May 1981 through December 1983, the time of the previous investigation. The comparison group of additives workers was defined as those workers currently working in the additives department with less than 180 days of known prior DAS production work during their entire employment. Because of missing company work history information, only 16% of the additives workers were confirmed as having no prior DAS production experience. Thirty-six percent of the additives workers had confirmed DAS production experience averaging a total of 85 days. Forty-eight per cent of the additives workers had some gaps in their work history information. Because length of DAS exposure could not be calculated for the workers with work history gaps, they were included in categorical exposure analyses only.

In order to consider the potential effect of duration of DAS exposure, in addition to the categorical exposure variables described above, we combined all available sources of work history information for each study participant to calculate total time working in DAS production during the previous study period (May 1981-December 1983) and total time as a DAS worker. Work history information was missing among 36.5% of the study group (primarily between 1966-1980), and these workers were dropped from analyses of exposure duration. Consequently, these duration of exposure indices were less powerful than the categorical descriptors.

b. Regression analysis. To assess the effect of DAS production work on continuous measures of hormone levels, we derived adjusted mean hormone levels, associated standard errors and 95% confidence intervals from least squares regression analysis. To take into account the potential interrelatedness of the individual hormone levels, a multivariate analysis of variance (MANOVA) was performed to examine all hormones simultaneously. The effect of DAS production work on hormone levels was also assessed by multiple logistic regression analysis of categorized quartiles of hormone levels. In the logistic regression analyses, the highest or lowest quartiles (corresponding to the lowest or highest 25% of study values in the direction of hypothetical pathology) were

considered the outcomes of interest. The relative odds of falling into the highest or lowest quartile and the 95% confidence interval for the odds ratio were calculated. PC-SAS^{TM} software was used for all statistical procedures (SAS Institute, 1989).

Regression modeling proceeded as a modified backwards selection using the results of prior stratified analyses to assess evidence of confounding and interaction. Age and exposure were retained in all models; other variables were retained at $p \leq 0.1$. Log transformations were used to normalize data for all hormones except the ratio of free:total testosterone, which was normalized with an arcsin transformation. The following variables were evaluated as potential confounders or effect modifiers: 1) demographic factors, including age, race, marital status, hourly/salaried status, education, and potential exposures from other employment or hobbies; 2) medical/physical factors, including body mass index (weight/height^2), presence of a varicocele, occurrence of mumps at age 11 or later, fever in the last month, report of a current cold, current use of analgesics, current use of blood pressure medication, and current use of allergy medication; 3) shift and sleep factors, including day/rotating shift status and indices of sleep quality and cumulative sleep debt in the week prior to the study; and 4) substance factors, including multiple indices of tobacco, caffeine and alcohol use. Residual, multicollinearity, and goodness-of-fit analyses were conducted to confirm that final models did not violate analytic assumptions.

c. Hormones and medical conditions. Serum levels of total testosterone (ng/dL), free testosterone (pg/mL), FSH (mIU/mL), LH (mIU/mL), prolactin (ng/mL), estradiol (ng/L), and the ratio of free: total testosterone were examined as outcomes in regression analyses. Additionally, hormone levels were categorized and odds ratios generated for the highest or lowest quartiles for each hormone (corresponding to the lowest or highest 25% of study values in the direction of hypothetical pathology). Physician findings of testicular abnormalities (testicular volume of $\leq$ 10 mL or missing testicle/s) or gynecomastia (presence of palpable breast glandular tissue) (Braunstein, 1993) were also considered study outcomes. Two or more self-reported adverse reproductive events for the pregnancies fathered during employment at the study company (spontaneous abortion, stillbirth, neonatal death, ectopic pregnancy, low birthweight, prematurity, and birth defects) was examined as a categorical variable. Adverse reproductive outcomes could not be examined individually due to small numbers.

RESULTS

1. Study group

Ninety-three (72%) of the 129 workers initially identified agreed to participate in the study. Of the current DAS workers, 29 (88%) participated, while 23 (88%) of the former DAS workers and 41 (59%) of the additives workers participated. After examination of detailed work histories and physical examination data, we excluded 13 participants because they did not satisfy the study eligibility criteria for exposure, and two participants on the basis of testicular abnormalities incurred prior to employment at the study company. An additional seven workers that we did not identify from company documents (three current DAS, one additives, and three former DAS workers) were allowed to participate after we confirmed eligibility. The final study group consisted of 85 participants: 30 current DAS workers, 35 additives workers, and 20 former DAS workers.

In general, current and former DAS workers were significantly older than the additives workers and had been employed longer at the company, although all three groups had worked at the facility for a considerable number of years (Table I). For those workers with complete work history data, current DAS workers had worked in the DAS area longer (mean ± SD, 15.9 ± 8.3 years) then former DAS workers (7.5 ± 7.1 years) and additives workers (0.13 ± 0.19 years). Although all three groups had similar proportions of drinkers, former DAS workers reported significantly less frequent drinking than current DAS or additives workers. Current DAS workers were more likely to be currently taking prescription medications than the additives workers. Current and former DAS workers were more likely than additives workers to report the belief that workplace exposures affect sex drive or fertility. Twenty-two (61%) of the 36 men who refused participation in the study completed a nonparticipant survey. Nonparticipants and participants did not differ on the basis of age, race/ethnicity, marital status, or the total number of pregnancies fathered.

2. Hormone analyses

The results of hormone analyses and variables included in final models are given in Tables II and III. Results of the stratified analyses and initial regression modeling provided strong evidence of confounding and/or effect modification with salary/hourly employment status. Because the number of salaried workers was small, and because the workers with the greatest potential for exposure were the hourly chemical operators, further analyses were restricted to hourly workers (N=63).

After adjustment for age and body mass index (BMI), mean total testosterone levels for current and former DAS workers were significantly lower than the mean for the comparison group of additives workers (458 and 442 ng/dL, respectively, vs. 556 ng/dL; Table II). Current DAS workers were 3.6 times and former DAS workers 2.2 times as likely as additives workers to have a testosterone level in the lowest 25% of study values (95% CIs: 0.5 - 24.4 and 0.3-18.0, respectively). For free testosterone, current DAS workers were 2.5 times and former DAS workers 2.3 times as likely to fall into the lowest 25% of study values (95% CIs: 0.5-13.1 and 0.4-13.1, respectively). Two additives workers had outlying testosterone values of 1440 ng/dL and 1510 ng/dL. When we excluded these two workers from the testosterone analysis, the adjusted mean testosterone level for additives workers decreased (507±1 ng/dL; p=0.22 and p=0.14 for the comparison of additives workers to current and former DAS, respectively). We examined all available medical, laboratory, statistical, and survey data to find any possible reason for the outlying testosterone results. None was found, and the outliers were retained in the analyses.

Models using measures of duration of employment in DAS production showed an inverse relationship between testosterone level and length of employment. For total testosterone age- and BMI-adjusted models, total duration of employment in DAS production and duration of employment in DAS production during the 1981-1983 investigation were inversely related to the natural log of testosterone levels. (For total duration of DAS employment: adjusted exposure coefficient = -2.9×10^{-5} log testosterone units per day of DAS employment, p=0.048; for duration of DAS employment during the 1981-1983 investigation: adjusted exposure coefficient = -2.0×10^{-4} log testosterone units per day of former DAS employment, p=0.039). The negative relationship between testosterone levels and duration of DAS employment is graphically illustrated in Figure 3, a plot of predicted testosterone values as a function of total years of DAS production work, after adjustment for age and

BMI.

Table III reports adjusted means and odds ratios for hormones other than testosterone. After adjustment for age, smoking, BMI, and alcohol consumption, the mean FSH level for former DAS workers was 6.7 mIU/mL, compared to 10.3 for additives workers (p=0.06). Other mean hormone levels did not differ by exposure group after adjustment for confounders, and 95% confidence intervals for all quartile odds ratios included unity. A multivariate ANOVA test of exposure effect on the seven hormones/hormone ratio tested was not significant (Wilks' Lambda p=0.57).

In another set of analyses, current and former DAS exposure groups were pooled and compared to the additives workers. Combining these exposure groups did not significantly change the hormone analysis results described above.

3. Medical status

All but one study participant underwent the study's limited medical examination. The number of men with testicular abnormalities (testicle/s with a estimated volume of 10 mL or less) was too few to analyze (current DAS workers, N=0; former DAS workers, N=2 (15%); additives workers, N=1 (4%)). We also compared testicular volumes of current and former DAS workers and additives workers, which did not differ from each other in a MANOVA (Wilks' Lambda=0.95; p=0.6). Bilateral gynecomastia was similarly found in small numbers in all groups (current DAS workers, N=2 (8%); former DAS workers, N=3 (23%); additives workers, N=2 (8%)). The self-reported condition of two or more adverse reproductive outcomes during company employment was similar in the three groups (23% in DAS workers, 14% in former DAS workers, and 13% in additives workers).

DISCUSSION

In this cross-sectional survey of reproductive hormones and physical status, we found significantly decreased mean testosterone levels in both current and former DAS workers when compared to a group of additives workers at the same company. Current and former DAS workers were 2.2-3.6 times as likely as the additives workers to fall in the lowest quartile of testosterone values (<386 ng/dL). We also found that duration of employment in DAS production was negatively related to the testosterone levels of these workers.

The finding of modest mean hormone level differences between DAS exposure groups requires interpretation beyond direct comparison to clinical normal ranges for the respective hormones. Hormonal group means for this study were generally within the range of values considered clinically normal (although normal clinical ranges for testosterone vary considerably, most ranges include a lower normal level of approximately 200-350 ng/dL and a higher normal limit of 1000-1200 ng/dL). In the context of a male reproductive epidemiologic study, however, modest group differences in reproductive biomarkers such as hormone levels, even within clinically normal ranges, can indicate the potential dysfunctional impact of a reproductive toxicant (Katz, 1991).

Former DAS workers had lower testosterone, lower FSH, and possibly higher prevalence of

abnormally reduced testicular size and gynecomastia (based on extremely small numbers). Current DAS workers only showed differences in testosterone levels. The observations of decreased FSH levels, small testicular volume, and gynecomastia are of interest because they are biologically consistent with previous reports of sequelae of occupational and clinical male estrogenic exposures.

Selection bias might be a possible explanation for our findings. While participation rates among current and former DAS workers were high (88%), only 59% of additive workers participated. If these additives workers who participated were "overly healthy" compared to the non-participants, an artifactual increase in adverse outcomes and conditions would appear among DAS workers. However, among additive workers, participants and non-participants reported similar demographics and number of pregnancies fathered. It is also possible that DAS workers who were unhealthy at the time of the 1981-1983 study left DAS production, resulting in an overestimate of risk in the group of former DAS workers. We collected no data on reasons for transferring out of DAS production, but it is possible that in some cases, workers who experienced potency, libido, or other reproductive problems while manufacturing DAS were motivated to seek a transfer, and workers who perceived fewer health effects remained in the DAS area. However, testosterone level findings were similar in the current DAS worker group, many of whom had long duration of employment in the DAS manufacturing area. Thus it does not appear that selection bias is a likely explanation for the decreased testosterone levels observed among DAS workers in this study.

It is also possible that positive findings might be underestimated. Many of the additives workers had some prior DAS area work experience. Inclusion of these workers in the comparison group would tend to diminish any positive effects of exposure. Examination of cumulative DAS employment data for all workers can address this potential underestimate. Even though work history information for our study group was incomplete, examination of available data indicates a significant decrease in predicted testosterone levels with increasing duration of DAS production work, which is consistent with the results of the categorical analysis.

Our ability to associate specific health effects with either the old or the new DAS production process is limited. Seventy-five per cent of the current DAS workers had worked in the older DAS production process at the time of the first study (1981-1983). Current and former DAS workers spent very similar amounts of time working in the older DAS production process during that period (mean ± SD, 1.9 ± 1.2 and 1.8 ± 1.0 years for current and former DAS, respectively). Total duration of work years in the DAS area, however, was approximately twice as long for current as compared to former DAS workers (15.9 ± 8.3 vs. 7.5 ± 7.1; $p=0.0002$). The number of current DAS workers with exposure limited only to the new process which we found at our 1991 visit was too small to analyze. Because of the long DAS work history of many current DAS workers, we could not address the question of whether the current DAS process is independently associated with adverse reproductive health effects, and it is possible that exposures in the current process are not related to the observed effects.

Since there were no NIOSH-approved methods for analyzing air samples or biological specimens for DAS, we were unable to implement environmental monitoring or biomonitoring for DAS or its metabolites in this study. Besides DAS, more than one of the process chemicals may have had potential for association with the adverse effects we found in the DAS manufacturing area. As

detailed below, the effects reported in this study are consistent with long term effects caused by one or more of these agents: the end product DAS; the feedstock paranitrotoluene (PNT); or the antifoaming agent 2-ethyl-1-hexanol (2EH), which was removed from the production process during the early 1980's.

DAS: Adverse male reproductive effects have been associated with occupational exposure to estrogenic-like substances such as oral contraceptives and DES. These effects include lowered testosterone levels, decreased libido, impotence, and testicular damage, gynecomastia resultant from estrogen imbalance, and lowered or normal gonadotrophin levels (Zaebst et al., 1980; Harrington et al., 1978; Shmunes and Burton, 1981). At least one clinical report indicates that prolonged estrogen therapy may result in irreversible testicular destruction and the loss of the feedback response of the hypothalamic-pituitary-gonadal axis. Contrary to expectations, FSH and LH were found to be at normal levels (Wortsman et al., 1989). In a report of estrogen therapy for cancer, low testosterone and normal LH levels were found three years after cessation of estrogen treatment (Tomic 1983). Low testosterone, FSH, and LH levels were reported in male estrogen pharmaceutical manufacturing workers (Willems 1981). Quinn et al. (1990) found serum testosterone levels of <350 ng/dL in 37% of current DAS workers. In the same workplace approximately ten years later, we also found lower mean testosterone levels in DAS workers when we compared their levels to those found in additives workers (27%, 14%, and 5% of our current DAS, former DAS, and additives workers had serum testosterone levels of <350 ng/dL, although testing was done by different laboratories.) The very limited data also suggest the possibility of decreased FSH, increased gynecomastia, and decreased testicular size in former DAS workers.

PNT: PNT, the starting material in the DAS process, has been reported to cause testicular atrophy and necrosis of the seminiferous tubules in rats. In a National Toxicology Program subchronic feeding study (1989), rat reproductive organ weights, epididymal sperm density, and testicular spermatid head counts were reduced at the 10,000 ppm level. Primary testicular failure of this type tends to be permanent and, if similar effects occurred in men, this would create a clinical picture of lowered testosterone, testicular atrophy, and elevated FSH levels due to insufficient negative testicular feedback from the germinal epithelium. Our finding of decreased testosterone levels is consistent with a possible effect of PNT, but we found marginally decreased rather than elevated FSH levels in these workers.

2EH: Some information is known about the toxicity of 2EH (the antifoaming agent used in the older DAS process) because of its metabolic role in the toxicology of the widely used plasticizer di(2-ethylhexyl)-phthalate (DEHP). DEHP is a well characterized animal teratogen. In rats, chronic administration of DEHP causes seminiferous tubule atrophy (Gray TJB and Gangolli SP, 1986) of limited reversibility (Oishi S, 1985). In humans and rodents, the primary metabolic products of DEHP are mono-2-ethyl-hexyl phthalate (MEHP) and 2EH. 2EH is metabolized further to 2-ethylhexanoic acid, which appears to be an active teratogen (Ritter et al., 1987). No information is available concerning the effects of 2EH or 2-ethylhexanoic acid on human male hormones, potency, or libido. Because the older DAS process was a more open system than the current process, and foaming, splashing and boiling over were reportedly common occurrences, the potential for dermal and perhaps oral exposure to DAS and 2EH at this reactor in the older process appears to have been high.

CONCLUSIONS

Our findings suggest that although mean group hormone values are within clinically normal ranges and sample sizes are limited, occupational DAS exposure may be associated with alterations in the male reproductive profile, specifically with decreased levels of serum testosterone. Additional biologically plausible findings based upon small numbers also suggest lowered levels of FSH, possibly increased gynecomastia, and decreased testicular size among the former DAS workers. Several chemicals currently or formerly present may be responsible for these findings, including DAS, PNT, and 2EH. In the aggregate, the findings are suggestive of a modest but prolonged effect upon the hypothalamic-pituitary-gonadal axis, and are most consistent with the published health effects of exposure to estrogen-like substances. We were unable to address the question of whether the current DAS process is independently associated with adverse reproductive health effects, and it is possible that exposures from the current process are not related to the observed effects.

Altered hypothalamic-pituitary-gonadal function has been associated with symptoms of impotency and lowered libido (Spark et al., 1980). An evaluation of perceived libido and potency and the relationship of reported symptoms to hormone results in this population is presented in the following report.

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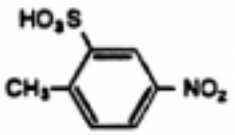
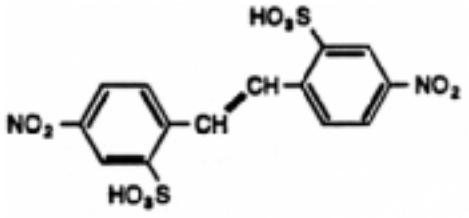
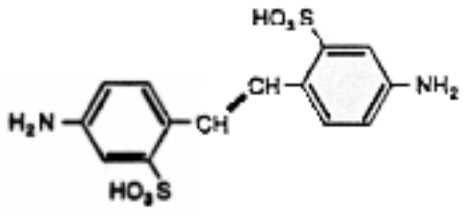
Figure 1. Chemical structures of primary raw materials, intermediates, and products in the Stilbene (DAS) production process. PNT=para-nitrotoluene; PNTSA=para-nitrotoluene sulfonic acid; DNS=4,4'-dinitrostilbene-2,2'-disulfonic acid; DAS=4,4'-diaminostilbene-2,2'-disulfonic acid; and E_2=estradiol. The structure of the synthetic estrogen diethylstilbesterol (DES) is shown for comparison. Modified from Quinn MM et al. (1990).

PNT

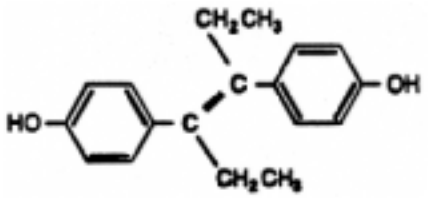


PNTSA





DAS



DES

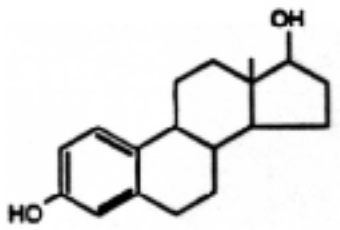


Figure 2. Overview of historical process differences in the stilbene (DAS) production process at the study company. Solid arrows in center of chart depict the raw materials, intermediates, and products of the process, which have not changed. Areas within dotted polygons indicate aspects of the process which differed between the time of the 1981-1983 study (left of chart) and the current study (1991, right of chart).

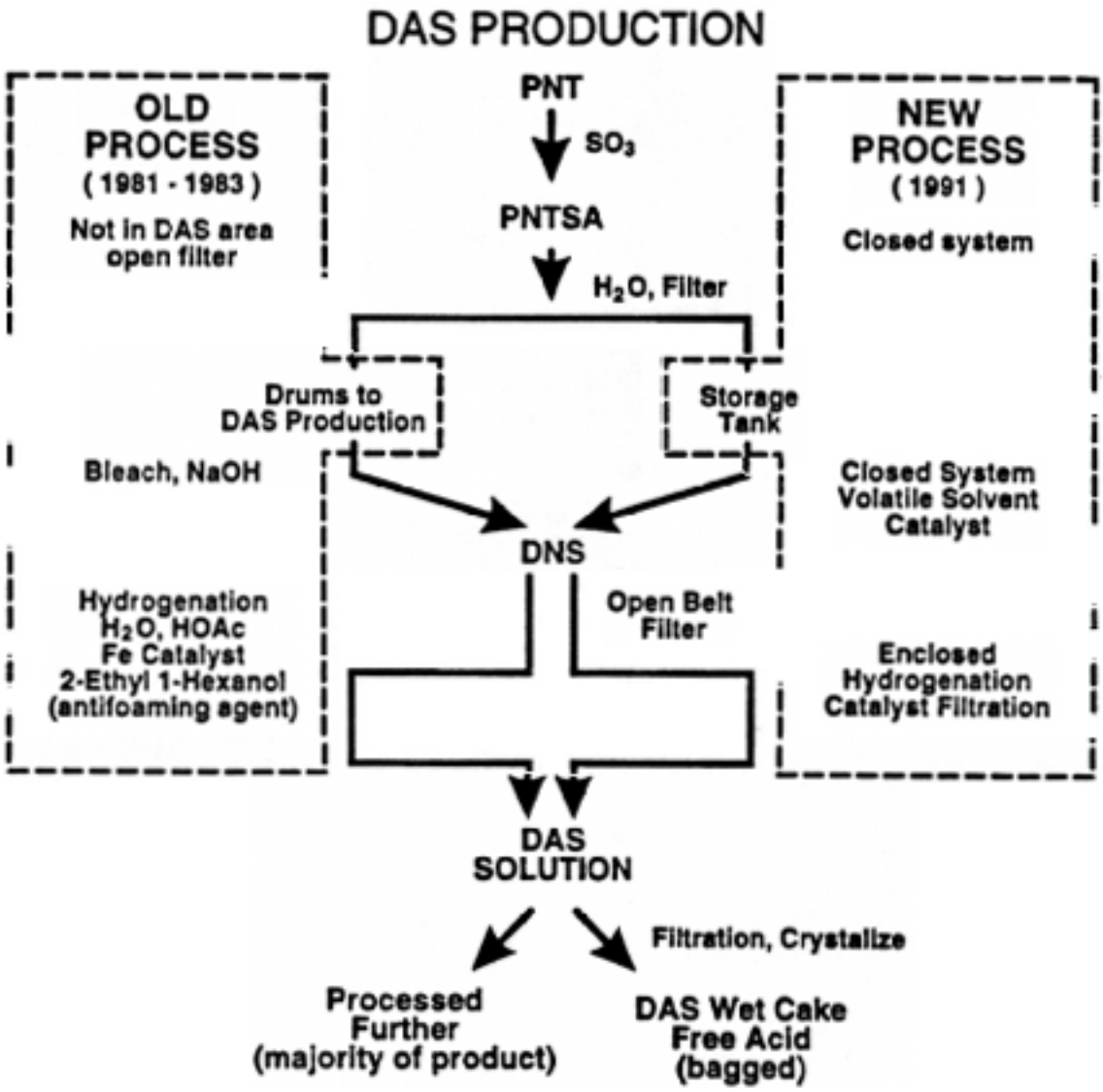


Figure 3. Plot of predicted testosterone values (ng/dL) as a function of total years of DAS production work, after adjustment for age and BMI. The original linear model was based on log testosterone values, which have been untransformed for interpretability; therefore, the values appear as a scatter plot rather than a line. Approximately 41% of the analysis group is missing due to incomplete work histories.

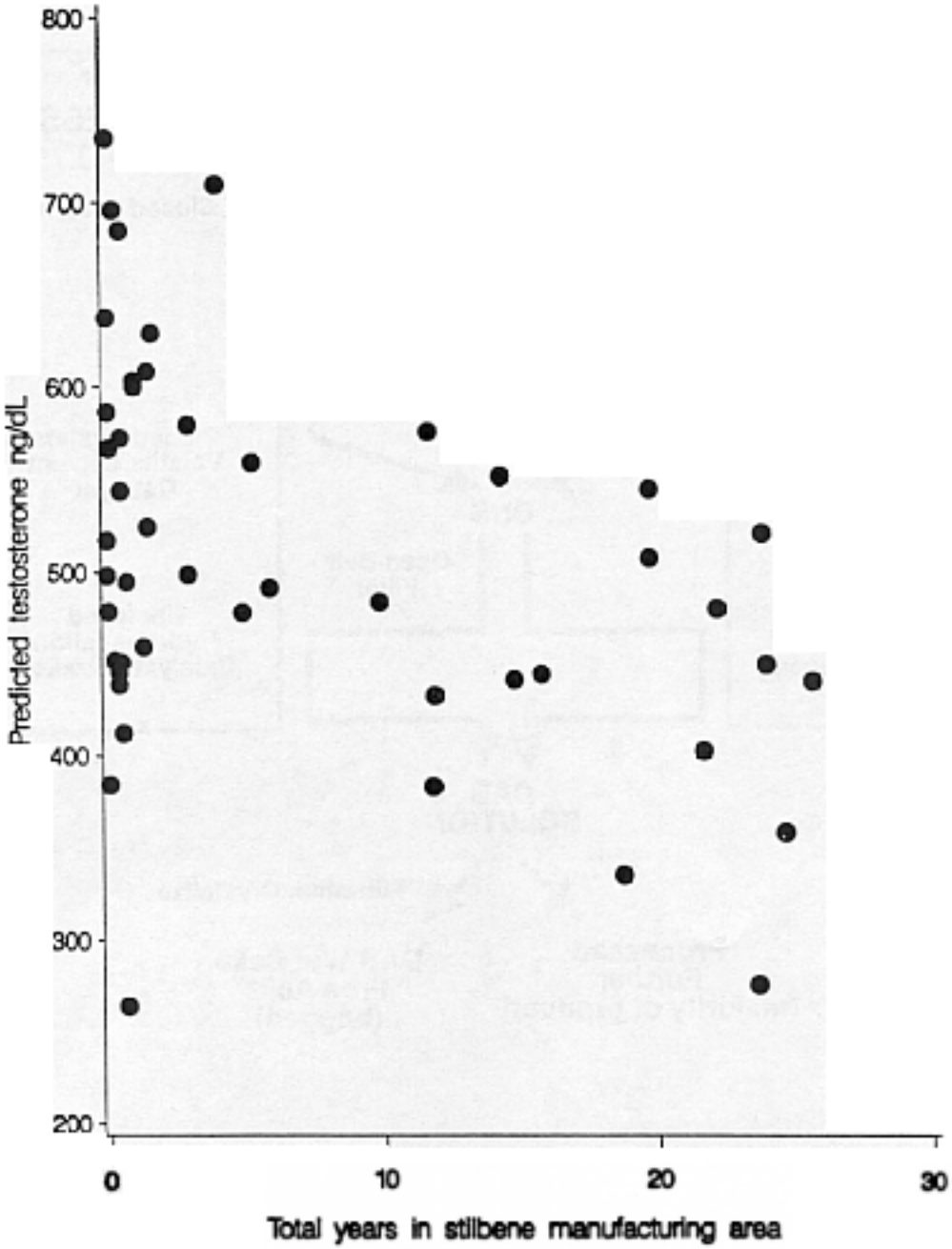


Table I. Characteristics of DAS Workers, Former DAS Workers, and Additives (unexposed) Workers

Characteristic	DAS Workers (N=30)	Former DAS Workers (N=20)	Additives Workers (N=35)
Age (years; mean±SD)[1]	45.9±8.8	45.2±6.4	39.0±7.3
Nonwhite (%)	43.3	45.0	34.3
Body Mass Index (kg/m^2; mean±SD)	28.0±4.0	27.8±2.3	27.2±3.8
Education: Greater than high school (%)	26.3	35.0	37.1
Married (%)	83.3	90.0	85.7
Duration of employment (years; mean±SD)[2]	20.3±6.6	22.2±5.2	15.1±8.4
Duration of employment in DAS area (years; mean±SD)[3]	15.9±8.3	7.5±7.1	0.13±0.19
Hourly workers (%)	80.0	70.0	71.4
Current tobacco user (%)	40.0	35.0	37.1
Cigarettes/day (current smokers; mean±SD)	22.1±11.7	18.8±10.3	14.8±10.8
Currently consume alcoholic beverages (%)	56.7	55.0	68.6
≥ 3 drinks/week (%)[4]	16.7	0.0	28.6
Caffeine consumption (estimated mg/day; mean±SD)	352.0±324.6	400.9±481.7	246.6±173.4
Current use of medications (%)[5]	56.7	42.1	30.3
Ever fathered a pregnancy (%)	90.0	95.0	88.6
Total number of pregnancies fathered (mean±SD)	3.2±2.3	3.5±1.8	2.6±1.7
Ever consulted an MD because of a fertility problem (%)	6.7	5.0	14.3
Reported belief that workplace exposures affect sex drive or fertility (%)[6]	68.0	60.0	23.3

[1] $p=0.0006$ (DAS vs. additives); $p=0.005$ (Former DAS vs. additives).
[2] $p=0.005$ (DAS vs. additives); $p=0.0008$ (Former DAS vs. additives).
[3] $p=0.0001$ (DAS vs. additives); $p=0.0005$ (Former DAS vs. additives); $p=0.0002$ (DAS vs. Former DAS). Up to 48% of these data are missing due to incomplete work histories. See text for details.
[4] $p=0.001$ (Former DAS vs. additives); $p=0.019$ (DAS vs. Former DAS).
[5] $p=0.11$ (all groups); $p=0.034$ (DAS vs. additives).
[6] $p=0.0007$ (DAS vs. additives); $p=0.009$ (Former DAS vs. additives).

Table 2. Adjusted Mean Testosterone Levels and Rates and Adjusted Odds Ratios for Lowest Quartile of Hormone Levels in Stilbene (DAS) Workers, Additives Workers, and Former DAS Workers[1]

Hormone	N	Adjusted Mean[2]	SE	Number (%) in Lowest Quartile[3]	OR (95% CI)[4]
Total Testosterone (TT) (ng/dL)					
DAS Workers	22	458[5]	1.1	8(36.4)	3.6(0.5 - 24.4)
Former DAS Workers	14	442[6]	1.1	3(21.4)	2.2(0.3 - 18.0)
Additives Workers	22	556	1.1	3(13.6)	---
Free Testosterone (FT) (pg/mL)					
DAS Workers	22	17.8	1.1	9(41.0)	2.5(0.5 - 13.1)
Former DAS Workers	14	17.4	1.1	5(35.7)	2.3(0.4 - 13.1)
Additives Workers	22	19.9	1.1	5(22.7)	---
FT:TT Ratio					
DAS Workers	22	0.40	0.0004	3(13.6)	0.3(0.4 - 2.0)
Former DAS Workers	14	0.40	0.0007	4(28.5)	1.3(0.2 - 8.8)
Additives Workers	22	0.37	0.0004	6(27.3)	---

[1] Additives workers are the comparison group for current and former DAS workers. All variables reported except free:total testosterone ratio (FT:TT) were normalized with natural log transformations prior to analysis, then untransformed for reported results. Standard errors of these untransformed means are analogous to adjusted geometric standard deviations. The FT:TT ratio was normalized with an arcsin transformation prior to analysis, then untransformed for reported results.

[2] Total testosterone was adjusted for age and body mass index (BMI). Free testosterone was adjusted for age and smoking. The FT:TT ratio was adjusted for age, smoking, and BMI.

[3] Lowest quartile for total testosterone was $\leq$ 386 ng/dL; for free testosterone, $\leq$ 15.0 pg/mL; for FT:TT ratio, $\leq$ 0.32.

[4] Additives workers are the comparison group for current and former DAS worker odds ratios. Hormones were adjusted for age and other factors as described in Note 2.

[5] $p = 0.05$.

[6] $p = 0.04$.

Table 3. Other Adjusted Mean Hormone Levels and Rates and Adjusted Odds Ratios for Highest Quartile of Hormone Levels in Stilbene (DAS) Workers, Additives Workers, and Former DAS Workers[1]

Hormone	N	Adjusted Mean[2]	SE	Number (%) in Highest Quartile[3]	OR (95% CI)[4]
Follicle Stimulating Hormone (FSH, mIU/mL)					
DAS Workers	22	11.8	1.1	8(36.4)	0.9(0.2 - 4.3)
Former DAS Workers	14	6.7[5]	1.2	3(21.4)	0.3(0.1 - 2.1)
Additives Workers	21	10.3	1.1	6(28.6)	---
Luteinizing Hormone (LH, mIU/mL)					
DAS Workers	22	12.0	1.1	5(22.7)	0.7(0.2 - 3.1)
Former DAS Workers	14	10.3	1.1	2(14.3)	0.4(0.1 - 2.3)
Additives Workers	21	11.7	1.1	7(33.3)	---
Prolactin (ng/mL)					
DAS Workers	22	9.7	1.1	5(22.7)	1.1(0.2 - 5.9)
Former DAS Workers	14	11.0	1.1	5(35.7)	1.9(0.3 - 9.9)
Additives Workers	22	9.4	1.1	4(18.2)	---
Estradiol (ng/L)					
DAS Workers	24	26.6	1.1	4(16.7)	1.0(0.2 - 5.2)
Former DAS Workers	14	28.7	1.2	2(14.3)	1.3(0.2 - 10.4)
Additives Workers	25	27.5	1.1	8(32.0)	---

[1] Additives workers are the comparison group for current and former DAS workers. All variables reported were normalized with natural log transformations prior to analysis, then untransformed for reported results. Standard errors of these untransformed means are analogous to adjusted geometric standard deviations.

[2] FSH was adjusted for age, smoking, BMI, and alcohol consumption. LH was adjusted for age. Prolactin was adjusted for age and smoking. Estradiol was adjusted for age and alcohol consumption.

[3] Highest quartile for FSH was $\geq$ 15.2 mIU/mL; for LH, $\geq$ 14.7 mIU/mL; for Prolactin, $\geq$ 13.5 ng/mL; and for Estradiol, $\geq$ 41.0 ng/mL.

[4] Additives workers are the comparison group for current and former DAS worker odds ratios. Hormones were adjusted for age and other factors as described in Note 2.

[5] $p = 0.06$.

REFERENCES

Abraham GE (1973): Radioimmunoassay of plasma steroid hormones. In Heftman E (ed): "Modern Methods of Steroid Analysis." New York: Academic Press Inc., pp 452-470.

Bartke A, Steele RE, Musto N, Caldwell BV (1973): Fluctuations in plasma testosterone levels in adult male rats and mice. Endocrinology 92:1223-1228.

Braunstein GD (1993): Gynecomastia. New Engl J Med 328:490-495.

Bremner WJ, Vitiello MV, Prinz PN (1983): Loss of circadian rhythmicity in blood testosterone levels with aging in normal men. Clin Endocrinol Metab 56:1278-1281.

Ciss M, Huyen N, Dutertre H, Phu-Lich N, Truhaut R (1980): Toxicological study of nitro-toluenes: long-term toxicity [French]. Dakar Med 25:293-302.

Gray TJB, Gangolli SD (1986): Aspects of the testicular toxicity of phthalate esters. Environ Health Perspectives 65:229-235.

Hammond SK, Smith TJ, Ellenbecker MJ (1987): Determination of occupational exposure to fabric brightener chemicals by HPLC. Am Ind Hyg Assoc J 48:117-121.

Harrington JM, Stein GF, Rivera RO, de Morales AV (1978): The occupational hazards of formulating oral contraceptives - a survey of plant employees. Arch Environ Health 33:12-15.

Hostetler KA, Leach MW, Hyde TE, Wei LL (1993): Evaluation of 4,4'-diamino-2,2'-stilbene-disulfonic acid (DAS) as a putative weak estrogen. Toxicologist [Abstract 676, Society of Toxicology 1993 Annual Meeting] 13:186.

Katz DF (1991): Human sperm as biomarkers of toxic risk and reproductive health. J NIH Res 3:63-67.

McCann D, Kirkish L (1985): Evaluation of free testosterone in serum. J Clin Immunoassay 8:234-236.

National Toxicology Program (1989): Para-nitrotoluene sperm motility vaginal cytology evaluation in rodents. NTP Contract No. NO1-ES-3-5026, March. Environmental Health Research and Testing, Inc., Lexington, KY. pp 29.

Odell WD, Rayford P, Ross GT (1967): Simple partially automated method for radioimmunoassay of human thyroid stimulating, growth, luteinizing and follicle stimulating hormones. J Lab Clin Med 70:973-980.

Odell WD, Ross GT, Rayford PL (1966): Radioimmunoassay of human luteinizing hormone. Metab Clin Exp 15:287-289.

Oishi S (1985): Reversibility of testicular atrophy induced by Di(2-ethylhexyl) phthalate in rats. Environ Res 36:160-169.

Quinn MM, Wegman DH, Greaves IA, Hammond SK, Ellenbecker MJ, Spark RF, Smith ER (1990): Investigation of reports of sexual dysfunction among male chemical workers manufacturing stilbene derivatives. Am J Indust Med 18:55-68.

Reynolds CF, Frank E, Thase ME, Houck PR, Jennings JR, Howell JR, Lilienfeld SO, Kupfer DJ (1988): Assessment of sexual function in depressed, impotent, and healthy men: factor analysis of a brief sexual function questionnaire for men. Psychiatry Research 24:231-250.

Ritter EJ, Scott WJ, Randall JL, Ritter JM (1987): Teratogenicity of Di(2-ethylhexyl) Phthalate, 2-ethylhexanol, 2-ethylhexanoic acid, and valproic acid, and potentiation by caffeine. Teratology 35:41-46.

Rodbard D, Jutt D (1974): Statistical analysis of radioimmunoassays and immunoradiometric (labeled antibody) assays, RIA and related procedures in medicine. Presented at the International Atomic Energy Agency Symposia, Istanbul, Turkey, September 1973. Vienna I:165-192.

SAS Institute, Inc. (1989): SAS/STAT User's Guide, Version 6, Fourth Edition, Volumes 1 and 2. Cary, NC: SAS Institute Inc.

Shmunes E, Burton DJ (1981): Urinary monitoring for diethylstilbesterol in male chemical workers. J Occup Med 23:179-182.

Sinha YN, Selby FW, Lewis UJ, Vanderlaan WP (1973): A homologous radio-immunoassay for human prolactin. J Clin Endocrinol Metab 36:509-516.

Smith ER, Quinn MM (1992): Uterotropic action in rats of amsonic acid and three of its synthetic precursors. J Toxicol Environ Health 36:13-25.

Spark RF, White RA, Connolly BP (1980): Impotence is not always psychogenic: newer insights into hypothalamic-pituitary-gonadal dysfunction. JAMA 243:750-755.

Takihara H, Sakatoku J, Fujii M, Cosentino MJ, Cockett ATK (1983): Significance of testicular size measurement in andrology. I. A new orchiometer and its clinical application. Fertil Steril 39:836-840.

Tomic R (1983): Some effects of orchiectomy, oestrogen treatment and radiation therapy in patients with prostatic carcinoma. Scand J Urol Nephrol Supplementum 77; Stockholm, Sweden: Almqvist and Wicksell.

Willems H (1981): Occupational exposure to estrogens and screening for health effects. J Occup Med 23:813-817.

Wortsman J, Hamidinia A, Winters SJ (1989): Hypogonadism following long-term treatment with diethylstilbestrol. Am J Med Sci 297:365-368.

Zaebst DD, Tanaka S, Haring M (1980): Occupational exposure to estrogens - problems and approaches. In McLachlan JA (ed): "Estrogens in the Environment." New York: Elsevier/North-Holland, pp 377-389.

4. REPORT II

Evaluation of Reproductive Function Among Men Occupationally Exposed to a Stilbene Derivative: II. Perceived Libido and Potency

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ABSTRACT

This is the second of two reports of a NIOSH Health Hazard Evaluation conducted in response to complaints of sexual dysfunction among men who manufacture the stilbene derivative 4,4'-diaminostilbene-2,2'-disulfonic acid (DAS; CAS 81-11-8), an intermediate in the manufacture of fluorescent whitening agents. The first report described results of the analysis of reproductive hormone levels. This second report provides results from the analysis of perceived libido and potency. In a cross-sectional design, self-reported sexual function of 30 male workers who manufacture DAS and 20 former DAS workers was compared to that of 35 workers who manufactured plastics additives in a different manufacturing area. Questionnaire items were examined by factor analysis, reducing the data to these components of sexual function: sexual activity/performance (two factors), interest, satisfaction, and physiologic competence. Adjusting for age, currently exposed workers were more likely than unexposed workers to have a value in the lowest quartile for interest (adjusted OR=1.9, 95% CI 0.5-7.2), physiologic competence (adjusted OR=1.9, 95% CI 0.6-6.4), and activity/performance factor II (adjusted OR=5.8, 95% CI 1.3-27.3). Former DAS workers reported problems associated with activity/performance factors I and II compared to unexposed workers (adjusted OR=2.2, 95% CI 0.5-10.1 and adjusted OR=6.7, 95% CI 1.2-35.9, respectively). Although the small study size limits the precision of the effect estimates, the pattern of results suggests a possible effect on sexual function of working in the DAS manufacturing area.

Key words: impotence, serum testosterone, occupational exposures, stilbene manufacture

INTRODUCTION

The stilbene derivative 4,4'-diaminostilbene-2,2'-disulfonic acid (DAS; CAS 81-11-8), an intermediate in the manufacture of fluorescent whitening agents is speculated to have estrogenic properties based on structural similarity with estradiol and with the potent synthetic estrogen diethylstilbestrol (DES). Adverse male reproductive effects from exposure to estrogenic substances such as oral contraceptives and DES have been reported to include lowered testosterone levels (Willems, 1981), decreased libido and impotence (Harrington et al., 1978). In a previous investigation at this plant in 1981, researchers found that 14% of the DAS workers experienced impotence (defined as a failure rate of 25% or greater to achieve erection during sexual activity) in the preceding six months, and 36% experienced decreased libido (defined as a decreased desire for sexual activity, leading to a 25% or greater decline in frequency) during the 2 years prior to the study (Quinn et al., 1990). The investigators were unable to conclude that DAS work was associated with reproductive impairment because of the absence of a comparison group. In the accompanying report of a 1991 study, both current and former DAS workers had lower mean testosterone levels than the unexposed group, after adjustment for age and body mass. We present results from the analysis of 1991 survey data on perceived libido and potency among current and former DAS workers and a group of unexposed workers from the same plant.

MATERIALS AND METHODS

Details of the study design and methods have been described in the accompanying report.

Study Population and Data Collection

A target study population of 129 male workers was identified. Thirty-three were currently working in the DAS manufacturing area ("DAS workers"); 26 were employees who worked in the DAS area at the time of the first investigation (May 1981 through December 1983) but were no longer working in the DAS area ("Former DAS workers"); and 70 were workers from a separate manufacturing area which produces plastics additives ("Additives workers").

Shift-standardized blood samples were analyzed for levels of the reproductive hormones free and total testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin, and estradiol.

A questionnaire obtained information on demographics, smoking and drinking habits, work history, exposures to chemical and physical agents, and medical history including factors that may be associated with depressed libido such as diabetes mellitus, thyroid disease, obesity, alcohol ingestion, use of certain medications, and neurologic disease. Questions to assess potency and libido were excerpted from the 21-item Brief Sexual Function Questionnaire of Reynolds et al. (1988), which has acceptable test-retest reliability and construct validity (Howell et al., 1987; Reynolds et al., 1988). The survey is designed to assess four principal aspects of

sexual function: interest, activity, satisfaction, and physiologic competence. We chose to include the 15 items that correlated highest with these four factors in a factor analysis conducted by Reynolds et al. (1988).

Definition of Exposure Groups

A current DAS worker was defined as an employee in the DAS production area who had worked there for a minimum of 30 days immediately previous to our study. Former DAS workers were employees currently working in other areas of the plant who had worked 30 days or more in the DAS area during the time of the first investigation (May 1981 through December 1983). The comparison group of Additives workers was defined as those workers currently employed in the Additives department who had spent less than 180 days in the DAS production area.

Data Analysis

Factor analysis was used to take into account the correlations inherent in answers to questions regarding symptoms of libido and potency. Factor analysis reduces a set of correlated variables to a smaller set of conceptually meaningful, relatively independent variables called "factors" (Rossi et al., 1983). A participant's questionnaire responses are then weighted by the factor loadings (or correlation coefficients) to derive individual factor scores so that each person has a summary score for each of the factors. Exposed and unexposed group factor scores were compared using analysis of variance (ANOVA). Stratified analyses were conducted for potentially confounding variables, and adjusted odds ratios and 95 percent confidence intervals were computed. Logistic regression was used to model the association between work in the DAS area and a low score (less than the 25th percentile) on each of the factors, controlling for potential confounding variables. The covariates considered as potential confounders were age, race, marital status, employment status (hourly/salaried), body mass index (kg/m^2), tobacco use, alcohol consumption, current use of medications, other exposures outside of work, history of diabetes, history of mumps at age 11 or later, history of neurological problems, and an index of sleep quality and cumulative sleep debt in the week preceding study data collection.

To examine the link between reported symptoms of libido/potency and levels of testosterone, two-way analysis of variance was used to calculate adjusted least squares mean hormone levels for each combination of exposure and dichotomized libido/potency factor. The libido/potency factor scores were dichotomized into those scores falling below the 25th percentile for that factor, and those scores falling into the upper three quartiles.

RESULTS

Participation rates and characteristics of the study population are described in detail in the accompanying report. The study population consisted of 30 current DAS workers, 35 additives workers, and 20 former DAS workers. In general, current and former DAS workers were significantly older than additives workers (mean ages 45.9 and 45.2, respectively vs. 39.0; $p<0.01$) and had been employed longer at the company (mean years 20.3 and 22.2, respectively vs. 15.1; $p<0.01$). Current and former DAS workers were significantly more likely than unexposed workers to report that they believed workplace

exposures affect sex drive and fertility (68% and 60%, respectively vs. 23%; $p<0.01$).

Responses to selected questions regarding sexual function are presented in Table I. Exposed workers were less likely to feel sexual drive more than once a week compared to unexposed men (70% and 86%, respectively; $p=0.12$), and less likely to have experienced full erections during sexual activity compared to unexposed (79% and 94%, respectively; $p=0.06$). Exposed workers were also more likely than unexposed workers to report improvements in sexual function (increased sex drive and better erections) when they are away from work for one or more days.

We used factor analysis to identify five sexual function factors. Three of these factors -- Interest, Satisfaction, and Physiologic Competence -- were also reported by Reynolds et al. (1988) from which the survey instrument was adapted. A fourth factor reported by Reynolds, "Activity/Performance", fell into two separate factors in our study. We called these factors Activity/Performance I and II. Table II shows the questionnaire content of the items that make up each of the five factors. For example, questions regarding length of intercourse and quality of erection were correlated with one another and, grouped together, form a factor called "Activity/Performance I."

Exposed and unexposed group mean scores were compared for each of the five factors, adjusted initially only for age. A lower mean score indicates lower reported libido or potency. Figure 1 shows the difference between the exposed and the comparison group means and the 95% confidence intervals for the difference between means. Although the confidence intervals are wide, indicating a lack of precision in the estimates, the exposed group had lower mean scores for the activity/performance factors than did the unexposed group. Because a comparison of means may mask an association at the extremes of libido/potency scores, we also examined the association between working in the DAS area and having a value in the lowest quartile (i.e., low reported libido/potency) for each of the five sexual function factors (Table III). Exposed workers were more likely than unexposed workers to have a value in the lowest quartile for interest (adj OR=1.9, 95% CI 0.5-7.2), physiologic competence (adj OR=1.9, 95% CI 0.6-6.4), and activity/performance II (adj OR=5.8, 95% CI 1.3-27.3).

Marital status, employment status (hourly/salaried), and use of certain prescription medications were considered potential confounders or effect modifiers of the association between exposure and low libido/potency. Because of the small number of workers in certain of these subgroups, a subanalysis was restricted to the relatively homogenous and larger group of married, hourly employees not currently taking prescription medications for hypertension or allergies. In this subgroup, exposed men were 5-17 times more likely to report problems with activity/performance compared to unexposed men (adj OR=5.3, 95% CI 0.8-37.2 for activity/performance I, and adj OR=17.8, 95% CI 1.5-217.4 for activity/performance II) (Table III). The confidence intervals are quite wide due to the small study size. Exposed workers were also twice as likely as unexposed workers to report problems with interest (adj OR=1.9, 95% CI 0.3-11.9).

Table IV presents the results for workers employed in the DAS area during the 1981-83 investigation, but no longer employed in that department. Age-adjusted odds ratios indicate an approximately 2- to 6-fold increase in reporting of problems associated with activity/performance compared to the additives workers (adj OR=2.2, 95% CI 0.5-10.1 for

activity/performance I, and adj OR=6.7, 95% CI 1.2-35.9 for activity/performance II), and almost a 3-fold increase in reporting problems associated with interest (adj OR=2.7, 95% CI 0.6-11.2). These odds ratios are larger, although less precise, in the restricted analysis. An elevated odds ratio for reported problems associated with physiologic competence (OR=3.7, 95% CI 0.5-29.5) was also observed among former DAS workers compared to additives workers (Table IV).

Finally, we examined the association between self-reported symptoms of low libido/potency and adjusted mean testosterone levels by exposure status. This analysis was restricted to the group of married, hourly employees not currently taking prescription medications for hypertension or allergies and was adjusted for age and body mass index as described in the accompanying report. Two Additives workers with outlying values for testosterone (1,440 ng/dL and 1,510 ng/dL) were excluded from this analysis. When each of the three exposure groups were considered separately, we found no clear relationship between testosterone level and reported symptoms of low libido/potency (lowest quartile versus all other quartiles). However, we observed somewhat lower adjusted mean testosterone levels in DAS workers in the lowest quartile for activity/performance I than in additives workers in the lowest quartile (382.9 vs. 628.1 ng/dL, respectively, p=0.10) (Figure 2). Former DAS workers in the lowest quartile for physiologic competence had a lower adjusted mean testosterone level than additives workers in the lowest quartile (382.2 vs. 603.5 ng/dL respectively, p=0.06). No clear patterns were found in adjusted mean testosterone levels for activity/performance II or for the other factors. Retaining the two Additives workers with outlying testosterone values in the analysis strengthened the results.

DISCUSSION

This is the second of two reports of a NIOSH investigation of workers who manufactured the stilbene derivative, DAS. The first report found lower mean testosterone levels in the current and former DAS workers than in a group of currently unexposed workers. The analysis presented here found that both current and former DAS workers report more symptoms of impotence and decreased libido than workers not exposed to DAS.

Selection bias may have occurred if study participation was correlated with the outcome of interest (i.e., if workers with symptoms were more likely to participate than those without symptoms). The high participation rates in the exposed groups (88% and 89% for current and former DAS workers, respectively) indicate that the probability of serious selection bias in these groups is relatively low. The participation rate of 59% in the unexposed comparison group is of some concern; if unexposed workers with symptoms were less likely to participate (e.g., because of difficulty discussing perceived sexual dysfunction), the bias would be away from the null. However, when we compared data from nonparticipants to similar data obtained from participants, no clear differences in demographics or in number of pregnancies fathered were observed. Among workers in the comparison group, participants were no more likely than non-participants to say that they believed workplace exposures affect sex drive or fertility (23% versus 24%, respectively).

The study was limited by the lack of established methods to analyze biological samples for DAS. The "measure" of exposure in this study is work area in the plant. However, since it

is unknown whether DAS is actually the etiologic agent, work area in general may serve as a reasonable proxy measure of exposure in this case. Based on an industrial hygiene evaluation and examination of material safety data sheets, the comparison group was not currently exposed to DAS or substantive quantities of any other known reproductive toxicant, thereby minimizing the potential for misclassification of exposure status.

Information used to derive the outcome variables was obtained by administering a questionnaire, so outcome measures were necessarily of a subjective nature. This subjectivity may have resulted in overreporting of symptoms in the exposed group and/or underreporting of symptoms in the unexposed group. Current clinical tools to measure erectile dysfunction were considered impractical for field applications. The similarity of the sexual function factors we identified with those reported by previous investigators in clinic populations of healthy, depressed, and impotent men using a similar survey instrument (Reynolds et al., 1988) strengthens our confidence in the reliability of the instrument, although the questionnaire data by themselves cannot be determined to be free of reporting bias. The fact that many more exposed workers than unexposed workers reported a belief that workplace exposures affect sex drive or fertility is difficult to evaluate since this could be a measure of reporting bias or of the existence of a true effect of exposure. Improvements in sexual function when the respondent was away from work for one or more days may be a psychological rather than a physiological effect.

We did not observe a clear association between testosterone levels and symptoms, although somewhat lower mean testosterone levels were observed for workers in the exposed groups who reported symptoms of lowered activity/performance (activity/performance I; current DAS workers) and physiologic competence (former DAS workers). We did not see a relationship between mean testosterone levels and activity/performance factor II, which was associated most strongly with both current and former DAS work. Impotence and lowered libido have a variety of potential causes, only some of which may affect the hypothalamic-pituitary-gonadal axis and result in altered testosterone levels (Spark et al., 1980; Tsitouras et al., 1982; Slag et al., 1983). The nature of the relationship between hormonal factors and sexual behavior remains to be elucidated.

Detailed information was collected on potential confounders and account taken in the analysis of their possible effects. It appears unlikely that any bias in the estimates of the odds ratios arose from known confounders. Although it is possible that unknown or unmeasured confounding factors may partially explain the observed effects, such a factor would have to have a very strong relationship with exposure and/or sexual function to account for the magnitude of our findings.

CONCLUSIONS

Although the small study size limits the precision of the effect estimates, the pattern of results suggest a possible effect on sexual function of working in the DAS manufacturing area. Both current DAS workers and men who formerly worked in the DAS area reported more symptoms of sexual dysfunction than comparison workers. The biological plausibility of an estrogenic effect of DAS exposure on reproductive function and consistency with prior reports of adverse male reproductive effects resulting from occupational and clinical estrogen exposure (Willems, 1981; Shmunes and Burton, 1981;

Wortsman et al., 1989; Harrington et al., 1978; Zaebst et al., 1980) provide evidence that this may be a meaningful association.

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REFERENCES

Hammond SK, Smith TJ, Ellenbecker MJ (1987): Determination of occupational exposure to fabric brightener chemicals by HPLC. Am Ind Hyg Assoc J 48:117-121.

Harrington JM, Stein GF, Rivera RO, de Morales AV (1978): The occupational hazards of formulating oral contraceptives - a survey of plant employees. Arch Environ Health 33:12-15.

Howell JR, Reynolds CF, Thase ME, Frank E, Jennings JR, Houck PR, Berman S, Jacobs E, Kupfer DJ (1987): Assessment of sexual function, interest and activity in depressed men. J Affect Dis 13:61-66.

Quinn MM, Wegman DH, Greaves IA, Hammond SK, Ellenbecker MJ, Spark RF, Smith ER (1990): Investigation of reports of sexual dsyfunction among male chemical workers manufacturing stilbene derivatives. Am J Indust Med 18:55-68.

Reynolds CF, Frank E, Thase ME, Houck PR, Jennings JR, Howell JR, Lilienfeld SO, Kupfer DJ (1988): Assessment of sexual function in depressed, impotent, and healthy men: factor analysis of a brief sexual function questionnaire for men. Psychiatry Research 24:231-250.

Rossi PH, Wright JD, Anderson AB (1983): Handbook of Survey Research. Academic Press, Inc. Page 269.

Shmunes E, Burton DJ (1981): Urinary monitoring for diethylstilbesterol in male chemical workers. J Occup Med 23:179-182.

Slag MF, Morley JE, Elson MK, Trence DL, Nelson CJ, Nelson AE, Kinlaw WB, Beyer S, Nuttall FQ, Shafer RB (1983): Impotence in medical clinic outpatients. JAMA 249:1736-1740.

Spark RF, White RA, Connolly MS (1980): Impotence is not always psychogenic. JAMA 243:750-755.

Tsitouras PD, Martin CE, Harman SM (1982): Relationship of serum testosterone to sexual activity in healthy elderly men. J Gerontol 37:288-293.

Willems H (1981): Occupational exposure to estrogens and screening for health effects. J Occup Med 23:813-817.

Wortsman J, Hamidinia A, Winters SJ (1989): Hypogonadism following long-term treatment with diethylstilbestrol. Am J Med Sci 297:365-368.

Zaebst DD, Tanaka S, Haring M (1980): Occupational exposure to estrogens - problems and approaches. In McLachlan JA (ed): "Estrogens in the Environment." New York: Elsevier/North-Holland, pp 377-389.

TABLE 1. Characteristics of libido and potency experienced during the past month by current DAS workers, former DAS workers, and Additives workers

Questionnaire item	Current DAS Workers (N=30)	Former DAS Workers (N=20)	Additives Workers (N=35)
Currently have a sex partner	30 (100%)	19 (95%)	35 (100%)
Have been sexually active in the past month	28 (93%)	19 (95%)	35 (100%)
Have felt sexual drive more than once a week	21 (70%)	12 (60%)[1]	30 (86%)
Increase in sex drive when away from work one or more days	13 (43%)	8 (40%)	12 (35%)
Have always felt pleasure during sexual activity	21 (75%)	15 (79%)	31 (89%)
Have experienced full erections during sexual activity	22 (79%)[2]	15 (79%)[3]	33 (94%)
Better erections when away from work one or more days	8 (30%)	6 (30%)	6 (18%)
Have not lost or can always regain lost erections	22 (79%)	14 (74%)	31 (89%)
Have had nocturnal erections once a week or more	17 (61%)	12 (60%)	19 (54%)
Have ejaculated once a week or more	21 (70%)	13 (65%)	28 (80%)

[1] $p=0.031$ (Former DAS vs. Additives)
[2] $p=0.063$ (Current DAS vs. Additives)
[3] $p=0.087$ (Former DAS vs. Additives)

TABLE 2. Question content of items that loaded highest on each of five dimensions of sexual function

Factor I **"Activity/Performance I"**
How erect penis during sexual activity
Pleasure from sexual experiences
Length of intercourse after insertion
Ejaculation without full erection
Factor II **"Interest"**
Frequency of sex drive
Frequency of sexual thoughts, fantasies, etc.
Frequency of sexual activities
Response to partner's sexual advances
Factor III **"Satisfaction"**
Satisfaction with sex life
Satisfaction with sexual relationship with partner
Factor IV **"Physiologic Competence"**
Ability to regain erection
A.M. erections
Problem ejaculating when aroused
Factor V **"Activity/Performance II"**
How often ejaculate
Premature ejaculation

FIGURE 1. Difference in age-adjusted mean factor scores (and 95% confidence intervals) between DAS workers and Additives workers

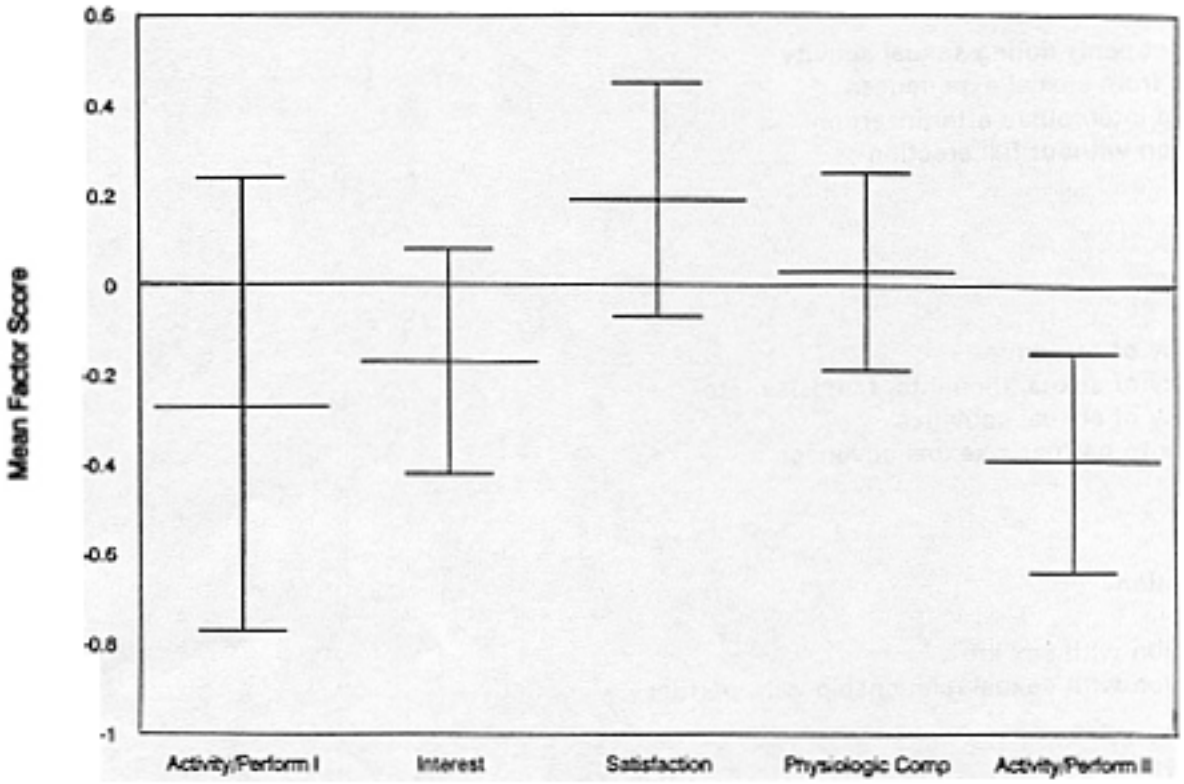


TABLE III. Odds of having a value in the lowest quartile for each sexual function factor: Current DAS workers compared to Additives workers

			Restricted Analysis**	
Factor	Total number of workers/Number in lowest quartile	Age-Adjusted OR (95% CI)*	Total number of workers/Number in lowest quartile	Age-Adjusted OR (95% CI)
Activity/performance I	49/12	1.3 (0.3-5.3)	22/8	5.3 (0.8-37.2)
Interest	46/15	1.9 (0.5-7.2)	22/8	1.9 (0.3-11.9)
Satisfaction	44/17	0.7 (0.2-2.6)	17/13	1.0 (0.2-4.9)
Physiologic competence	44/17	1.9 (0.6-6.4)	24/6	0.6 (0.1-5.4)
Activity/performance II	49/12	5.8 (1.3-27.3)	23/7	17.8 (1.5-217.4)

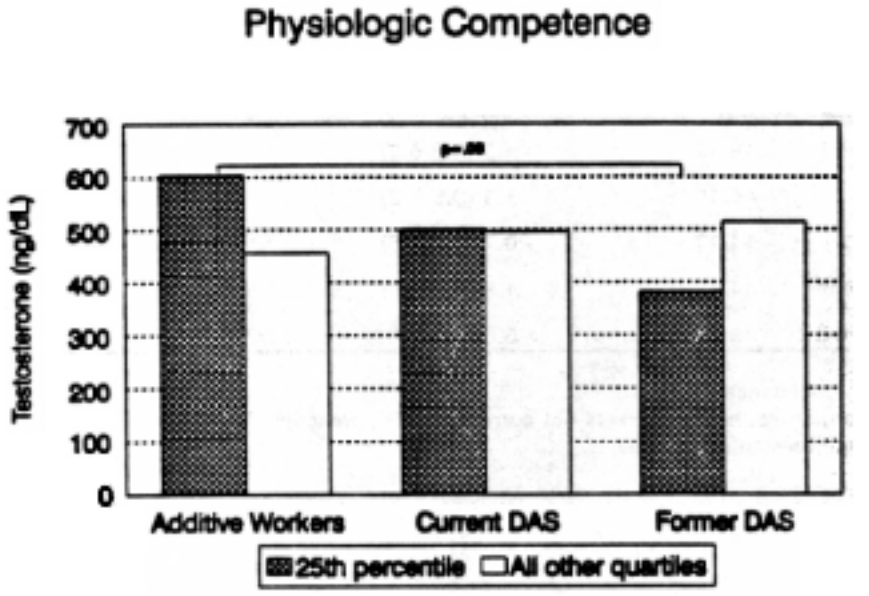
* OR, odds ratio; CI, confidence interval.
** Restricted to married, hourly workers not currently taking prescription medications for hypertension or allergies.

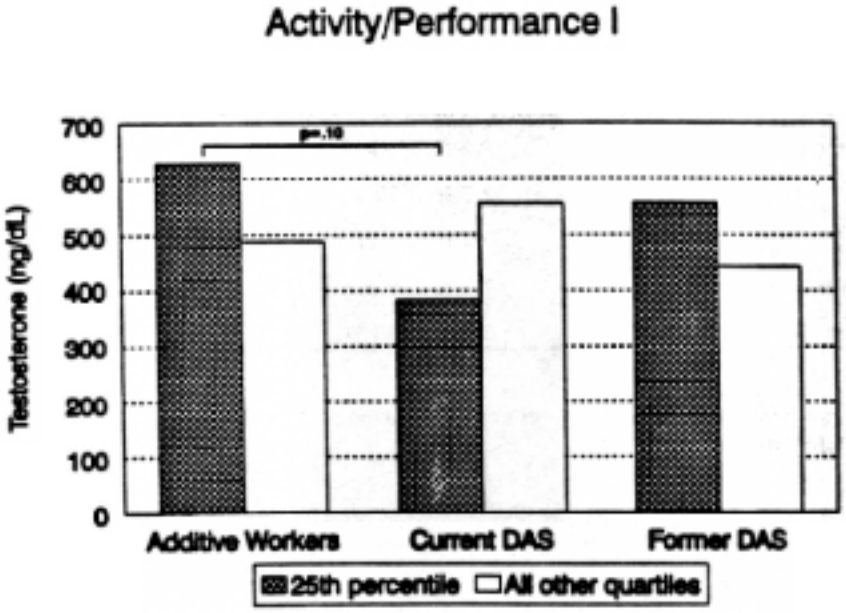
TABLE IV. Odds of having a value in the lowest quartile for each sexual function factor: Former DAS workers compared to Additives workers

			Restricted Analysis**	
Factor	Total number of workers/Number in lowest quartile	Age-Adjusted OR (95% CI)*	Total number of workers/Number in lowest quartile	Age-Adjusted OR (95% CI)
Activity/performance I	42/10	2.2 (0.5-10.1)	22/4	3.9 (0.3-44.5)
Interest	40/12	2.7 (0.6-11.2	19/7	3.4 (0.5-22.1)
Satisfaction	38/14	0.7 (0.2-2.7)	17/9	0.3 (0.0-2.2)
Physiologic competence	40/12	1.3 (0.3-5.3)	20/6	3.7 (0.5-29.5)
Activity/performance II	42/10	6.7 (1.2-35.9)	21/5	11.1 (1.0-129.4)

* OR, odds ratio; CI, confidence interval.
** Restricted to married, hourly workers not currently taking prescription medications for hypertension or allergies.

FIGURE 2. Mean testosterone levels by exposure status and quartile of perceived libido/potency (lowest quartile vs. all other quartiles); adjusted for age and body mass index.





5. RECOMMENDATIONS

Air sampling and biomonitoring for exposure to DAS were not conducted in this study because there currently are no NIOSH, OSHA, or any other fully validated methods for analyzing air samples or biological specimens for DAS. Besides DAS, more than one of the process chemicals or intermediates may have had potential for association with the adverse effects we found in the DAS manufacturing area workers. Although the exact relationship between DAS manufacturing and male reproductive health is not known, NIOSH considers that the DAS manufacturing process may contain one or more potential male reproductive toxicants and recommends that exposure to chemicals in the DAS manufacturing process be reduced to the lowest feasible concentration. Observations made during the survey suggest that direct skin contact with DAS or its precursors could be reduced by the routine use of gloves and the periodic cleaning of the yellow residue found on many surfaces in the DAS manufacturing area. Improved housekeeping in certain specific areas of the DAS production area such as the final bagging and filtration areas could also reduce exposures. Requiring showers and a clothing change area could reduce the possibility of wearing contaminated clothing out of the facility, and thus reduce the potential for contamination of the home environment.

Because the observations of reduced testosterone and symptoms of lowered potency and libido appear to be persistent, workers with low serum testosterone levels or symptoms of altered libido or potency are encouraged to consult with their personal physicians for followup examination.

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