# Occupational Exposure to Estrogens

## A Report of Two Pilot Medical and Industrial Hygiene Surveys

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Project Officers:
Shiro Tanaka, M.D.
Dennis D. Zaebst, M.S., C.I.H.

Industrywide Studies Branch
Division of Surveillance, Hazard Evaluations
and Field Studies
National Institute for Occupational Safety and Health
Centers for Disease Control
United States Public Health Service
Department of Health and Human Services

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DISCLAIMER

Mention of company names or products does not imply or constitute endorsement by the National Institute for Occupational Safety and Health.

## ABSTRACT

Pilot industrial hygiene and biomonitoring surveys of occupational exposure to estrogens were conducted at two pharmaceutical plants. Plant A* manufactures estrogen replacement tablets using natural conjugated estrogens. Plant B* manufactures oral contraceptive pills using synthetic estrogens and progestogens. The surveys were conducted to assess the necessity and feasibility of additional (or more extensive) medical and industrial hygiene surveys, to evaluate the characteristics of workers' biological responses to estrogen exposure, and the corresponding exposures of workers to estrogens.

Industrial hygiene data at Plant A indicated that exposures to total conjugated estrogens in air ranged from 0.48 to 5.32 $ug/m^3$, as compared to the derived target exposure limit (DTEL)** of 0.4 $ug/m^3$. At Plant B, geometric mean exposure to mestranol ranged from 0.052 to 1.04 $ug/m^3$ as compared to a DTEL of 0.06 $ug/m^3$. However, these exposure levels were well below the known daily clinical effect dose of 40 $ug/m^3$ for total conjugated estrogen and 6 $ug/m^3$ for mestranol, respectively. (The concept of the DTEL with reference to pharmaceutical workers exposed to biologically active chemicals is discussed in the Discussion Section of the report.)

Air samples obtained inside respirators at Plant B suggested less effective workplace protection factors than expected. These results may indicate the need for further evaluation or modification of respirators used. Wipe sampling results at Plant B also indicated that skin absorption may be a source of exposure.

Biomedical tests revealed no clinically significant differences between the exposed and control groups. However, some biological indices such as serum ethynylestradiol levels appeared to show possible estrogen exposure and absorption despite the use of respirators.

It is concluded from the results of these surveys and the small size of the directly exposed worker populations available for study that a full scale medical and industrial hygiene effort would not be very productive at this time.


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*Plant A is Ayerst Laboratories, Incorporated, Rouses Point, New York
*Plant B is Syntex Inc., Palo Alto, California

**Derived Target Exposure Limit (DTEL) This term has been arbitrarily used in this report to characterize the method by which such a value is calculated. Therefore, it does not reflect or imply official policy or procedure of NIOSH.

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University of Cincinnati Medical Center
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Dr. John A. McLachlan
National Institute of Environmental Health Sciences
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Mr. Rafael Moure
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Dr. Edwin Ortiz
Food and Drug Administration
Rockville, Maryland

Dr. Alan G. Robinson
University of Pittsburgh School of Medicine
Pittsburgh, Pennsylvania

Dr. Emery Wilson
University of Kentucky College of Medicine
Lexington, Kentucky

Dr. Bernett Zumoff
Montefiore Hospital and Medical Center
Bronx, New York

Within NIOSH/CDC:

Mr. Charles Neumeister
Dr. JeAnne Burg
Mr. Martin Berkeley
Ms. Marie Haring
Mr. Robert Schutte
Dr. Sherry Selevan
Dr. Robert N. Ligo
Dr. Larry K. Lowry
Dr. Channing Meyer
Dr. Howard N. Ory
Ms. Shiu T. Lee

Report preparation by:
Mrs. Rita Conners
Mrs. Ruth McGrath
Mrs. Marianne E. Fleckinger

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# INTRODUCTION

## HISTORICAL BACKGROUND OF OCCUPATIONAL ESTROGEN EXPOSURE

"Estrogen" is a generic term for estrus-producing compounds. Either biologically produced or chemically manufactured, they promote growth and development of the vagina, uterus, and fallopian tubes in mammals (Murad & Gilman, 1975). Estrogens used in the pharmaceutical industry may be generally classed as natural or synthetic, and as steroidal or non-steroidal (Figure 1). All steroidal estrogens, both natural (e.g. estrone) and synthetic (e.g. mestranol) possess the typical multi-ring structure depicted in the top four examples in Figure 1. The most important non-steroidal estrogens are the stilbestrols, examples of which are dienestrol and diethylstilbestrol (DES).

Shortly after the initiation of commercial production of estrogens, numerous reports of their effects such as gynecomastia and decreased libido among male workers, and menstrual disorders (increased flow or inter-menstrual spotting) among female workers, began to appear. The incriminated agents were diethylstilbestrol (Scarff & Smith, 1942; Fitzsimmons, 1944; Watrous & Olsen, 1959), hexestrol (Pagani, 1953), natural conjugated estrogens (Goldzieher & Goldzieher, 1949; Fisk, 1950), and synthetic steroidal estrogens such as ethynylestradiol (EE2) or mestranol (Harrington, et al, 1978). Reports of occupational exposures were reviewed in detail in a recent publication (Zaebst, et al, 1980). During the late 1960's and throughout the 1970's, the use of oral contraceptives became widespread. Many epidemiological studies were published indicating associations of oral contraceptive use with thromboembolic diseases such as coronary occlusions, cerebral ischemia and thrombophlebitis (Inman, et al, 1970; Vessey and Mann, 1978).

Occupational exposures to estrogens and their potential health effects have been an on-going concern of health professionals both within and outside of government. NIOSH's involvement in occupational estrogen exposure was prompted by a report by Harrington, et al, in 1978, in which both female and male workers of an oral contraceptive manufacturing plant were shown to have signs and symptoms of over-exposure to synthetic estrogens.

## PURPOSE AND SCOPE OF PILOT STUDIES

This study was conducted to:

1. Obtain estimates of environmental levels of estrogens to which workers were exposed;

2. Evaluate the degree and characteristics of exposed workers' biological responses;

3. Based on the above, to more accurately assess the necessity and feasibility of additional (or more extensive) medical or industrial hygiene surveys.

Two plants were selected for this pilot study. The first produced tablets containing natural, conjugated estrogens, which are used to treat post-menopausal symptoms (Plant A). The second produced oral contraceptive tablets containing mestranol and norethindrone (Plant B). Both were chosen because of the relatively large pool of workers potentially exposed and because of the expected differences in potency and possible differences in mode of action between the two types of estrogen.

At both plants, environmental sampling and medical tests were limited to production employees (process operators) engaged in tablet compounding and compressing, because exposures of process operators to estrogens were expected to be higher than those of other production employees.

## DESCRIPTION OF STUDY FACILITIES

Plant A is a medium sized firm of 20 or so buildings and approximately 840 employees, of whom 588 are hourly. Only about 75 workers were involved in the manufacture of estrogenic products. Of these, about 30 on the average are assigned to work involving intermittent exposure to NCE during blending, granulation, and compression several days per month. The firm manufactured a variety of products, some of which contained natural conjugated estrogens (NCE's). No synthetic estrogens were used or manufactured at this facility. In 1978, NIOSH conducted a walk-through survey of the facility, results of which were summarized in a 1979 report. Refer to Appendix A for a detailed description of the facility, workforce, process, and safety and health programs abstracted from that report.

The raw material used in Plant A is a mixture of 10% natural conjugated estrogens and excipients. The main estrogenic constituents are estrone (51%), equilin (24%), 17-alpha dihydroequilin (15%) and other estrogens (10%). Pharmacologically, the estrogenic activity of this material is not much different from synthetic estrogens, but its estrogenic potency is said to be about 1/30 to 1/100 of ethynyl estradiol and 1/5 to 1/12 of diethylestilbestrol (Wilson, 1978; Speroff et al, 1973).

Plant B is a large firm which consists of two major buildings and an offsite warehouse. The company employed approximately 1200 people, of which 200-250 were involved in the manufacture of "estrogen" products. The firm manufactured oral contraceptive tablets, steer and heifer pellets, and a number of other steroidal products. The oral contraceptive products, (the manufacturing of which was the focus of the study) contain the active ingredients mestranol (3-methyl-ether of ethynyl estradiol) and norethindrone, a steroidal progestogen. Refer to Appendix A for a detailed description of the facility.

## METHODS

### ENVIRONMENTAL SAMPLING PROCEDURES

Environmental sampling was conducted on one shift only at Plant A, and for six shifts (three days) at Plant B. Personal and area air sampling, and skin wipe sampling were conducted to evaluate process operators' exposures to NCE at Plant A, and to mestranol and norethindrone at Plant B. At Plant B, additional sampling was conducted inside the air supplied respirators worn by the workers. For comparative purposes, tandem outside respirator samples were also obtained. A limited comparison of results obtained by radioimmunoassay (RIA) vs. high performance liquid chromatography (HPLC) was also conducted using replicate area samples obtained at Plant B. This was done since the tandem sample sets obtained inside and outside the workers' helmets were analyzed by RIA to improve sensitivity, but at some loss of confidence in the accuracy of the results as compared to the HPLC method. The techniques used to collect and analyze the air and wipe samples are described in detail in Appendix B.

### BIO-MEDICAL PROCEDURES

A short questionnaire was used to obtain information regarding the participant's name, current job title, length of employment, length of current position, length of work with estrogen manufacturing, exposure to other drugs manufactured, history of heart attack, stroke, thyroid disease, liver disease, heavy drinking and any sex organ problems. These conditions were asked since they may either be precipitated by estrogen or are known to influence estrogen metabolism.

Blood test items analyzed are listed in Tables 3 (Plant A) and 13 (Plant B). Appendix D describes the rationale for selection of each of the test items. Blood chemistry analysis of Plant A samples was conducted by Laboratory Procedures South, Inc., Decatur, Georgia. Plant B samples were analyzed by Laboratory Procedures, Inc., Woodland Hills, California.

At Plant A, blood samples of estrogen workers were obtained before the shift (in the morning after a fast of at least eight hours), while control subjects (packaging and warehouse) were sampled after the shift (about four hours after lunch).

At Plant B, blood samples of estrogen workers were obtained as follows:

Day 0 = Before the shift on the first day of estrogen rotation.
Day 3 = After the shift of the third day in rotation.
Day 19 = Before the shift of the 19th day in rotation.

Blood samples from control subjects (packaging and sterile products area workers) were taken only once in the morning of Day 2. When the samples were taken in the morning, the subjects were asked not to eat or drink other than water after dinner the night before. However, by necessity, more than several hours of fasting could not be imposed prior to the post-shift sampling.

## STATISTICAL ANALYSIS

### Environmental Data

Due to of the small number of samples, Plant A environmental data were not subjected to statistical analysis. Plant B personal breathing zone sample results were grouped by job-type. For each job-type, summary statistics were computed, including the range, the geometric mean, and the 95% confidence interval for the mean. The confidence intervals were computed by taking the logarithm of the individual data values, computing the confidence intervals on the log-transformed data, and finally calculating the antilog of these values. The job means were then subjected to a single-classification Analysis of Variance (ANOVA) using the log-transformed data. The assumption of homogeneity of variances was tested using the "F-max" test (Sokal and Rohlf, 1969).

A series of paired, full shift samples (N = 14) obtained by NIOSH and Plant B were compared using regression and correlation analysis and the non-parametric Wilcoxon Signed Ranks Test for paired sets of data. In the regression analysis, the line of best fit was calculated using the method of least squares. Student's t-test was used to compare the slope and intercept to their expected theoretical values ( 1 and 0, respectively). Finally, Pearson's Product Moment Correlation Coefficient was calculated to assess the degree of association between the analytical methods. In the regression/correlation analysis, values reported as non-detectable were excluded from the analysis. The Wilcoxon Signed Ranks Test was carried out twice, first assuming the non-detectable values were equal to zero, and second, assuming these values were equal to the limit of detection. This was done to provide assurance that the "non-detectable" values were not influencing the test.

### Medical Data

Data from the exposed and the control groups were compared by sex using Student's t-test. In Plant A, there was only one female exposed and two female control workers. Females were thus excluded from the analysis.

In Plant B, female workers who reported the use of either an estrogen - replacement drug or an oral contraceptive were excluded from statistical analysis. The blood Ethynyl Estradiol (EE2) and Estrogen Stimulated Neurophysin (ESN) concentrations obtained from exposed workers on Days 3 and 19 were compared to those obtained on Day 0 using the paired t-test. In this case, the Day 0 concentration for each person was used as the reference (or control) value.

## RESULTS

### PLANT A

#### Environmental

Personal breathing zone air sampling was attempted on a total of five persons. One worker was engaged in blending operations; two were engaged in granulation, and two were engaged in compression of tablet cores. However, the two samples obtained from the compression operators were lost due to pump failure.

The blending operator's estimated eight hour, time weighted, average exposure to total conjugated estrogen was 0.48 ug/$m^3$, expressed as the sodium (estrogen) sulfate (Table 1). The corresponding eight hour, time weighted, average exposures of the two operators in granulation were 5.06 and 5.32 ug/$m^3$ of air.

During the shift sampled, the blending operator was engaged in preparation and dry milling of the dessication* by dry mixing NCE raw material with excipients (all non-active compounds). The operator was observed wearing a disposable, single-use Glendale GR 500 dust respirator (TC-21C-195), approved for use against pneumoconiosis and fibrosis producing dusts. He also wore white dacron coveralls, rubber gloves, and a hair cover.

The granulation operators were engaged in steps subsequent to the dry milling of the dessication. These operators were observed wearing the same personal protective equipment as the blending operator.

Table 1 also presents the results of three stationary air samples located in the granulation processing area next to the fluid bed dryer. Two samples were obtained using the personal sampling apparatus, and the third was obtained at a higher flow rate (5 cfm) using four inch diameter FALP filters. Air concentrations of total estrogens in this area as indicated by the two personal pumps (operated at 4 lpm) were 1.10 and 0.82 ug/$m^3$ of air. The high volume sample result was 13.8 ug/$m^3$.

Table 2 presents the results of wipe samples obtained from the hands of four workers processing estrogens after lunch, and near the end of the shift. For control purposes, two samples were obtained before the shift from two workers packaging coated tablets. As indicated in Table 2, positive results were found in all samples. Although the results of the samples obtained from estrogen workers appeared to be somewhat higher than the controls, the

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"Dessication" is the company's term for the first stage blend of raw materials.

data pose some difficulty in interpretation. In some of the samples, the ratios of estrogens quantitated were not consistent with the known compositions as they were in the air samples. Thus, it is likely that positive interferences (e.g. unidentified substances from worker's skin) were contributing to some of the elevated results.

Medical

Table 3 presents the results of analysis of blood samples obtained from 11 male workers at Plant A. Between the groups of 6 exposed and 5 non-exposed male employees, there were no significant differences in values of estrogen stimulated neurophysins (ESN), corticosteroid binding globulin (CBG), and testosterone (total and percent free). Hematocrit (HCT) was significantly higher ( p 0.001) among the exposed group (50.3%) than the non-exposed group (41.9%).

Of the coagulation factors only antithrombin III was significantly lower in the male exposed group ($p = 0.024$). Each individual value was within the normal range of 80 - 120%. Blood chemistries, including high density lipoprotein (HDL), low density lipoprotein (LDL), cholesterol, and cholesterol/HDL ratio were not significantly different between exposed and non-exposed males.

PLANT B

Environmental

Tables 4-11 present the results of the environmental sampling conducted during the survey. Table 7 presents a summary of the personal air sampling with descriptive statistics by job type. Table 12 is a statistical comparison of side by side sampling conducted by NIOSH and Plant B personnel.

Based on the data in Tables 4 - 6, and the results of a single classification Analysis of Variance (Table 8), it appears that the granulation job poses the greatest potential for exposure to airborne mestranol among those jobs sampled. The geometric mean of the eight hour, time weighted average exposures to mestranol in this job was 1.04 ug/$m^3$, compared to 0.16 ug/$m^3$ in compression of tablets (Table 7). In tablet testing, exposures to mestranol were non-detectable at a detection limit of about 0.02 micrograms of mestranol per cubic meter of air.

Table 7 also indicates the granulation operators' geometric mean "inside respirator" exposure to mestranol (0.075 ug/$m^3$) and the corresponding 95% confidence interval (0.0008-6.9 ug/$m^3$). Comparison of this with the "outside respirator" geometric mean indicates that in granulation, the inside respirator concentration averaged approximately 7% of the outside respirator concentration.

Calculation of the protection factors for two sets of paired (outside-inside respirator) samples (Table 9), indicates protection factors of 10 and 40, far below what is normally expected (1000) of the type of respiratory protective equipment used. The third set of paired samples (obtained on employee A on 11/19/80) was not used to calculate a protection factor. This employee changed jobs midway through the shift, and in moving to the second job, removed the respiratory protective equipment. At this time, the inside respirator sample was removed, and the outside respirator sample was left in place for the remainder of the shift so that a calculation of the employee's time weighted average exposure could be made. However, in this case, the "inside respirator" concentration for the duration of the sample was also relatively high (0.73 ug/$m^3$).

Particularly noteworthy are the results of sampling on employee C, as indicated in Tables 5 and 6. On all three shifts, this person's potential exposures (exclusive of respiratory protection) were substantially higher than would be expected on the basis of the jobs performed and the results of other personal samples in those jobs. This strongly suggests, along with subjective observations made during the survey, that poorer work practices played a role in this employee's elevated exposures.

Table 10 indicates the results of wipe samples obtained from the employees' hands and faces. The most notable result was that obtained from the hands of an employee (both hands, all surfaces) just after adding pure mestranol to the blender. A total weight of 198 ug of mestranol was obtained from this sample.

Table 11 lists the results of a series of replicate area samples obtained over three shifts in the granulation area milling room, and the results of control samples obtained in the dispensary, which was located in a separate building some distance (several hundred yards) from the estrogen processing facility. Extensive statistical analysis was not performed on these samples due to the limited range of concentration. The RIA technique, however, appeared to give slightly higher results. The mean multiple* of the HPLC technique was approximately 1.3. Neither mestranol nor norethindrone was detected from the sample obtained in the dispensary (limit of detection, 0.02 $ug/m^3$).

Table 12 lists the results of fourteen side by side, full shift, personal samples obtained by NIOSH and Company B personnel, and the results of statistical analysis of the paired samples. The NIOSH samples were analyzed by HPLC, and the Plant B samples by RIA. Over a concentration range of 0.02 to 18.0 $ug/m^3$, the mean difference between pairs was 0.55 $ug/m^3$. The results of a regression analysis (least squares) indicated that the slope of the calculated regression line was 0.93, and the y-intercept was -0.33 $ug/m^3$. Neither of these values were significantly different from their expected, theoretical values ( 1 and 0, respectively). The correlation (r) was 0.98, and the results of the Wilcoxon Signed Ranks Test (non-parametric test designed for matched pairs) indicated no significant difference between the paired values.

Bio-Medical Results

Table 13 lists the results of analysis of blood samples obtained from a total of 14 employees (eight males and six females) at Plant B. Of the 8 males, three were on a 3-week rotation for manufacturing of oral contraceptives, and the remaining five were warehouse workers serving as controls. Three of the six female subjects worked on the estrogen rotation and the remaining three were in packaging. (One female estrogen worker and two female packaging workers reported that they were on estrogen therapy and thus were excluded from the statistical analysis).

Ethynylestradiol (EE2): Although the number of subjects is small, it appears that the EE2 level in the exposed males increased from Day 0 to Day 3 and remained somewhat elevated on Day 19. The difference was significant using the paired t-test (p 0.05). On day 0, EE2 levels were slightly higher in the control group than in the exposed group. Samples were not obtained from the control group on Days 3 and 19. However, assuming that EE2 levels in the controls remained unchanged through Day 3 and Day 19, the difference in blood EE2 concentrations between the exposed and control groups was not statistically significant. A similar moderate elevation was evident in female workers, although only two observations are available for Day 19 of the exposed.

* Calculated as the arithmetic average of the ratios of individual data pairs.

Estrogen Stimulated Neurophysin (ESN): The ESN levels in the two exposed male workers remained essentially the same for Days 0, 3, and 19. In the exposed female workers, the values increased from Day 0 to Day 3 but the numbers are too small to be treated statistically. The ESN values in both male and female exposed groups were not different from those in the respective controls.

Corticosteroid Binding Globulins (CBG): In both male and female exposed workers, there was no difference between the Day 0 and Day 19 results. Neither were these values different from the control groups. Female workers using estrogenic drugs showed larger values than other female workers (not using estrogenic drugs) on Day 0. One female worker using oral contraceptive pills had a markedly elevated value on Day 19.

Testosterone (Total and Percent Free): There were no statistically significant differences in these values between Day 0 and Day 19 in either male or female exposed workers, although there was a very slight decrease toward Day 19. There was a definite difference between the male and female workers, which merely reflected the sex difference in normal values for each sex.

Routine Blood Chemistry: There were no statistically significant differences between the exposed and control groups in all of the tested items except that exposed females had significantly elevated values for thyroxin and T-4 index when compared to the control group. However, the reason for this difference is not clear. Since the test samples were taken on Day 0, it is not considered to be a result of exposure to estrogen.

ENVIRONMENTAL MESTRANOL LEVEL AND SERUM ETHYNYLESTRADIOL

An attempt was made to determine whether an index of measured serum ethynylestradiol levels could be related to an index of exposure to airborne mestranol in "estrogen" area workers. For an index of mestranol absorption in each worker, the serum EE2 level obtained on Day 3 was divided by that obtained on Day 0. For an exposure estimate of mestranol, inside respirator concentrations were used, if available. If only breathing zone (outside respirator) concentrations were available, they were divided by a respirator protection factor (R.P.F.) of 25 for those workers in granulation or 5 for those in tablet compression. It was assumed that each worker's respiratory volume was 1 $m^3$ of air/hour and the value was multiplied by the hours of work in that job.

Therefore: Exposure dose D = M (mestranol) $ug/m^3$ x 1 $m^3/hr$ x hours of work per day divided by R.P.F. Furthermore, based on de la Pena's report (1975) on the metabolism of mestranol, it was assumed that 80% of Day 3 mestranol exposure contributed to Day 3 serum (end shift) ethynylestradiol, and only 10% and 20% of Day 1 and Day 2 (respectively) exposure contributed to Day 3 EE2. These values were plotted on semi-log graph paper and subjected to correlation analysis. However, no significant correlation was established between these two indices. The lack of correlation may be due

either to incorrect assumptions at several points, or to the fact that inhalation exposures are only one factor in estrogen absorption by workers. This analysis did not include possible absorption by ingestion or through the skin.

## DISCUSSION

Unlike studies using laboratory animals conducted under controlled conditions, studies of workers in their places of employment inevitably encounter many constraints and conditions that are beyond the researchers' control. Examples of these constraints are availability of a sufficient number of exposed workers and suitable non-exposed workers to serve as controls, time and duration of exposure, and type of other products workers may be handling. Cross-sectional surveys of this kind are particularly vulnerable to daily variations of industrial operations and the data must be interpreted with this in mind. A basic, and hopefully reasonable assumption is that the situation during the survey was typical of daily operations, and that workers' measured biological responses were typical of their average responses. With these caveats in mind, these two pilot studies seem to provide some useful information.

### Plant A

Although exposure limit values are best established on the basis of demonstrated health effects and exposure levels measured in field studies, the paucity of such data in workers exposed to natural conjugated estrogens requires a different approach. In this report, we used a method which may be termed "derived target exposure limit (DTEL)". A similar method was previously reported (Harrington, et al, 1978). A DTEL for natural conjugated estrogen may be calculated as follows: the minimum effective therapeutic dose* for women is approximately 300 micrograms (0.3 milligrams) of total natural conjugated estrogen. Employing a safety factor of 100, a safe daily dose for occupational exposure would be on the order of 3 micrograms. Assuming an inhaled volume (in eight hours of work) of eight cubic meters of air, and further assuming absorption of 100% of the inhaled dose, this results in an eight hour, time weighted, average target concentration of approximately 0.4 ug/$m^3$. Similarly, it can be postulated that subclinical or even clinical effects would occur with repeated exposures to an eight hour TWA concentration of approximately 40 ug/$m^3$.

This calculation, of course, does not take into account other possible side effects related to estrogen absorption, such as circulatory or endocrine system disorders, or the possible effects in males, for which little dose-response data exists. Although a number of estrogenic side effects have been seen (in both men and women) at therapeutic dose levels, it is not known at present whether subclinical adverse effects occur at lower dose levels such as those which may occur in occupational environments. Given the current lack of solid information, therefore, the calculated target concentration given here should be interpreted and used with some caution.

As seen in Table 1, the results of very limited sampling during the pilot survey indicates that eight hour TWA exposures at Plant A were on the order of 0.5 - 5 ug of total conjugated estrogen per cubic meter of air. Thus,

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* for treatment of atrophic vaginitis and/or kraurosis vulvae.

potential exposures were approximately equal to (or slightly in excess of) the above calculated target of 0.4 ug/$m^3$, but were not near the calculated "therapeutic effect" level of 40 ug/$m^3$. Firm conclusions regarding levels of exposure in relation to any exposure limit criterion, however, are not justified strictly on the basis of this limited sampling. It should be noted that sampling data obtained by the company (in 1978) indicated that exposures were frequently in excess of 40 ug/$m^3$, ranging up to 200 ug/$m^3$ over a full shift of sampling. However, even if airborne exposures are currently in the clinical effect range, this would not necessarily result in the appearance of symptoms in employees due to several factors. First, respiratory protection (and other protective clothing) is currently used by all employees working in "estrogen" areas. Actual exposures, therefore, are likely to be lower than those measured by routine personal sampling. Second, the company reported that the involved employees only spent approximately 10% of total working hours in production of NCE products.

Results of limited wipe sampling from the hands of employees (Table 2) tend to indicate that estrogen workers' hands are contaminated at a low level (9.6 ug - 35 ug) when compared to control samples (4ug - 8.7 ug). At this time, the overall contribution of this potential route of exposure is difficult to assess. However, because of the possibility of positive analytical interferences alluded to earlier, one could not conclude that skin contamination and subsequent absorption of the dry estrogen dust was a significant route of exposure at this plant.

Based on the above data and considerations, one would not expect to see remarkable results from analysis of blood samples obtained from these employees. In general, this was the case. Admittedly, this was a small pilot study and only compared groups of male workers, since there were only 3 female workers to be studied. No pre- and post-shift comparison was made, because (1) at the time of the survey, there was no perfected radioimmunoassay method available for equilin or dihydroequilin; and (2), NCE workers were not on rotation, eliminating the need for pre- and post-rotation measurement.

Only two test items showed statistically significant differences between the exposed and control groups. First, hematocrit (HCT) was significantly higher for the NCE exposed group. This difference was still significant even after removing a worker who had a history of colitis and sarcoidosis from the non-exposed group. This result is very difficult to explain. Estrogen is not known to increase hematocrit. In the Coronary Drug Project Report (1973), it is reported that the study group treated with 2.5 mg of conjugated estrogen (ESG1) had lower levels of serum bilirubin, SGOT, alkaline phosphatase, uric acid, and hematocrit. Our HCT result is contrary to this.

One procedural difference was that blood drawing was done in the morning (7 - 8am) before the shift for the NCE group with at least eight hours of fasting, while the control group was tested in the afternoon (4 - 5pm) after the shift and with up to five hours of fasting. Although this was not an

ideal control group, it is doubtful that the difference in sampling time and fasting period can account for this much change in HCT. The manufacturing was done in an air conditioned building and it is unlikely that the estrogen workers were dehydrated. Since a small sample group was involved, this could be just a spurious occurrence, although the p-value is very small.

Among the coagulation factors tested, antithrombin III (At-III) was lower in the male NCE exposed group, suggesting that this group's balance of bleeding vs clotting was shifted toward clotting when compared to the control. Antithrombin III is part of a protective hematologic mechanism guarding against intravascular thrombin production. Oral contraceptive administration has a significant depressant effect on At-III activity (von Kaulla, et al, 1971), but conjugated estrogens administered in a dose of 1.25 mg daily did not alter the At-III level (Notelovitz & Greig, 1976). The latter study was done on menopausal women whereas the current study involved males with an average age of 33.5 years (exposed) and 30.2 (not exposed).

It is possible that the low antithrombin III may be related to the higher HCT value of the exposed group. The correlation coefficient (r) between hematocrit and At-III was -0.749 ($p = 0.0080$) for the 11 subjects tested, meaning that the higher the HCT value, the lower the At-III value. No such relationship has been found in the literature related to antithrombin III.

Plant B

In order to obtain a derived target exposure limit (DTEL) applicable to industrial exposures to mestranol, the principal exposure of interest at Plant B, assumptions similar to those used for the natural conjugated estrogens can be made. The usual oral daily dose of mestranol used in O.C. tablets is 50 ug, which causes a depression of follicle stimulating hormone in women. On this basis, and assuming a range of respiratory volume of 8 cubic meters for 8 hours, the calculated "no effect" concentration for an eight hour shift is on the order of 0.06 ug/$m^3$ of air. Similarly, clinical or subclinical estrogenic effects would occur with repeated eight hour exposures on the order of 6 ug/$m^3$. (This, as discussed previously, does not consider the other possible adverse effects of mestranol, or the potential effects in males, for which no dose-response data exists.)

In comparison with these estimates, the geometric mean exposures (outside helmets) of granulation operators were greater by a factor of approximately seventeen (1.0 ug/$m^3$ compared to 0.06 ug/$m^3$), and that of the compression operators, greater by a factor of about 2.7 (0.16 ug/$m^3$ vs. "no-effect" concentration of 0.06 ug/$m^3$)

Perhaps the most meaningful comparison, however, is with the inside helmet samples. Measured exposures inside the 3M Company Whitecap helmets worn by granulation operators ranged from 0.01 - .35 ug/$m^3$ on an eight hour time weighted average basis, and the geometric mean of these exposures was 0.075 ug/$m^3$, which is on the order of the "target" concentration.

However, if one were to apply the worst case protection factor (x10) found in this survey to the highest recorded outside helmet exposure (18 ug/$m^3$), one could postulate that actual exposures in the range of 1.8 ug/$m^3$ (18/10) could easily occur, which is in excess of the previously estimated target limit by a factor of 30. However, this is still well below the calculated "clinical effect" concentration of 6 ug/$m^3$.

Although no actual exposures of this magnitude were recorded during the survey, it does point out that the respiratory equipment was apparently not performing as well as designed. Two observations were made during the survey which would support this conclusion. First, at least twice during the survey, processing operators' air supply tubes (connecting the filter backpack to the helmet) inadvertently became disconnected from their helmets. Although these were quickly reconnected by the operator or by a nearby operator, this could compromise the integrity of the respiratory protective equipment. This situation could be remedied by using some form of positive, quick release locking device. Second, several operators were observed working in the compression area communicating with other workers with their visors raised. This could be remedied by permanently sealing the visors. Optionally, a radio headset could also be used to improve communications between workers.

One notable observation was made regarding the potential for skin contact and subsequent absorption of estrogen. An extremely high mestranol value was detected on a hand wipe sample from a granulation operator immediately following addition of mestranol to the blender. This result indicates substantial contamination of the hands, and the possibility of an important route of exposure other than by inhalation. During this survey, the gloves of this operator, and those of several others, were checked for leakage just after the addition of the mestranol. It was discovered that the gloves in each case were torn, apparently due to abrasion during handling of heavy drums and other rough surfaced equipment. Since the mestranol at this stage of operations is handled in alcoholic solution, the potential for skin absorption was undoubtedly enhanced.

The limitations of the biomedical survey must be borne in mind. The exposed population at Plant B was even smaller than at Plant A. There were only eight estrogen workers in the rotation and the group was composed of three males and five females. The latter included two women taking estrogenic drugs. With such distinctly different characteristics of each group, they could not be grouped conveniently as "exposed". In addition, their number was further diminished when some workers could not return for Day 3 or 19 due to various reasons. As a result, the number of samples in some groups dwindled to 1. Within the control groups, no sampling was conducted on Day 3 or 19. It was assumed that since they were not exposed to estrogens, factors dependent on estrogens would remain unchanged from Day 0. Finally, male exposed workers are all fairly new employees, suggesting a high turnover rate in this job category. This was not the case with female workers.

EE2 levels were elevated on Day 3 and Day 19 compared to Day 0 in male exposed workers. A similar pattern was seen in female exposed workers, although their Day 19 levels were back to Day 0 level. It is most likely that this elevation is a result of occupational exposure. Exposed or non-exposed, females had higher EE2 values than males and this is considered to be partly due to the higher endogenous estrogen levels in females cross reacting in RIA.

It is probable that the absorption of mestranol by the exposed workers was not sufficient to cause a measurable rise in their blood serum levels of Estrogen Stimulated Neurophysin (ESN). None of the samples from the male exposed workers indicated an elevation of ESN, while samples from females indicated ESN elevations on Day 3, and some decline on Day 19. In contrast to these results, the ESN levels in the samples from the female exposed worker using an oral contraceptive were markedly elevated throughout the testing period. Although this was a single case, the ESN elevations in this worker paralleled the reported behavior of ESN in response to estrogen (oral contraceptive) intake.

A similar pattern was observed in the case of the CBG results. CBG plasma levels were elevated only in one female worker using an oral contraceptive. It is again probable that environmental exposures were not sufficient to

cause a significant elevation of CBG at either Plant A or Plant B. The threshold dose resulting in a detectable CBG response to diethylstilbestrol is reported to be about 0.1 mg (Seal and Doe, 1969). The corresponding dose for the conjugated estrogen (Plant A) is about 0.25 mg (or 250 ug) and that for mestranol (Plant B) is about 0.01 mg (or 10 ug), using the comparison of potencies of these estrogens (Wilson, 1978).

At Plant A, inhalation exposures to conjugated estrogen did not reach this level. If the highest concentration value of 13.8 $ug/m^3$ (total estrogens in air by high volume sample) is multiplied by 8 $m^3$ of air inhaled by a worker during an eight hour period, the resulting dose (110 ug) is about one-half of the threshold dose.

At Plant B, the highest potential mestranol exposure measured was 18.0 $ug/m^3$ (estimated eight hour, time-weighted average for Operator C) in Table 5. This is approximately equivalent to a mestranol dose of 144 ug (18 $ug/m^3$ x 8 $m^3$). Obviously, this is far above the therapeutic dose and could have produced signs and symptoms of over-exposure, if the operators were not protected by a respirator or reduced exposure time (workers usually do not work in one area for 8 hours a day). Similar reasoning can also be applied to the ESN results. Unfortunately, Operator C did not return for blood drawing on Day 19 and it is not known if the concentration of CBG or ESN in the employee's blood was elevated as a result of the potentially high exposure to mestranol.

ESN and CBG respond to estrogen administration at a therapeutic dose level, such as that achieved with the use of oral contraceptive pills. These tests, however, may not be sensitive enough to respond to doses typically received in the occupational setting, even though the exposures may be slightly above the calculated target exposure level.

## SUMMARY AND RECOMMENDATIONS

1. Historical industrial hygiene data for exposures to natural conjugated estrogens (generated by Plant A) indicate that exposures (in 1978) were potentially in the clinical effect range. However, limited data obtained during this survey indicated exposures were substantially below this level, although at or somewhat above the "target" level calculated in this report. These data, although very limited, suggests that it would have been unlikely that observable clinical changes in workers would be detectable at the time of the study. In general, this was the case.

2. There was an unexplainable increase of hematocrit among estrogen-exposed workers at Plant A. This may be a chance occurrence.

3. There was a decrease in Antithrombin III among the exposed group at Plant A, which may be a chance occurrence or a possible effect of chronic estrogen exposure. The decrease may also be related to hematocrit values which showed a significant negative correlation with At-III.

4. Potential exposures measured at Plant B were high enough to be of some concern, particularly during granulation operations, and it is obvious that protective equipment must be worn by workers while working in the steriod areas. However, based on measurements during the survey, the respiratory equipment (and other protective equipment, i.e. gloves) used was not giving the degree of protection expected. Exposures measured inside the respiratory protective devices of several granulation operators were at or slightly above the range of "target" exposure (.06 $ug/m^3$), and averaged 0.075 $ug/m^3$. In addition, calculations based on measured protection factors and exposures during the survey suggest that inhalation exposures might occur at a level up to approximately 30 times the calculated target exposure level, but still well below the clinical effect level of 6 $ug/m^3$.

5. The results of skin wipe sampling at Plant B showed substantial contamination of the skin of the hands with mestranol following the handling of the pure chemical. Although dermal absorption of estrogen cannot be adequately quantitated at present, such exposure should be considered additive to the respiratory absorption of airborne dust.

6. Among estrogen workers at Plant B, indices for estrogen absorption (EE2 and ESN) showed a marginal pattern of elevation as predicted but it was not, as expected, of the same magnitude as seen in oral contraceptive administration.

7. These results and the small size of the exposed population available indicated that a full scale medical/industrial hygiene study would not be warranted.

8. The results of the environmental sampling at these two plants indicate strongly that additional evaluations of work practices and control technology at other plants would be valuable. Such evaluations would serve to identify both the strengths and weaknesses of specific control systems in place, and would allow the documentation, transfer, and application of the best of these principles throughout the industry.

9. In general, evaluation of the occupational exposure and health status of pharmaceutical workers is complicated by the following factors:

    a. workers are usually exposed to multiple drug products and ingredients;

    b. workers are rotated to various products which are manufactured on a periodic or sporadic basis;

    c. the small numbers of exposed workers makes it infeasible to study them epidemiologically with an adequate statistical power; and

    d. toxicological data on pharmaceuticals is usually available from therapeutic trials in which drugs are administered by mouth, skin application, injection, etc. but rarely by inhalation.

The quality of pharmaceutical products and their therapeutic uses are regulated by the Food and Drug Administration. The health of pharmaceutical workers comes under the jurisdiction of the Occupational Safety and Health Administration. No specific pharmaceutical products have been regulated under OSHA other than through the general duty clause, probably due to the difficulties cited above. To protect the health of pharmaceutical workers who are exposed to biologically active chemicals, it may be necessary to use the concept of a derived target exposure limit (DTEL). As explained in the discussion section, DTEL is calculated from the known minimum clinical effective dose, an inhalation factor and a safety factor (e.g. 100). In view of the scarcity of occupational toxicological information, this approach would be a reasonable alternative until more definitive information becomes available.

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TABLE 1
PLANT A
AIR SAMPLING RESULTS
SAMPLING DATE: 7/1/80

| JOB/AREA | OPERATOR | SAMPLE VOLUME (LITERS) | CONCENTRATION ($ug/m^3$) SODIUM (ESTROGEN) SULFATE | | | | ESTIMATED 8 HR. TWA* CONCENTRATION ($ug/m^3$) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | ESTRONE | EQUILIN | DHE | TOTAL** | ESTRONE | EQUILIN | DHE | TOTAL |
| Blending Premarin #866, cleanup | A | 1772 | 0.17 | 0.17 | 0.15 | 0.54 | 0.15 | 0.15 | 0.14 | 0.48 |
| Loading raw materials, granulation, milling Premarin #866 | B | 1824 | 2.76 | 1.14 | 0.88 | 5.31 | 2.62 | 1.09 | 0.84 | 5.06 |
| Loading raw materials, granulation, milling Premarin #866 | C | 1801 | 2.65 | 1.41 | 1.07 | 5.70 | 2.47 | 1.31 | 1.01 | 5.32 |
| Area sample; processing room, near fluid bed drier control panel | --- | 2129 | 0.58 | 0.26 | 0.15 | 1.10 | --- | --- | --- | --- |
| Area sample; processing room, near fluid bed drier control panel | --- | 2136 | 0.39 | 0.21 | 0.14 | 0.82 | --- | --- | --- | --- |
| High volume area sample; processing room, near fluid bed drier control panel | --- | 74,000 | 6.22 | 3.31 | 2.91 | 13.8 | --- | --- | --- | --- |

*Assuming no exposure during non-sampled period.
**Total concentration of estrone, equilin, dihydroequilin divided by 0.9

TABLE 2
PLANT A
WIPE SAMPLING RESULTS - HANDS
SAMPLING DATE: 7/1/80

| JOB/OPERATION | SAMPLE NUMBER | HANDS (MICROGRAMS) SODIUM (ESTROGEN) SULFATE ESTRONE | EQUILIN | DHE* | TOTAL** | COMMENTS |
|---|---|---|---|---|---|---|
| Control-operator packaging coated tablets | AYW-1 | 2.5 | 0.07 | 1.1 | 4.0 | Prior to beginning work |
| Control-operator packaging coated tablets | AYW-3 | 7.3 | 0.07 | 0.55 | 8.7 | Prior to beginning work |
| Process operator, granulation | AYW-4 | 24 | 4.0 | 3.3 | 35 | Near end of shift, before cleaning up-washed hands 5 min. prior to sampling |
| Process operator, granulation | AYW-10 | 2.5 | 2.3 | 5.1 | 11 | Near end of shift, before cleaning up; removed gloves by pulling on fingers prior to sampling |
| Process operator, granulation | AYW-8 | 4.4 | 2.3 | 1.9 | 9.6 | Just after lunch, before starting afternoon work |
| Process operator, blending | AYW-5 | 13.8 | 3.2 | 2.5 | 22 | Near end of shift, before cleaning up |
| Process operator, compression of tablet cores | AYW-9 | 13.8 | 6.3 | 8.6 | 32 | Just after lunch, before starting afternoon work |
| Process operator, compression | AYW-11 | 0.41 | 0.83 | 22.1 | 26 | Near end of shift, before cleaning up; removed gloves by pulling on fingers prior to sampling |

* DHE = 17-alpha dihydroequilin

** Sum of estrone, equilin and 17-alpha dihydroequilin weights divided by 0.9

TABLE 3
PLANT A
BLOOD TEST RESULTS
(JUNE, 1981)

| | MALE EXPOSED MEAN ± S. D. | MALE NOT EXPOSED MEAN ± S. D. | UNITS |
|---|---|---|---|
| Number of Subjects | 6 | 5 | Person |
| Age | 33.5 ± 10.4 | 30.2 ± 8.6 | Years |
| Years of Employment | 12.4 ± 11.7 | 2.9 ± 2.2 | Years |
| Estrogen Stimulated Neurophysins (ESN) | 0.72 ± 0.60 | 0.64 ± 0.33 | ng/ml |
| Corticosteroid Binding Globulins (CBG) | 21.8 ± 3.4 | 22.3 ± 2.6 | mg/ml |
| Testosterone (Total) | 4.81 ± 1.19 | 5.03 ± 1.17 | ng/ml |
| Testosterone (% Free) | 3.61 ± 0.70 | 4.13 ± 0.48 | % |
| Hematocrit (Hct) | 50.3 ± 2.5 * (p <0.001) | 41.9 ± 2.4 | % |
| Coagulations Factors | | | |
| Prothrombin Time (PT) | 10.6 ± 0.3 | 11.0 ± 0.5 | sec. |
| Fibrinogen | 299.2 ± 50.9 | 275.0 ± 54.5 | mg/dl |
| Factor VII | 122.0 ± 26.0 | 125.8 ± 38.2 | % |
| Factor IX | 112.2 ± 14.8 | 102.4 ± 13.7 | % |
| Factor X | 121.2 ± 14.3 | 115.2 ± 15.5 | % |
| Antithrombin III (At - III) | 102.2 ± 7.1 * (p = 0.025) | 112.4 ± 4.9 | % |
| Plasminogen | 3.50 ± 0.54 | 3.50 ± 0.97 | ug/ml |
| Routine Blood Chemistry | | | |
| Gamma Glutamic Transpeptidase (GGTP) | 32.2 ± 49.5 | 13.8 ± 4.6 | mu/ml |
| Triglycerides | 72.5 ± 37.6 | 80.8 ± 36.6 | mg/dl |
| Cholesterol | 194.7 ± 50.5 | 170.0 ± 24.1 | mg/dl |
| High Density Lipoprotein (HDL) | 46.7 ± 6.9 | 47.2 ± 14.7 | mg/dl |
| Low Density Lipoprotein (LDL) | 133.3 ± 45.1 | 106.6 ± 27.3 | mg/dl |
| Cholesterol/HDL | 4.2 ± 1.3 | 3.9 ± 1.7 | ----- |
| Bilirubin, total | 0.7 ± 0.3 | 0.6 ± 0.2 | mg/dl |
| Glucose | 89.0 ± 9.3 | 84.4 ± 4.7 | mg/dl |
| T-3 Uptake | 38.9 ± 3.4 | 40.5 ± 4.2 | % |
| Tyroxine | 8.3 ± 1.5 | 7.6 ± 2.0 | ug/dl |
| T-4 Index | 3.2 ± 0.4 | 3.0 ± 0.5 | ----- |

*This difference is statistically significant at the p - value listed.

TABLE 4
PLANT B
AIR SAMPLING RESULTS - GRANULATION

| JOB/AREA | DATE (1980) | OPERATOR | SAMPLE VOLUME (LITERS) | CONCENTRATION ($ug/m^3$) MESTRANOL | CONCENTRATION ($ug/m^3$) NORETHINDRONE | ESTIMATED 8HR. TWA CONCENTRATION ($ug/m^3$)* MESTRANOL | ESTIMATED 8HR. TWA CONCENTRATION ($ug/m^3$)* NORETHINDRONE |
|---|---|---|---|---|---|---|---|
| Process operator outside helmet** | 11/17 | A | 964 | 2.49 | 33.0 | 1.25 | 16.6 |
| Process operator inside helmet ** | 11/17 | A | 956 | 0.25 | 1.25 | 0.12 | 0.60 |
| Process operator outside helmet | 11/17 | B | 760 | 1.18 | 6.03 | 0.47 | 2.39 |
| Process operator outside helmet ** | 11/18 | E | 820 | 1.6 | 29.6' | 0.68 | 10.4 |
| Process operator inside helmet ** | 11/18 | E | 828 | 0.04 | 0.65 | 0.01 | 0.06 |
| Process operator Outside helmet | 11/18 | F | 900 | 3.15 | 140 | 1.48 | 65.5 |
| Process operator Outside helmet | 11/19 | A | 1108 | 1.95 | 31.2 | 1.13 | 18.0 |
| Process operator Inside helmet ** | 11/19 | A | 916 | 0.73 | 16.0 | 0.35 | 7.63 |
| Process operator Outside helmet | 11/19 | B | 552 | 6.68 | 82.6 | 1.92 | 23.7 |

* Assuming no exposure during non-sampled periods.
**Analysis by RIA. The remaining unmarked samples were analyzed by HPLC.

TABLE 5
PLANT B
AIR SAMPLING RESULTS
COMPRESSION

| JOB/AREA | DATE (1980) | OPERATOR | SAMPLE VOLUME (LITERS) | CONCENTRATION ($ug/m^3$) MESTRANOL | CONCENTRATION ($ug/m^3$) NORETHINDRONE | ESTIMATED 8HR. TWA CONCENTRATION ($ug/m^3$) * MESTRANOL | ESTIMATED 8HR. TWA CONCENTRATION ($ug/m^3$) * NORETHINDRONE |
|---|---|---|---|---|---|---|---|
| Process operator | 11/17 | E | 1320 | 0.10 | 1.29 | 0.07 | 0.89 |
| Process operator | 11/17 | F | 1268 | 0.16 | 3.39 | 0.11 | 2.24 |
| Process operator | 11/17 | G | 1180 | 0.10 | 0.64 | 0.06 | 0.39 |
| Process operator | 11/18 | A | 1236 | 0.40 | 7.71 | 0.26 | 4.96 |
| Process operator | 11/18 | G | 1440 | <0.03 | 1.32 | <0.02 | 0.99 |
| Process operator | 11/18 | B | 1400 | <0.03 | 0.16 | <0.02 | 0.12 |
| Process operator | 11/18 | C | 1484 | 11.7 | 158 | 9.04 | 122 |
| Process operator | 11/19 | F | 1184 | 0.47 | 8.61 | 0.29 | 5.31 |
| Process operator | 11/19 | G | 1324 | <0.04 | 1.94 | <0.03 | 1.34 |
| Process operator | 11/19 | C | 1516 | 22.8 | 270 | 18.0 | 213 |

*Assuming no exposure during non-sampled periods.

TABLE 6
PLANT B
AIR SAMPLING RESULTS
TABLET TESTING AND MIXED JOBS

| JOB/AREA | DATE (1980) | OPERATOR | SAMPLE VOLUME (LITERS) | CONCENTRATION ($ug/m^3$) MESTRANOL | CONCENTRATION ($ug/m^3$) NORETHINDRONE | ESTIMATED 8HR. TWA CONCENTRATION ($ug/m^3$) * MESTRANOL | ESTIMATED 8HR. TWA CONCENTRATION ($ug/m^3$) * NORETHINDRONE |
|---|---|---|---|---|---|---|---|
| Process operator granulation and compression; 80% compression | 11/17 | C | 1620 | 1.76 | 28.3 | 1.49 | 23.9 |
| Process operator granulation and tablet testing; 80% tablet testing | 11/17 | D | 1572 | <0.03 | <0.03 | <0.02 | <0.02 |
| Process operator compression and tablet testing 80% tablet testing | 11/18 | D | 1484 | <0.03 | 0.04 | <0.02 | 0.03 |
| Process operator tablet testing 100% | 11/19 | D | 1492 | <0.03 | 0.15 | <0.02 | 0.12 |

*Assuming no exposure during non-sampled periods.

TABLE 7
PLANT B

SAMPLING SUMMARY, MESTRANOL DATA
BY JOB TYPE

| JOB/AREA | NUMBER OF SAMPLES (N) | RANGE ($ug/m^3$) | GEOMETRIC MEAN ($ug/m^3$) | 95% CONFIDENCE INTERVAL ($ug/m^3$) |
|---|---|---|---|---|
| Granulation processing; Outside helmet | 6 | 0.47-1.92 | 1.04 | 0.6-1.7 |
| Granulation processing; Inside helmet | 3 | 0.01-0.35 | 0.075 | 0.0008-6.9 |
| Compression; Outside helmet | 10 | < 0.02-18.0 | 0.16 | 0.025-1.02 |
| Compression; Outside helmet* | 8 | < 0.02-0.29 | 0.052 | 0.016-0.16 |

* Excluding two largest data points as outliers (9.04 $ug/m^3$ and 18.0 $ug/m^3$)

TABLE 8
PLANT B
ONE WAY ANOVA COMPARING GEOMETRIC
MEAN EXPOSURES TO MESTRANOL FOR
GRANULATION AND COMPRESSION OPERATIONS

| SOURCE | DF | SS | MS | Fs |
|---|---|---|---|---|
| Among jobs | 1 | 5.8269 | 5.8269 | 25.5*** |
| Within jobs | 12 | 2.7404 | 0.22837 | |
| Total | 13 | 8.5673 | | |
| $F_{.05(1,12)} = 4.75$ | | $F_{.01(1,12)} = 9.33$ | | $F_{.001(1,12)} = 18.6$ |

*** $p<0.001$

Conclusion: There is a highly significant (p 0.001) added variance component among jobs.

TABLE 9
PLANT B
COMPARISON OF OUTSIDE-INSIDE
RESPIRATOR MESTRANOL CONCENTRATIONS;
AND CALCULATED PROTECTION FACTORS

| JOB/AREA | CONCENTRATION ($ug/m^3$) OUTSIDE HELMET | INSIDE HELMET | PROTECTION FACTOR (O/I)* |
|---|---|---|---|
| Granulation process Operator A | 2.49 | 0.25 | 10 |
| Granulation process Operator E | 1.60 | 0.04 | 40 |

* The protection factor is calculated as the outside helmet concentration divided by the inside helmet concentration.

TABLE 10
PLANT B
WIPE SAMPLES-HANDS AND FACE
SAMPLING DATE: 11/19/80

| | Packaging Mechanic | Warehouse lead operator | Area (1) Coordinator | Process operator (Op. A) | Process operator (Op. B) | Process operator (Op. F) | Process operator (Op. G) |
|---|---|---|---|---|---|---|---|
| | Control | Control | Control | Granul. | Granul. | Compres. | Compres. |
| HANDS (MICROGRAMS) | | | | | | | |
| BEFORE SHIFT | | | | | | | |
| MESTRANOL | <1.0 | <1.0 | <1.0 | <1.0 | 2.0 | 2.0 | 3.0 |
| NORETHINDRONE | <0.25 | <0.25 | <0.25 | 17.2 | 4.99 | 18.2 | 3.10 |
| MID SHIFT | | | | | | | |
| MESTRANOL | | | | 198* | | | |
| NORETHINDRONE | | | | 132* | | | |
| AFTER SHIFT | | | | | | | |
| MESTRANOL | | | | <1.0 | 4.0 | 9.0 | <1.0 |
| NORETHINDRONE | | | | 47.2 | 141 | 33 | 19.9 |
| FACE (MICROGRAMS) | | | | | | | |
| BEFORE SHIFT | | | | | | | |
| MESTRANOL | <1.0 | <1.0 | <1.0 | <1.0 | <1.0 | 3.0 | 1.0 |
| NORETHINDRONE | <0.25 | <0.25 | <0.25 | 14.6 | 0.80 | 5.54 | 1.32 |
| AFTER SHIFT | | | | | | | |
| MESTRANOL | | | | <1.0 | | <1.0 | |
| NORETHINDRONE | | | | 6.19 | | 10.9 | |

Granul. = Granulation
Compres. = Compression

* This sample obtained from operator immediately following addition of mestranol to blender.

TABLE 11
PLANT B
SAMPLING SUMMARY
AREA SAMPLES-MILLING AREA AND CONTROLS

| AREA | ANALYSIS METHOD | DATE (1980) | SAMPLE VOLUME (LITERS) | CONCENTRATION ($ug/m^3$) MESTRANOL | NORETHINDRONE |
|---|---|---|---|---|---|
| Milling on top of exhaust hood | HPLC | 11/17 | 1908 | 0.09 | 2.27 |
| Milling on top of exhaust hood | HPLC | 11/17 | 1932 | 0.14 | 5.16 |
| Milling on top of exhaust hood | HPLC | 11/17 | 1900 | 0.17 | 4.71 |
| Milling on top of exhaust hood | RIA | 11/17 | 1900 | 0.13 | 6.05 |
| Milling on top exhaust hood | RIA | 11/17 | 1904 | 0.15 | 8.51 |
| Milling on top of exhaust hood | RIA | 11/17 | 1924 | 0.12 | 7.38 |
| Milling on top of exhaust hood | HPLC | 11/18 | --- | Sample voided; pump stopped | |
| Milling on top of exhaust hood | HPLC | 11/18 | 1836 | 0.12 | 4.90 |
| Milling on top of exhaust hood | HPLC | 11/18 | 1832 | 0.10 | 4.56 |
| Milling on top of exhaust hood | RIA | 11/18 | 1824 | 0.18 | 8.60 |
| Milling on top of exhaust hood | RIA | 11/18 | 1828 | 0.08 | 3.91 |
| Milling on top of exhaust hood | RIA | 11/18 | 1836 | 0.13 | 6.64 |

TABLE 11 (cont.)
PLANT B
SAMPLING SUMMARY
AREA SAMPLES-MILLING AREA AND CONTROLS

| AREA | ANALYSIS METHOD | DATE (1980) | SAMPLE VOLUME (LITERS) | CONCENTRATION ($ug/m^3$) MESTRANOL | NORETHINDRONE |
|---|---|---|---|---|---|
| Milling on top of exhaust hood | HPLC | 11/19 | 1880 | 0.13 | 4.80 |
| Milling on top of exhaust hood | HPLC | 11/19 | --- | Sample voided; pump stopped | |
| Milling on top of exhaust hood | HPLC | 11/19 | 1884 | 0.27 | 5.79 |
| MIlling on top of exhaust hood | RIA | 11/19 | 1856 | 0.32 | 7.65 |
| Milling on top of exhaust hood | RIA | 11/19 | 1880 | 0.33 | 8.78 |
| Milling on top of exhaust hood | RIA | 11/19 | 1868 | 0.35 | 11.5 |
| Dispensary | HPLC | 11/18 | 1640 | 0.03 | 0.03 |
| Dispensary | RIA | 11/18 | 1640 | Sample lost in analysis | |

TABLE 12
COMPARISON OF AIR MONITORING RESULTS
NIOSH AND PLANT B MESTRANOL DATA

| NIOSH EIGHT HOUR TWA CONCENTRATION ($ug/m^3$) | PLANT B EIGHT HOUR TWA CONCENTRATION ($ug/m^3$) |
|---|---|
| <0.02 | 0.01 |
| <0.02 | 0.056 |
| <0.02 | 0.083 |
| <0.02 | 0.087 |
| 0.06 | 0.028 |
| 0.07 | 0.041 |
| 0.11 | 0.149 |
| 0.146 | 0.121 |
| 0.26 | 0.064 |
| 0.29 | 0.247 |
| 1.49 | 1.41 |
| 1.92 | 0.598 |
| 9.04 | 5.417 |
| 18.0 | 17.766 |

Average difference between pairs: 0.55 $ug/m^3$

Regression/Correlation: N = 10*
r = 0.98
Slope = 0.93
Intcp. =-0.33

Wilcoxon signed ranks test: N = 14

a) Assigning zero to "less than" values: Not different
b) Assigning limit of detection to "less than" values: Not different

* Concentration reported as less than the limit of detection were excluded from the regression/correlation analysis.

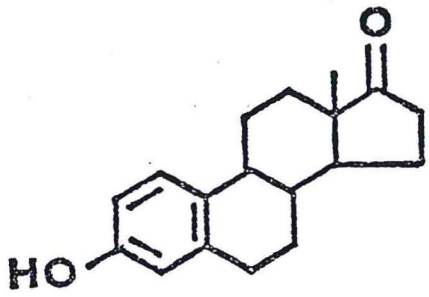
TABLE 13 BLOOD TEST RESULTS - PLANT B (NOVEMBER, 1980)

| | | MALE | | FEMALE | | |
|---|---|---|---|---|---|---|
| | | EXPOSED MEAN ± S.D. | NOT EXPOSED MEAN ± S.D. | EXPOSED MEAN ± S.D. | NOT EXPOSED MEAN ± S.D. | UNIT |
| Number of Subjects | | 3 | 5 | 3 | 3 | Person |
| Age | | 28.3 ± 9.5 | 30.2 ± 8.6 | 35.3 ± 15.6 | 43.7 ± 16.5 | Years |
| Years of Employment (Not necessarily exposure) | | 0.5 ± 0.4 | 5.2 ± 4.0 | 7.5 ± 4.5 | 9.7 ± 2.1 | Years |
| $EE_2$ | Day 0 | 10.7 ± 1.2 | 12.8 ± 3.1 | 19.3 ± 7.8 | 16.3 ± 8.5 | ng/ml |
| | 3*[0.05] | 17.3 ± 2.1 | ----- | 28.0 ± 9.5 | ----- | |
| | 19*[0.05] | 15.0 ± 1.4 (2) | ----- | 19.5 ± 12.0 (2) | ----- | |
| ESN | Day 0 | 1.9 ± 0.70 | 2.6 ± 3.2 | 1.9 ± 1.8 (2) | 1.3 ± 0.6*[0.01] | ng/ml |
| | 3 | 1.4 ± 0.57 (2) | ----- | 4.1 ± 2.0 | ----- | |
| | 19 | 2.0 ± 1.41 (2) | ----- | 2.7 ± 3.0 (2) | ----- | |
| CBG | Day 0 | 27.0 ± 5.5 (2) | 23.9 ± 3.7(4) | 24.9 ± 1.7(2) | 24.8 ± 1.9 | mg/ml |
| | 19 | 25.7 ± 5.9(2) | ----- | 24.4 ± 1.2(2) | ----- | |
| T-total | Day 0 | 4.15 ± 0.42(2) | 4.09 ± 0.86(4) | 0.78 ± 0.21(2) | 0.92 ± 0.25 | ng/ml |
| | 19 | 3.98 ± 0.16(2) | ----- | 0.57 ± 0.13(2) | ----- | ----- |
| T % free | Day 0 | 2.62 ± 0.06(2) | 2.88 ± 0.15 | 2.91 ± 0.45(2) | 2.47 ± 0.08 | % |
| | 19 | 2.65 ± 0.04(2) | ----- | 2.74 ± 0.22(2) | ----- | |
| Routine Blood Chemistry - Day 0 Only | | | | | | |
| GGTP | | 19.3 ± 3.5 | 12.2 ± 5.9 | 17.7 ± 3.1 | 16.7 ± 4.9 | mu/ml |
| Triglycerides | | 124.7 ± 91.8 | 100.0 ± 29.2 | 82.0 ± 73.5 | 101.3 ±65.2 | mg/dl |
| Cholesterol | | 169.3 ± 33.8 | 170.8 ± 49.9 | 186.0 ± 74.8 | 218.3 ± 55.3 | mg/dl |
| HDL | | 37.3 ± 8.5 | 44.8 ± 9.5 | 65.7 ± 16.7 | 61.3 ± 10.8 | mg/dl |
| LDL | | 107.0 ± 27.1 | 106.0 ± 48.3 | 104.0 ± 54.8 | 136.7 ± 39.5 | mg/dl |
| Cholersterol/HDL | | 4.8 ± 1.7 | 4.0 ± 1.8 | 2.8 ± 1.0 | 3.6 ± 0.8 | ----- |
| Bilirubin, total | | 0.37 ± 0.06 | 0.66 ± 0.25 | 0.27 ± 0.15 | .0.3 ± 0.0 | mg/dl |
| Glucose | | 101.7 ± 8.4 | 95.8 ± 8.5 | 102.3 ± 4.7 | 94.3 ± 10.5 | mg/dl |
| T-3 Uptake | | 40.8 ± 3.0 | 40.2 ± 3.3 | 37.9 ± 1.2 | 37.4 ± 2.0 | % |
| Thyroxine | | 10.2 ± 2.9 | 9.5 ± 1.8 | 9.6 ± 0.1 *[0.03] | 7.7 ± 0.2 | ug/dl |
| T-4 Index | | 4.2 ± 1.3 | 3.8 ± 0.5 | 3.7 ± 0.2 *[0.03] | 2.9 ± 0.2 | ----- |

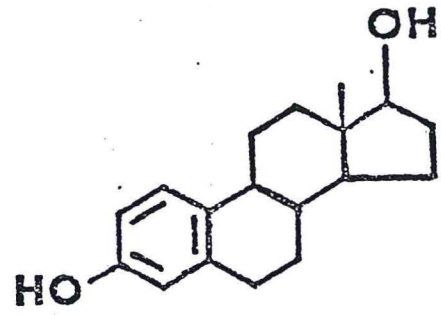
( ) Numbers in parenthesis indicate the number of test subjects when it is different from that listed on the top of the column.

* The difference is statistically significant at the p-values shown in [ ].

Fig. 1 CHEMICAL STRUCTURE OF REPRESENTATIVE ESTROGENS

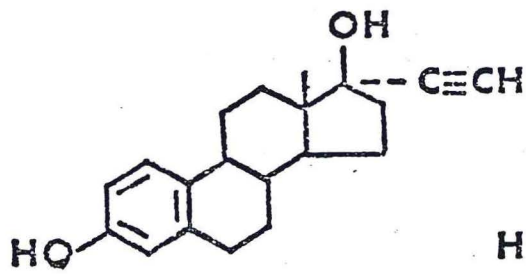



Estrone ($E_1$)

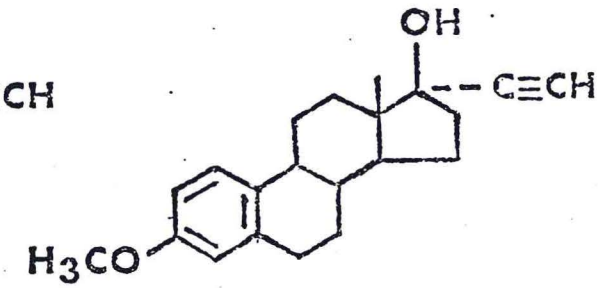



Estradiol ($E_2$)

Natural Estrogens

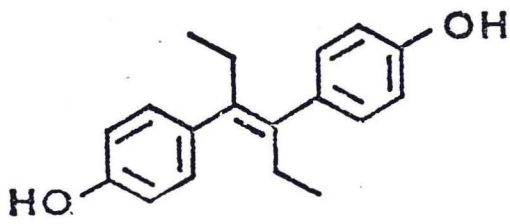



17α-Ethynylestradiol ($EE_2$)

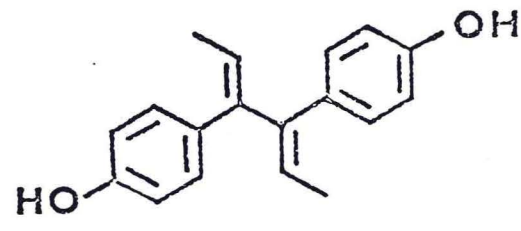



Mestranol (ME)

Ethynylated Estrogens




Diethylstilbestrol (DES)




Dienestrol

Non-steroidal Estrogens

# APPENDIX A

## DETAILED DESCRIPTION OF STUDY FACILITIES

### PLANT A

Plant A manufactures a variety of pharmaceutical products, including many different preparations containing natural conjugated estrogen (NCE). NCE is the sole active ingredient in this firm's major product for estrogen replacement but is also used in other preparations in combination with vitamins, methyl testosterone, and meprobamate, and is distributed in the form of tablets, capsules, liquids, creams, parenterals, and powders, both sterile and non-sterile. Of these, the tablets containing NCE (alone or in combination) are manufactured in the largest volume.

#### Workforce

Of approximately 450 hourly employees, about 75 employees are involved in the manufacture of products containing NCE, from granulation through packaging. Of these, approximately 30 are assigned to tasks where the company has detected measurable concentrations of NCE in the air. These 30 employees are on the average assigned to these tasks for approximately 10% of their work time. Although few in number, women do perform those tasks where the company has detected NCE in the air. A large number of women traditionally work in the packaging area, where potential for exposure is considered low. The overall employee turnover is below 10% per year.

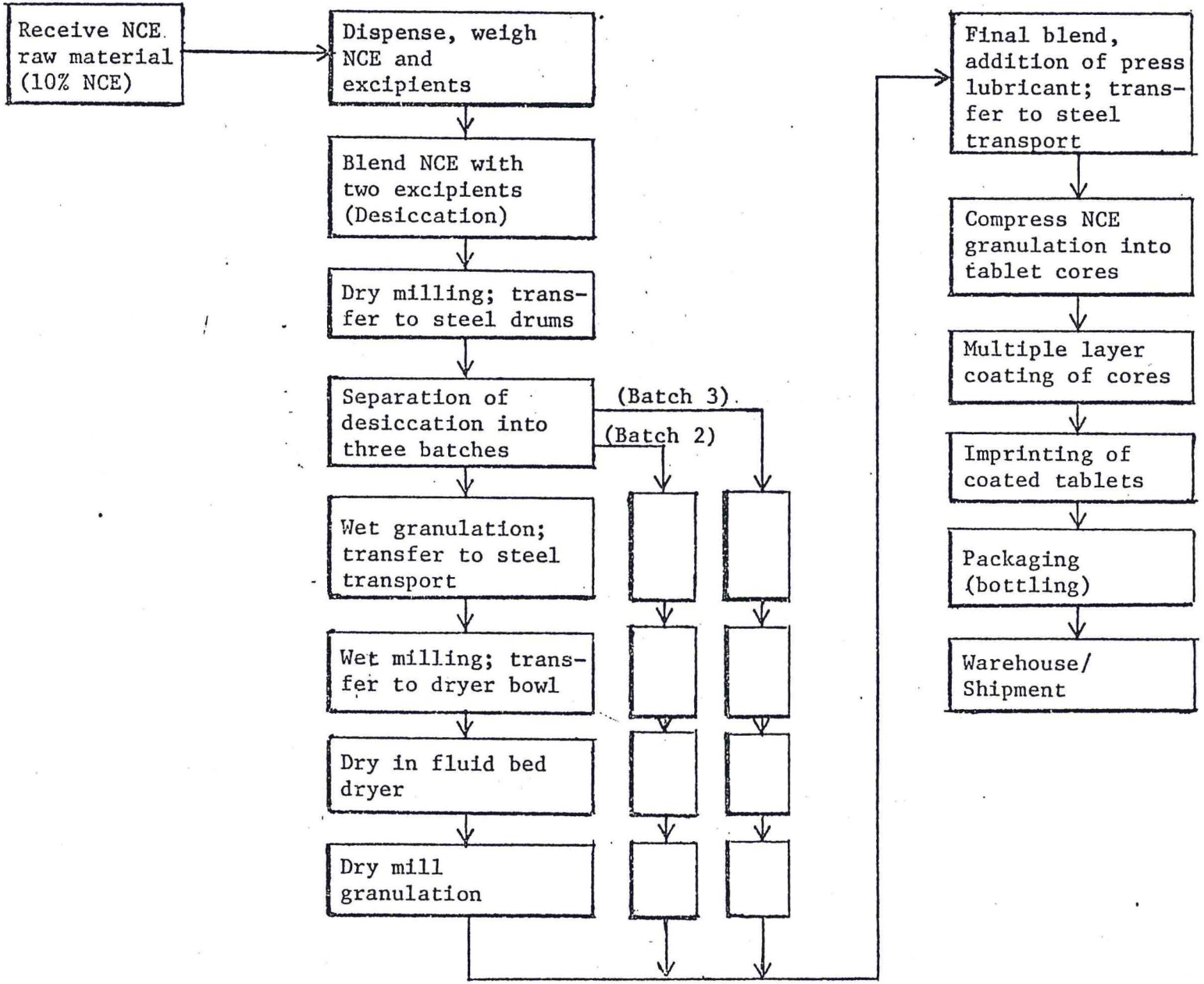
#### Description of Manufacturing Process

The following will be limited to a description of the manufacture of estrogen replacement tablets, since this process is representative of the tablet manufacturing process, is the single product manufactured in largest quantity, and was the focus of the study at this site.

The active ingredient is the NCE which constitutes approximately 10% of the basic raw material, which has been extracted from pregnant mare's urine, partially purified, concentrated, and dried on an inert carrier. The dried concentrate contains, in addition to the estrogens, non-estrogenic organic compounds, inorganic salts, and the inert carrier. The estrogenic component is a mixture of at least 9 conjugated estrogens, of which approximately 90% are the sodium (estrogen) sulfates of estrone, equilin, and 17-alpha dihydroequilin. This raw material is not manufactured at this site but is received as a pre-mixed concentrate.

The manufacture of estrogen replacement tablets at this site is essentially a step wise process involving batch mixing of ingredients followed by compression of the finished granulation into tablets. The tablets are then coated, imprinted, and stored prior to packaging. Figure 2 is a process flow diagram for the manufacturing of NCE tablets.

FIGURE 7
PROCESS FLOW DIAGRAM - PLANT A
NCE COATED TABLETS



Both active components and excipients (components other than the actives) are weighed out on a batch basis in a central weighing area under local exhaust ventilation. Assembled ingredients for specific batches are then moved as necessary to the compounding area for mixing.

The formulation steps typically begin with preparation of a pre-mix (dessication) by combining the NCE raw material with two excipients (non-active tablet components), followed by dry milling. During this process, the pre-weighed raw materials are loaded manually from storage containers into a large "V" blender, and dry mixed. The loading point (from a platform located over the mixer) is fitted with a local exhaust system. After mixing, the dessication is dropped from the bottom of the mixer through a "mill", where it is sized and directed into a metal drum. A polyethylene sleeve connects the bottom of the mixer and the mill. The mill and drum loading points are also fitted with local exhaust hoods. The operator wears a disposable, single-use Glendale GR500 dust respirator (TC 21C-195) approved for use against pneumoconiosis and fibrosis producing dusts, when working in the mixing room. He also wears white dacron coveralls, rubber gloves, and a hair cover.

Next, the dessication is wet blended with the remainder of the excipients. This process involves the loading and transfer of dessication to a mixing vessel, preparation of an organic solvent paste (added to the mixing vessel with the dessication to assure more even dispersion of components), wet mixing of the granulation, wet milling, drying in a fluid bed dryer, dry milling, and final blending with a lubricant to facilitate compression. These steps are repeated three times per eight hour shift (more or less) to complete one batch. The operators wear the same personal protective equipment as the blending operators. The process involves considerable open manual handling (loading, etc.) of various powders (wet and dry) containing the conjugated estrogens. Local exhaust hoods are placed at loading and unloading points (mixer, mills).

The rooms in which all of these steps are carried out are located adjacent to a central hallway in the estrogen processing area. The rooms are reportedly under negative pressure relative to the hallway, and are fitted with electrically operated sliding doors.

The granulation is then placed in stainless steel transports, sampled and assayed by quality control, and staged for the tableting operation. The process up to this point usually takes approximately 8 hours per batch.

Transports containing the granulation are then moved as needed to the second floor and are positioned directly over one of the tablet presses. The bottom of each transport is connected directly to a pipe which extends through the floor to the hopper of the press. The granulation is then fed automatically at a controlled rate to the tablet press. Formed cores exit the press through evacuated "deduster" tubes and are collected in open drums lined with plastic bags. The area above the tablet presses also contains an area in which the transports are manually washed down with detergent and

water. The waste water is flushed into a sanitary drain through a connecting hose. The tableting process is continuously monitored by the press operator; he inspects the cores for physical attributes and collects samples for laboratory assay.

Pressed cores are then removed to a separate area in which they receive a multiple layer coating. The cores first receive an imprevious coating, followed by many other coatings. The coating is done by manually loading the tablets into one of several ventilated rotating pans. The appropriate coating material is then added by an automated system and the batch is allowed to tumble in the pan for a length of time sufficient to assure even coating of the tablets. The tablets are then polished, imprinted with the appropriate marking, inspected, and staged for packaging operations.

The majority of estrogen replacement products are bottled. In the main packaging area (where all products are packaged), the coated tablets are manually loaded into the hopper of a bottle filling machine. The machine automatically counts and feeds the tablets to the bottles. The filled bottles are then conveyed down a line where they are plugged with cotton inserts, inspected, capped, and labeled. After packaging, the tablets are quarantined, sampled by quality control, and when released, are moved to the warehouse and shipped.

Safety and Industrial Hygiene Programs

Personal protective equipment worn by operators during NCE processing activities (i.e. weighing through tablet compression, but not packaging) included launderable coveralls, rubber gloves, safety shoes, hair covering, and several types of air purifying respirators. These included either single use, disposable respirators (e.g. 3M Company, 8710) or half mask, cartridge respirators (e.g. Glendale Company, GR500). Although facilities were available for changes of clothing, showers etc., there were no specific requirements prepared and communicated to employees on these specific practices or for entry and exit to areas where potential for exposure to estrogens exists. Written standard operating procedures for estrogen processing operations did, however, specify safety equipment requirements for specific jobs.

Although NCE product formulation rooms, compression cubicles, and similar areas were reportedly under negative pressure relative to access hallways, (following good manufacturing procedures specified by FDA), these areas were not totally isolated from adjacent areas (for example, by means of airlocks). It is conceivable that some migration of air and process generated dust to adjacent areas might have occurred, possible resulting in some degree of inadvertent exposure to employees not working directly in the manufacturing area.

Until 1978 there were no requirements for the use of respiratory protection or other personal protective equipment by employees working in the NCE product formulating and tableting areas, although various types of

respiratory equipment were said to be provided. In May, 1978, however, the company began requiring the use of respirators (and gloves) for high potential exposure jobs such as blending, granulation, tablet inspection, and tablet core coating as well as for quality control technicians and roving inspectors, and for other personnel required to enter the estrogen manufacturing areas.*

Personal and area sampling for NCE has been conducted at this site since 1978. Environmental data obtained prior to 1978 are not available. Results of sampling in 1978 indicated highly variable concentrations of total conjugated estrogens in air (expressed as the sodium estrogen sulfate), ranging from below the limit of detection (0.1 - 1.0 micrograms per filter) to approximately 200 micrograms per cubic meter over a full shift, or up to 550 $ug/m^3$ for shorter periods (1/2 hour). These results suggest the actual extent of exposure of workers to estrogens prior to 1978, since respirators were not required prior to this time.

Medical Program

Two local physicians alternately attend the plant dispensary once a week, about two hours each time. In addition, the plant employs a full-time and a part-time nurse.

Pre-employment physical examinations include history, physical examination, ventilatory function test, audiometry, EKG, blood chemistry (at employees' expense) and pap smear for females. Voluntary periodic medical examinations are offered once every two years for those over age 40 and every three years for those under 40 years old. Biennial serology tests and chest x-rays are mandatory.

No particular examination or inquiry is conducted on estrogen workers to detect signs or symptoms of overexposure. There is no formal worker education program as to the potential toxicity of estrogens in the workplace. No scheduled rotation system is currently in effect at this plant. Company officials and one of the physicians stated that they have not experienced a single case of adverse estrogen effects from occupational exposure.

* The company had indicated that since the time of the 1978 walk-through survey, a respiratory protection program, including training, has been instituted. Also added were a gowning room and an a shower.

APPENDIX A (continued)

PLANT B

Plant B manufactures oral contraceptive tablets and a variety of other pharmaceutical products containing some form of estrogenic steroid. Steroidal ingredients used in various products include: mestranol (3-methyl ether of ethynyl estradiol), norethindrone, esterified estrogens (water soluble estrogens expressed as sodium estrone sulfate), testosterone propionate and estradiol benzoate.

Workforce

Approximately 125-150 employees are involved with estrogen product manufacturing. At least eighteen percent of the employees are of a minority race. Twenty five people work in the processing department, which handles all granulation and tableting. Of this group, 6 workers are female. The packaging department consists of 115-120 people of which about 80% are female. Six quality control people rotate into the oral contraceptive area on a monthly basis. The overall plant turnover has been about 16% a year. However, turnover in the oral contraceptive manufacturing area is reportedly lower.

Description of Manufacturing Process

The manufacture of pharmaceuticals containing estrogens and/or progestogens at this site is essentially a batch mixing of ingredients followed by compression of finished granulation into tablets (or pellets) by means of a tablet press. Tablets are then blister packaged (oral contraceptive only) or bottled. Pellets for cattle are individually placed in injection cartridges and are then boxed in the packaging area. Figure 3 is a block flow diagram which depicts the major steps involved in the production of the oral contraceptive tablets.

Both active components (mestranol and norethindrone) and excipients are weighed out on a batch basis in a central weighing area under local exhaust ventilation. Assembled ingredients for specific batches are then moved to the granulation area for mixing.

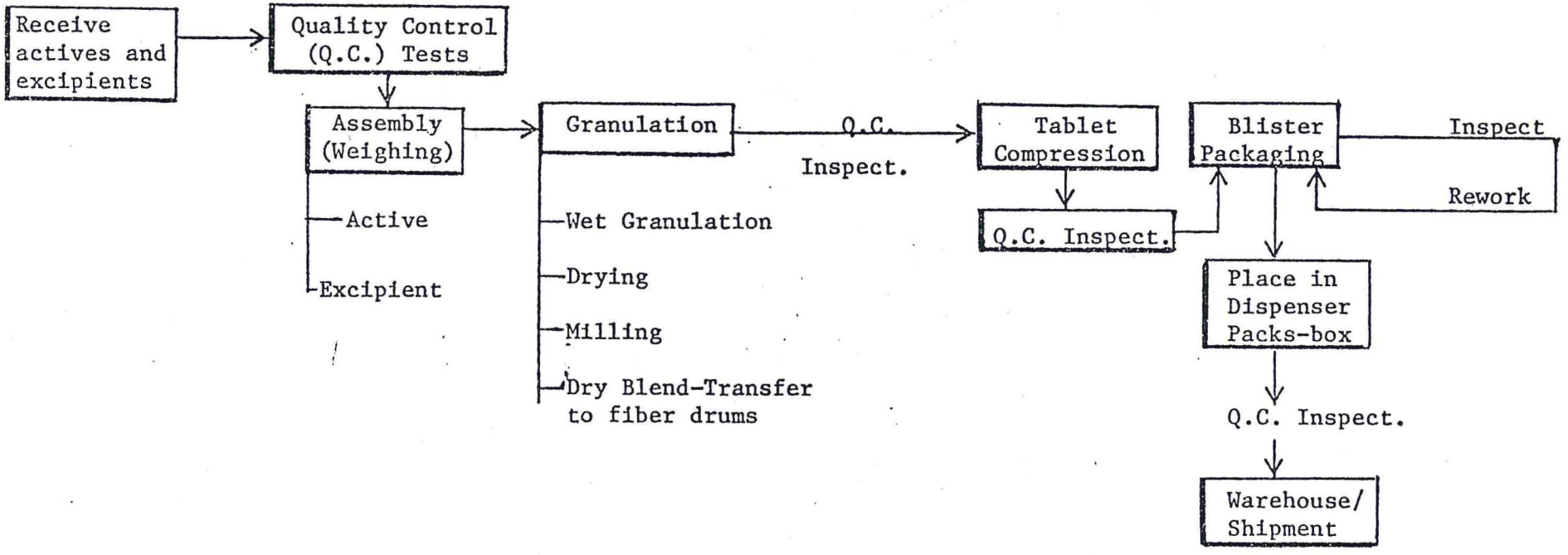
Preparation of the oral contraceptive granulation (compounding step) involves wet mixing of the tablet components, drying in a fluid bed dryer, milling, addition of a lubricant, and reblending prior to the compression step. The granulation is then placed and stored in drums, assayed by quality control, and staged for the tableting operation. The process up to this point (granulation) takes approximately three to four hours. This is done an average of four to five times per week for two weeks out of every month.

Granulation which has been tested and cleared by quality control is moved as necessary from a storage area to the compression rooms. The granulation is

FIGURE 3

PROCESS FLOW BLOCK DIAGRAM - PLANT B

ORAL CONTRACEPTIVE TABLETS



pneumatically fed from storage drums into the hopper of the tablet presses using a vacuum wand. Formed tablets exit from the machine through two tubes (equipped with mechanical de-dusters) at the side and drop into plastic lined drums. To suppress dust during the compression phase, the housing on the tablet presses are connected to the central vacuum line and are thus under negative pressure. Drums filled with tablets are sealed and stored, inspected, assayed by quality control, and staged for packaging operations.

The oral contraceptive products are blister packaged. This involves manually scooping tablets into blister pack filling machine hoppers. The hoppers and filling machines are fitted with local exhaust systems to suppress dust. The tablets are then immediately sealed between layers of foil and plastic film. The blister cards are then conveyed along a line on which they are inspected (rejected cards are opened and tablets are recycled or discarded), and are then placed in pouches and boxed with appropriate inserts.

Safety and Industrial Hygiene Programs

Personal protective equipment used in the "steroid" area during processing or testing of estrogenic compounds (including chemical weighing through tablet compression and quality assurance testing), includes launderable coveralls, separate steroid area safety shoes, rubber gloves and disposable (single use) air purifying respirators. During the jobs with the greatest potential for exposure (sampling or weighing of steroids, granulation phase and tablet compression), the operators are also required to wear a 3M #8710 mask plus a self-contained, air purifying respirator (3M Co. "Whitecap" helmet) connected to a battery operated blower and an absolute filter worn as a backpack (3M Co. "Whitecapsule II").

Male and female employees enter the granulation and compression area through separate locker rooms. In the locker room they remove street clothes and put on working uniforms, which include pants, shirts, coveralls, shoes, etc. Before entering the next area all personnel are required to don a 3M #8710 disposable mask. The next area is the personal protective equipment room. There, employees who are going to enter steroid containing areas obtain a "Whitecap" helmet, connected to a "Whitecapsule II". This area also contains special equipment for checking the performance of the filter packs and battery charge. Employees then enter the processing area through an air shower.

Upon leaving the area on breaks or lunch, operators are required to pass back through the air shower, remove coveralls and other equipment and wash their faces and hands. After completion of processing or at the end of the shift, they are also required to take soap and water showers prior to changing into street clothes or regular white uniforms. The coveralls are changed after operators have finished processing.

Medical Program

There is a full-time physician who is medical director. Other medical staff include one full-time and two part-time nurses. The pre-employment medical examination given to estrogen workers, consists of history, physical (emphasis on breast and testicles), CBC, urinalysis, SMA-16 and chest x-ray. This is repeated annually. In addition, females working in estrogen areas are required to have an annual pap test and pelvic examination to be performed by their personal physician but paid by the company.

Routine screening examination of estrogen workers is done every four to eight weeks. This includes review of symptoms (breast tenderness, libido change, etc.) and examination of the breast and axillary nodes, and measurement of areolae.

# APPENDIX B

## ENVIRONMENTAL SAMPLING AND ANALYSIS PROCEDURES

### Air Sampling Procedures

Full shift personal (breathing zone) samples were obtained by drawing air at a flow rate of about 3 1/2 to 4 lpm through 37mm millipore FALP Fluoropore™ filters (in two piece polystyrene cassettes) using Dupont P-4000™ personal sampling pumps. The pumps were attached to the operator's belt, and the closed face filters were clipped on the lapel or shirt collar in a downward facing position. Stationary full shift area samples were obtained in a similar manner using the same apparatus and filters, except that they were placed at strategic locations in the process areas.

At Plant A, one stationary high volume air sample was obtained in the same area as the area samples obtaned with personal sampling pumps. This was obtained by drawing air at a flow rate of 5 cfm through a four inch diameter Millipore FALP filter, using a Staplex model TF-1A pump equipped with a by-pass rotameter. This was done since it was not certain at the time that the samples obtained at the lower flow rate (4 lpm) would collect sufficient sample for analysis.

At Plant B, in addition to the regular personal samples, three sets of paired breathing zone samples were obtained on process operators engaged in granulation. In each of these cases, one of the cassettes was located in the normal lapel position, and the other inside the breathing apparatus (3M Company "Whitecap" helmet). The purposes were to estimate the operator's actual exposure, and to estimate the degree of protection afforded by the respiratory protective equipment. These samples were subsequently sent to a laboratory for radioimmunoassay (RIA) of mestranol and norethindrone. This was done because of the expected low concentrations inside the respiratory equipment, and because of the reported greater sensitivity of the RIA method. The outside respirator sample of each set was also analyzed by RIA so that a direct calculation of the protection factor afforded could be made.

Half of the stationary air samples obtained at Plant B were also analyzed by RIA. Replicate sets of six samples were obtained in one location on each of three shifts. These samples were then split, half being analyzed by RIA, and the remainder by HPLC. This was done to determine whether results were comparable when analyzed by the two methods. This was not intended to be a field validation of either of the two methods. However, gross comparability of results would help to confirm that the results of breathing zone samples analyzed by RIA were supportable.

### Wipe Sampling Procedures

Skin wipe samples were obtained using cotton gauze pads pre-extracted with 70/30 isopropanol and water. At the time of sampling, the pads were removed

from the scintillation vial (in which they were transported) with tweezers and dampened with an isopropanol and water (70/30) mixture from a squeeze bottle. The operator then used it to wipe the skin area of interest (hands or face), and subsequently placed the pad back in the glass vial, which was then recapped and labeled.

Analytical Procedures

Most filter samples and all of the wipe samples were analyzed using extraction and high pressure liquid chromatography (HPLC) techniques developed by NIOSH specifically for this project.

Samples obtained at Plant A were analyzed for the three major constituents, 17-alpha dihydroequilin, equilin, and estrone, which collectively comprise approximately ninety percent of the total estrogens in the NCE material. The samples were extracted from the sampling media (filters and gauze pads) with methanol using sonification for one hour. The samples were then filtered through FALP filters and concentrated by heat and a nitrogen stream. The sodium salts were hydrolyzed with concentrated sulfuric acid, and the samples were injected into the HPLC system. Separation was accomplished on a C18 radial compression column. The mobile phase was 40% acetonitrile/60% water isocratic for 10 minutes, 100% acetonitrile for 10 minutes to wash the column, and a 20 minute re-equilibration period. Detection was accomplished with a Waters 450 Variable Wavelength Detector set at 190 nm. Spiked filters and gauze pads were analyzed in the same manner, and the recovery efficiencies were incorporated in the reported concentrations.

Laboratory results were reported as nanograms per sample of free estrogens. In the case of the air samples, these results were used to calculate concentrations of each of the three estrogens in terms of micrograms of sodium (estrogen) sulfate per cubic meter of air. Total estrogen exposure was also estimated by totaling the results for each of the three estrogens. This estimate would very likely be low by a factor of about ten percent since the three estrogens comprise only ninety percent of the total estrogens present in the NCE mixture. Thus, the total concentration was divided by 0.9 to arrive at an estimate of total NCE exposure.

Samples obtained at Plant B were analyzed for mestranol and norethindrone, and synthetic estrogen and progestogen respectively. The filter samples were extracted with 2 ml. of acetonitrile using sonification, and an aliquot injected into the HPLC system. Separation was achieved by a Waters Radial Compression C18 column and a 75/25 acetonitrile/water mobile phase. Quanititation of norethindrone was done using a UV fixed wavelength detector monitoring at 254 nm, while mestranol was analyzed using a variable wavelength detector set at 206 nm. The limit of detection was reported as 0.05 ug/filter for both compounds.

Wipe samples obtained at Plant B were estracted with 10 ml of acetonitrile and analyzed in the same manner as the filters, except that interferences

necessitated modification of the mobil phase to 60%/40% acetonitrile/water. The detection limits increased to 1 ug/swipe for mestranol and to 0.25 ug/swipe for norethindrone.

Appendix C contains a detailed description of the RIA analytical procedure supplied by the contracting laboratory. The reported limits of detection were 0.01 nanograms per filter for mestranol and 0.01 micrograms per filter for norethindrone. The analyses were performed by the Endocrinology Laboratory, Department of Medicine, Emory University School of Medicine, Atlanta, Georgia.

## Radioimmunoassay (RIA) Procedures

### 1. Materials

Nonradioactive steroids, norethindrone (NET), ethinyl estradiol ($EE_2$) and mestranol ($MEE_2$) were obtained from Sigma Chemical Company, St. Louis, MO. 6,7-$^3$H-Ethinyl estradiol (SA=55 Ci/mmole) was obtained from New England Nuclear Corp., Boston, MA. 6,7-$^3$H-Mestranol was prepared by mild methylation of $^3$H-$EE_2$ with dimethyl sulfate in borate buffer. The synthesized mestranol was purified by thin layer chromatography on instant thin layer sheets (ITLC - SC, Gelman Inst. Co.) in ethyl acetate:cyclohexane (1:15). 6,7-$^3$H-Norethindrone (SA = ~5 Ci/mmole) was obtained from Amersham Searle Corporation, Chicago, IL. LH-20-100 Sephadex as well as thimerosal, bovine serum albumin (BSA), Norit A charcoal and dextran were purchased from Sigma Chemical Co. Buffer reagents and solvents were purchased from Fisher Scientific.

### 2. Extraction

The filter was extracted 1 x 5 ml with ethanol. One-half of the sample was taken for chromatography.

### 3. LH-20 Chromatography

Sephadex LH-20 was equilibrated in methanol and packed by gravitation in glass columns (1 x 11 cm). The columns were then equilibrated with 30 ml of the appropriate solvent system:

Benzene:Methanol (95:5) (for separation of ethinyl estradiol and norethindrone)

The dried extract was redissolved in 1 ml of the solvent system and applied onto the LH-20 column. The tubes were rinsed with two successive 1 ml aliquots of the solvent and similarly applied to the column. Samples were eluted with benzene:methanol (95:5) and fifteen 3 ml fractions were collected (Fig. A-1).

Aliquots of the eluates were taken and counted for radioactivity to locate the steroid peaks. Radioactive peaks corresponding to each of the steroids were pooled and evaporated to dryness under $N_2$. The dried steroid pools were redissolved in 1 ml of 95% ethanol and aliquots taken for radioimmunoassay.

4. Assay Procedure

Phosphate buffered saline [(PBS), 0.1 M dibasic sodium phosphate pH 7.2 containing (wt/vol) 0.9% NaCl; 0.1% ethylmercurinthiosalicylate (thimerosal) and 0.1% bovine serum albumin (Sigma RIA grade)] was used in all solutions. Antibody bound and free steroids were separated using 1% solution of dextran-coated charcoal (1% Norit; 0.1% Dextran T-70 in PBS).

a. Standard Curve

Ethanolic solutions of $EE_2$ (1.238 ng/ml); $MEE_2$ (2.513 ng/ml) and NET (1.040 ng/ml) were used as standards. Triplicate aliquots of the standard solutions were pipetted into series of 9 standard samples as follows:

For NET, the standard samples ranged from 10-416 pg;

For $EE_2$, the standard samples ranged from 10-248 pg, and

For $MEE_2$, the standard samples ranged from 25-502 pg.

Triplicate aliquots (200 µl) of each of the reconstituted sample extracts were pipetted into assay tubes and a further 200 µl aliquot was counted to determine recovery.

The samples for assay as well as the standard curve samples were dried under $N_2$ at 40°C. PBS buffer (0.1 M, 500 µl) was added to each tube followed by 100 µl of the appropriate antiserum (see below) (except tubes #9 of the standard curve which received an additional 100 µl of PBS). Appropriate radioactive steroid (100 µl) was added to each tube, vortexed and incubated overnight at 4°C.

b. Antisera

$EE_2$-A antibody (9/15/77)

$MEE_2$ antibody (RTIMEE)

NET antibody (RTI) Drone A/S

c. Labeled Steroids

| | |
|---|---|
| $EE_2$ assay; 6,7-$^3$H-$EE_2$ in PBS | 3000 cpm/100 µl |
| $MEE_2$ assay; 6,7-$^3$H-$MEE_2$ in PBS | 4000 cpm/100 µl |
| NET assay; 6,7-$^3$H-NET in PBS | 7000 cpm/100 µl |

d. Separation Procedure

The tubes were placed in an ice bath and 1 ml of cold 1% dextran coated charcoal solution was added and vortexed. The tubes were incubated at 4°C for 30 min followed by centrifugation at 1000 xg for 10 min at 4°C. The supernatant (containing the bound fraction) was then decanted into scintillation vials and counted. Triplicate 100 µl aliquots of the radioactive steroids were added to 1.5 ml of PBS in scintillation vials and similarly counted as an estimate of total radioactivity.

e. Special Considerations

The volume of ethanol used to redissolve the NET extract depended upon whether the expected value was high, medium or low. Low samples were assayed using 200 µl of 1.0 ml ethanol solution in triplicate. Medium range samples were dissolved in 2 ml ethanol and assayed at two levels using 50 and 100 µl or 100 and 200 µl aliquots in triplicate. Samples expected to be high were dissolved in 4 ml ethanol and assayed at two levels using 100 µl and 200 µl or 5 ml ethanol and assayed at 50 and 100 µl in triplicate. Recoveries for medium and high extracts were assessed with 1 ml aliquots. For $MEE_2$ and $EE_2$ 200 µl of the 1 ml ethanol solution were assayed in triplicate.

High and low standard controls of the appropriate steroid were included in each batch assay as a measure of reproducibility and precision.

ELUTION PATTERN OF $^{3}$H-NET AND $^{3}$H-EE$_2$ ON LH-20 SEPHADEX COLUMN CHROMATOGRAPHY USING BENZENE:METHANOL (95:5) AS ELUTING SOLVENT SYSTEM

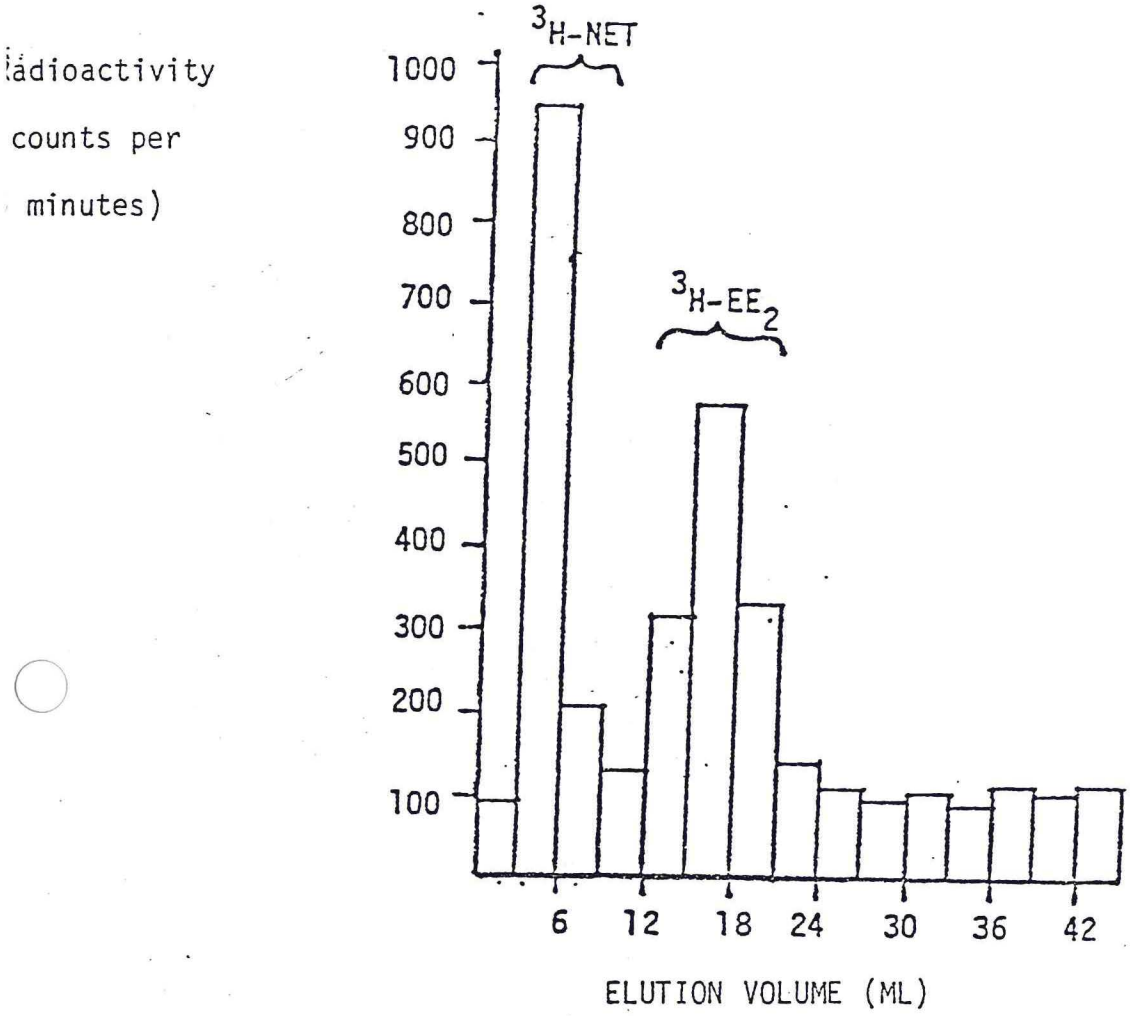



FIGURE A-1. A typical histogram of radioactivity (counts per 5 minutes of a 1/6 aliquot volume of the collected eluting fraction) versus eluting volume. The pattern of eluting radioactivity associated with $^{3}$H-NET and $^{3}$H-EE$_2$ was obtained by LH-20 sephadex column chromatography of an extracted plasma sample previously treated with a mixture of the two tritiated steroids. The solvent system used to separate these steroids was benzene:methanol (95:5). Three ml fractions were collected and a 0.5 ml aliquot of each fraction was assayed for radioactivity.

TABLE A-3. Antisera Cross Reactivities[a]

| Competing Steroid | Percent Cross Reaction | | |
|---|---|---|---|
| | $EE_2$ Antisera | $MEE_2$ Antisera | NET Antisera |
| Norethindrone | 0.8 | <0.6 | 100 |
| Mestranol | 1.5 | 100 | <3.2 |
| Ethinyl Estradiol | 100 | 29.6 | <4.8 |
| Cortisol | <0.6 | <0.6 | <0.2 |
| Estrone Sulfate | <0.6 | <0.6 | <0.2 |
| 17-β-Estradiol | 3.3 | <0.6 | <0.2 |
| 2-OH-Estradiol | <0.4 | <0.4 | <0.2 |
| Dehydroepiandrosterone | <0.6 | <0.6 | <0.2 |
| Progesterone | <0.6 | <0.6 | <0.2 |
| Testosterone | <0.6 | <0.6 | <0.8 |
| Cholesterol | <0.6 | <0.6 | <0.2 |
| Dihydrotestosterone | <0.6 | <0.6 | 0.8 |
| Estrone | <0.5 | <0.6 | <0.2 |
| 2-OH-Estrone | <0.5 | <0.6 | <0.2 |
| Estriol | <0.5 | <0.5 | <0.9 |
| Androstendiol | <0.5 | <0.5 | <0.9 |

a. Each substance tested for cross reactivity was examined at four levels ranging from approximately 0.5 ng to 5.0 ng. Percent values are < the value based on the largest amount tested for that substance.

# APPENDIX D

## RATIONALE FOR SELECTION OF CERTAIN BIOCHEMICAL INDICES IN SERUM OR PLASMA

### Glucose and Lipids

Glucose, cholesterol, triglycerides and high and low density lipoproteins were measured. These items were selected since absorption of exogenous estrogens may alter their blood levels. (Russ, et al, 1955; Oliver and Boyd, 1961; Stokes and Wynn, 1971; Kalkhoff, 1972; Davidoff, et al, 1973).

### Liver Function

Liver function tests included albumin, globulin, A/G ratio, bilirubin (direct, indirect and total), GGTP, SGOT, SGPT, LDH, and alkaline phosphatase. Measurement of liver functions were necessary because it has been established that estrogens are metabolized in the liver, and liver dysfunction will enhance estrogenic effects; diseased liver cells appear to have an ability to aromatize androgens to estrogens (Siiteri, 1977). There have been reports of cholestatic jaundice (Drill, 1974), nodular hyperplasia of the liver (Stauffer, et al, 1975), and hepatomas (Baum, et al, 1973), among oral contraceptive users.

### Thyroid Function ($T_3$ and $T_4$)

Plasma thyroid hormone concentration is closely related to the metabolism of estrogens and androgens via its influence on plasma sex hormone binding globulin (SHBG). In general, increased plasma thyroid hormone increases SHBG, which subsequently reduces the metabolic clearance rate of estrogens and androgens (Yen and Yaffe, 1978). Therefore, hyper-or hypothyroidism is a confounding factor in this study. Basic thyroid function tests were performed to detect such abnormalities.

### Creatinine and BUN

These tests were conducted to screen for renal dysfunction, since it is reported that estrogen stimulated neurophysins are increased in patients with renal failure (Amico et al, 1979). It is recognized that various other pathologic conditions are known to alter creatinine and BUN levels and the BUN/creatinine ratio. However, in a field study where elaborate tests are not feasible, these tests were considered adequate for screening purposes.

### Blood Clotting Factors (Plant A only)

As discussed previously, users of oral contraceptives experience higher incidence of thromboembolism. Poller's review (1978) indicates that the use of oral contraceptives accelerates the clotting of the procoagulants in the extrinsic and intrinsic clotting systems as well as reduction of antithrombin-III levels. Oral contraceptive workers were reported to have abnormalities in various clotting factors (Poller, et al, 1979). Therefore, the following tests were done to measure abnormalities of blood clotting:

measure abnormalities of blood clotting: prothrombin time, partial thromboplastin, fibrinogen, antithrombin III, factors VII, IX and X. Blood samples were collected using citrated plastic syringes. After separation, serum samples were transferred to plastic vials, frozen in dry ice, and shipped. Analyses were performed by the Coagulation Laboratory, Hematology Department, University of Cincinnati Medical Center. Clotting factors were not measured on Plant B workers due to technical difficulties in sampling.

Corticosteroid Binding Globulin (CBG)

Among the serum proteins which are known to increase in response to the administration of exogenous estrogens, the one most directly related in a dose response manner is corticosteroid binding globulin (Musa, et al, 1965). The elevation of CBG concentration is dose dependent for ethynylestradiol, diethylstilbestrol and conjugated estrogens (Seal & Doe, 1969). CBG in adults is not influenced by age. Therefore, it is considered to be a good biological index for estrogen exposure. Because of its slow increase after estrogen administration and relatively long half-life (Musa, et al, 1965), CBG was measured on Day 0 and 19 at Plant B. The analysis was conducted by the Endocrine Laboratory, University of Kentucky Medical Center, Lexington, Kentucky.

Testosterones (total and free percent)

Serum testosterone levels were measured as an index of male gonadal function. Testosterone is also present in females but in much smaller quantity. When a large dose of estrogen is given to males, testosterone levels decline due to negative feed-back to pituitary gonadotropins. This, together with direct actions of estrogen, will result in feminization of males (impotence, breast enlargement, etc.). However, it is also conceivable that a small amount of estrogen would increase testosterone-estrogen binding globulins (TEBG) which would cause an increase in total testosterone, but the free (not bound to proteins) testosterone concentration would probably remain the same. Therefore, both total and free testosterone were measured. Naftolin et al (1973) reported that serum testosterone levels fluctuate, that peaks appeared to follow an LH elevation by 45 to 80 minutes, but that fluctuations of T levels were much smaller (3-6 ng/ml) compared to the LH levels (5-20 MIU.ml). Total and free testosterones were measured by the Endocrine Laboratory, University of Kentucky Medical Center, Lexington, Kentucky.

Neurophysins

Neurophysins are defined as cysteine-rich protiens present in neurosecretory cells which specifically bind the neurohormones oxytocin, vasopressin, and certain of their analogs. Robinson (1975) reported the presence of two types of neurophysins; one is estrogen-stimulated neurophysin (ESN) and the other is nicotine-stimulated neurophysin (NSN). According to their analysis using radioimmunoassay (RIA), ESN levels were elevated 2 to 6-fold in women taking oral contraceptive pills when compared to non-pill users. In 5 male

subjects, ESN levels were sharply elevated 4 to 25-fold after DES administration (5 mg). These levels declined to the pre-DES level in 4 to 5 days. Cigarette smoking (nicotine) did not influence ESN levels. In view of these findings, measurement of ESN was considered to be a good method to evaluate worker exposure/absorption of estrogens. The ESN radioimmunoassay was conducted by the Endocrinology and Metabolism Laboratory, Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania.

Ethynylestradiol (EE2) (Plant B only)

Radioimmunoassay of EE2 was done on plasma samples. Mestranol, which is used in Plant B, is the 3-methyl ether of EE2. It is demethylated in the body to become EE2. Since EE2 and mestranol (methylated EE2) are exogenous estrogens, the presence of EE2 in the serum sample means that it has been absorbed from the outside by some route, namely, oral administration of contraceptive pills or occupational exposure through inhalation, skin absorption or inadvertent ingestion. Metabolic turnover of mestranol (measure as EE2) was slower than that of EE2 (de la Pena, et al, 1975). Radioimmunoassay of EE2 was done by Endocrinology Laboratory, Department of Medicine, Emory University School of Medicine, Atlanta, Georgia.

Gonadotropin Assays

Secretion of gonadotropic hormones such as FSH and LH receives negative feed-back from both endogenous and exogenous estrogen/progesterone. As a result, secretion of androgens including testosterone will be reduced when workers are exposed to estrogens on the job. However, it is reported that these gonadotropins are secreted in a spiked manner several times a day (Naftolin, et al, 1973). Thus, a single measurement of gonadotropins may not yield representative values. A more desirable procedure is to take 3 or 4 blood samples 30 minutes apart to get an average for the individual. However, in an industrial survey situation, it is very difficult to obtain successive multiple samples from each worker. Therefore, no gonadotropins were measured in this study.