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Testicular function of men occupationally exposed to para-tertiary butyl benzoic acid

by Whorton MD, Stubbs HA, Obrinsky A

Affiliation: Environmental Health Associates, Inc; Berkeley; CA 94704, United States.

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Table 3. Frequency and percentage distribution of exposure indices by time period for 51 participants with semen samples and 31 [a] nonparticipants and nonvasectomized participants without semen samples. (Source: Shell p-TBBA data, 1980)

Exposure points	Number of participants		Number of nonparticipants [a]	
	Frequency	%	Frequency	%
1954—1979				
< 500	43	84.3	25	89.3
500—999	4	7.8	1	3.6
1,000—1,499	3	5.9	1	3.6
≥ 1,500	1	2.0	1	3.6
1969—1979				
< 50	29	56.9	20	71.4
50—99	10	19.6	3	10.7
100—149	6	11.8	0	0
150—199	5	9.7	3	10.7
≥ 200	1	2.0	2	7.1
1978—1979				
< 5	26	51.0	17	60.7
5—9	15	29.4	5	17.9
10—14	6	11.8	4	14.3
15—19	3	5.9	0	0
20—24	1	2.0	2	7.1

[a] Includes three of six individuals who were classified as participants but who did not provide semen samples. The exposures of the remaining three could not be quantified and are therefore not included in either category.

Table 4. Mean sperm-count results (million cells/ml) by years of exposure and years since last exposure for the 51 participants who provided semen specimens. (Source: Shell p-TBBA data, 1980)

Length of exposure (a)	Number of years since last exposure						
	0 (Currently exposed)		1	2—3	4—5	6—10	> 10
≥ 10	55 58 181 6.5 53 57	9.5 71.5 125.5 210.5 3 84					
5—9	31 112 96 105	72 40.5 73 185.5					
4	107 54						
3	87 123						0
2	55.5 101					337.5	60
1	17.5		51	62		55 72	
< 1	82 121 84 95 36 99	86 48.5 4		87.5	4 23	135.5 253.5 3	53 29

of oligospermic men in the p-TBBA group (13.7 %) was somewhat higher than that in the reference group (4.9 %), the proportions of oligospermic individuals in the two groups were calculated, and the hypothesis of no difference between the two proportions was tested by a two-sample test for the difference of two proportions. No significant excess of oligospermic individuals was found in the p-TBBA-exposed group at the 5 % significance level.

Table 4 presents individual mean sperm counts classified by number of years of exposure (duration) and years since last exposure. This table was designed to array the individual sperm-count data in such a way that, if sperm-count depression were a function of years of exposure to p-TBBA, one would expect to see lower individual mean sperm counts in the upper cells of the matrix. Moreover, some notion of recovery — or lack of it — might be revealed by an inspection of the cells in the upper right portion of the matrix. Relatively low counts in these cells would be consistent with an absent or prolonged recovery phase. The data arrayed in this table do not suggest an association between mean sperm-count levels and years of exposure to p-TBBA. The number of study subjects was considered too small to evaluate the sperm-count results by job category.

More quantitative evaluations of these data for the presence of associations between exposure index scores and sperm count were performed. Two transformations of sperm count were examined to ascertain which transformation, log base 10 or square root, yielded the more normal distributions. The square root provided the most normal distributions of first, best, worst, and mean sperm count, and the logarithm was found to normalize exposure index scores. The Pearson correlation coefficients between the square roots of the four sperm-count measures and the log exposure index scores for the three time periods are shown in table 5. There were no statistically significant ($p < 0.05$) relationships found between square-root mean or worst sperm count and the log exposure index score for any of the time periods. The log exposure index score was found to be positively associated with square-root best sperm count for the time period 1954—1979 ($p = 0.049$) and with square-root first sperm count for the periods 1954—1979 ($p = 0.043$), and 1969—1979 ($p = 0.042$).

Table 5. Pearson correlation coefficients (r) and significance probabilities (p) for the square root of various sperm-count measures with log exposure points for three time periods for the 51 participants who provided semen specimens. (Source: Shell p-TBBA data, 1980)

Sperm-count measure	Exposure period		
	1954—1979	1969—1979	1978—1979
Mean			
r	0.219	0.208	0.149
p	0.061	0.072	0.148
Best			
r	0.234	0.203	0.126
p	0.049	0.076	0.189
Worst			
r	0.189	0.213	0.179
p	0.092	0.066	0.105
First			
r	0.243	0.245	0.188
p	0.043	0.042	0.093

Sperm cell morphology was evaluated for all the semen specimens. The mean value for normal morphology was 94.9 % with a standard deviation of 4.8 %. The morphology was less than 90 % in 10 samples: In seven samples the sperm counts all exceeded 55 million/ml, while the other three had sperm counts of less than 5 million cells/ml.

Few individuals had indicated a history of infertility. There was no relationship of work in the p-TBBA unit and infertility problems among the participants.

In the animal toxicology study, p-TBBA had the ability to affect the kidneys and liver adversely, as well as hematopoiesis. Thus results from the blood chemistry panels and hemograms were correlated with the exposure index scores, as were the blood hormone results. The sperm-count results and age were also correlated with the same laboratory test results. These laboratory tests were FSH, LH, testosterone, creatinine, blood urea nitrogen (BUN), serum glutamic oxalacetic transaminase, bilirubin, alkaline phosphatase, cholesterol, trigylceride, red blood cell count, and white blood cell count. Some variables were transformed into the logarithm or reciprocal when necessary.

The Pearson correlation coefficients be-

tween each of the 12 laboratory test results and exposure indices are shown in table 6 by time period for all 90 participants. For the exposure period 1954—1979, the statistically significant correlations ($p < 0.05$) with log exposure were LH (positive), testosterone (negative), BUN (positive), and cholesterol (positive). For the exposure period 1969—1979, the statistically significant correlations were FSH (positive), LH (positive), testosterone (negative), and cholesterol (positive). For the exposure period 1978—1979, statistically significant correlations with log exposure were negative for both alkaline phosphatase and triglyceride.

Correlation coefficients for these same 12 laboratory tests with age were calculated for all 90 participants. The statistically significant correlations ($p < 0.05$) with age were positive for FSH ($r = 0.38$), LH ($r = 0.21$), and cholesterol ($r = 0.22$) and negative for testosterone ($r = -0.19$) and white blood cell count ($r = -0.18$).

Finally, for the 51 participants providing semen samples, correlation coefficients for the same 12 laboratory tests and age with the square root of the mean sperm count were examined. The statistically significant correlations ($p < 0.05$) with square-root mean sperm count were FSH ($r = -0.36$) and cholesterol ($r = 0.31$).

Table 6. Pearson correlation coefficients (r) and significance probabilities (p) between 12 laboratory blood test results and log exposure index scores for three exposure periods for all 90 exposed participants. (Source: Shell p-TBBA data, 1980)

Laboratory test	Exposure period		
	1954—1979	1969—1979	1978—1979
Follicle stimulating hormone [a]			
r	0.124	0.212	0.035
p	0.122	0.022	0.373
Luteinizing hormone [a]			
r	0.229	0.238	0.129
p	0.015	0.012	0.113
Testosterone			
r	0.180	0.184	—0.077
p	0.045	0.041	0.235
Creatinine [b]			
r	0.140	0.155	0.152
p	0.094	0.073	0.077
Blood urea nitrogen			
r	0.179	0.082	0.127
p	0.046	0.220	0.116
Serum glutamic oxalacetic transaminase [a]			
r	0.020	0.022	0.029
p	0.425	0.417	0.393
Bilirubin			
r	0.156	0.069	0.046
p	0.071	0.260	0.334
Alkaline phosphatase			
r	0.087	—0.051	—0.209
p	0.208	0.318	0.024
Cholesterol			
r	0.271	0.214	—0.075
p	0.005	0.021	0.240
Triglycerides			
r	0.034	0.058	—0.176
p	0.375	0.294	0.048
Red blood cell count			
r	0.143	0.09	0.061
p	0.092	0.201	0.285
White blood cell count			
r	0.016	0.108	0.033
p	0.440	0.157	0.380

[a] Logarithm.
[b] Reciprocal.

Discussion

Earlier studies (1, 6) have indicated that chemically related interference with the male reproductive process is likely to be expressed in at least one of the following three ways: (i) demonstrable suppression of sperm count, (ii) infertile marriage, or (iii) abnormal gonadotropin levels. The sample of 90 male p-TBBA workers was evaluated with these three possible exposure-related outcomes in mind.

The concept of a "normal" sperm count has little meaning. Sperm counts ranging from a few million to more than 600 million have been encountered in groups of men judged to be free from exposure to chemicals that might affect sperm production. Thus, for a determination of whether men exposed to a chemical are experiencing sperm-count suppression as a result of that exposure, the distribution of sperm counts must be examined for the group. Additional information can be developed if the sperm-count distribution of the exposed group is compared with a similar distribution representing a population known to be free from exposure to known testicular toxins. In the present study, the sperm-count distributions of 51 p-TBBA-exposed men and 103 men in the nonexposed reference group were not significantly different. Nor was there a significant difference in the proportion of oligospermic men in the exposed and reference groups.

Statistical significance notwithstanding, the p-TBBA-exposed population included almost 16 % oligospermic men, while in the comparison population, only 6.5 % fell into this category. To assist in the interpretation of this observation, various exposure categories were examined for associations with mean sperm count. Mean sperm-count results were compared to years of p-TBBA exposure and years from last exposure (table 4). The data did not suggest an association between mean sperm-count values and p-TBBA exposure.

A more quantitative evaluation of exposure and sperm-count results (table 5) showed that the log exposure score was significantly related to the square-root best sperm count for the time period 1954—1979 and to the square root of first sperm count for the periods 1954—1979 and 1969—1979. No other statistically significant relationships were found. All three positive correlation coefficients can be interpreted as suggesting that an increase in exposure is related to an increase in the best (or the first) sperm count for these time periods.

From the analysis of all the data related to sperm production, we have concluded that there is no evidence of sperm-count suppression associated with p-TBBA exposure at the levels experienced in this plant. This conclusion is based on the following observations: (i) there was no significant difference between the proportions of oligospermic men in the reference and exposed populations; (ii) the sperm-count distributions for these two populations were not dissimilar; and (iii) there were no significant negative associations between sperm count and any of the several meaningful measures of exposure intensity and duration that could be formulated.

In contrast to earlier studies of DBCP (1, 6), there were few complaints of infertile marriages in the full cohort of 90 men. The percentage of men with infertile marriages was less than the 10—15 % expected in the general population.

The results of the hormone immunoassays were, for the most part, unremarkable. All values were within established laboratory normal ranges, except for three elevated FSH values. Two of these FSH values were associated with sperm counts of less than 5 million cells/ml. The other elevated FSH level occurred in a vasectomized man. These results are consistent with the data of Whorton et al (6) suggesting that FSH, LH, and testosterone together are not well correlated with chemically induced sperm-count suppression except in azoospermic men.

The significant correlations noted between the two exposure periods (1954—1979 and 1969—1979) and the specific laboratory tests are the same as those noted between age and laboratory results. The positive correlation of the exposure period 1954—1979 with BUN alone is not indicative of renal disease; only a simultaneous positive correlation with creatinine would signify renal disease. In our opinion the observed negative correlations for the exposure period 1978—1979 with alkaline phosphatase and triglycerides probably have no biological importance.

An elevation of FSH and LH and decreased testosterone may be seen in elderly men. An elevation in cholesterol levels with age would generally be expected.

The negative correlation between sperm count and FSH levels should be expected given the known relationship between the testis and the pituitary (4). The positive correlation between cholesterol and sperm count is interesting, but we know of no biological explanation for this observation.

Caution should be exercised in the interpretation of these correlation results since they are presented for descriptive purposes and the problem of multiple comparisons is introduced in the presentation of significance probablities from a large number (61) of comparisons. For example, approximately three p-values of less than 0.05 would be expected due to chance if all coefficients were independent. In addition these results should be evaluated with the knowledge that two of the three log exposure variables (1954—1979 and 1969—1979) were found to be highly correlated with age ($r = 0.55$, $p < 0.0001$; and $r = 0.32$, $p < 0.003$, respectively).

In conclusion, p-TBBA, at the levels of exposure experienced at this plant, does not have an apparent clinical or epidemiologic effect on testicular function. Furthermore there is no evidence that exposure to p-TBBA has caused infertility among the male employees who participated in this study. Finally, no adverse effects were seen in hepatic or renal function or hematopoiesis.

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