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An environmental survey of a plant formulating oral contraceptives demonstrated considerable variation in possible synthetic estrogen exposure to the plant personnel. Clinical epidemiological studies showed evidence of increased absorption of estrogens in some employees at the plant. In view of the company's considerable efforts to suppress dust from work areas, new approaches to containment may be necessary with biologically active dusts — including the establishment of a dust standard.

Occupational exposure to synthetic estrogens — the need to establish safety standards

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introduction

The purpose of this paper is to outline the methodology used in the investigation of suspected hyperestrogenism in a pharmaceutical plant formulating oral contraceptives consisting of a mixture of mestranol and norethindrone and to describe ways of refining the environmental and biological monitoring techniques used in this study. Furthermore, in view of positive clinical findings, consideration needs to be given to establishing occupational health standards for estrogens in air.

Synthetic estrogens have been employed therapeutically for contraception, menopausal symptoms, senile and atrophic vaginitis, and a variety of menstrual disorders.[1] Considerable research has been expended on an evaluation of the side effects of oral contraceptives.[2] Adverse effects include cerebrovascular and cardiovascular disease, migraine, sterility, and menstrual disorders such as amenorrhea, intermenstrual bleeding, and irregular bleeding.[3] Male usage of estrogens has been advocated for prostatic cancer and the prevention of ischemic heart disease. In the latter case, the coronary drug project discontinued its trial of estrogens because of adverse side effects including thromboembolic episodes and a possible increased risk of myocardial infarction.[4,5] Less severe side effects have been previously described — again in association with long-term estrogen therapy for coronary heart disease.[6] Men given 5-10 mg/day of conjugated equine estrogens or 5-25 mg/day of diethylstilbesterol equivalent to 200-1000 μg of ethinyl estradiol, the active metabolite of mestranol) (DES) had side effects within a few months. Eventually, 100% had breast enlargement, 82% had reduced testicular size, 77% had increased areolar pigmentation, and 74% had decreased or absent libido.

In view of these findings, it is surprising that virtually no information is available on the effects of occupational exposure to synthetic estrogens. Two studies of DES formulators — one in Polana[7] and the other in Newport, Tennessee[8] — demonstrate the ability of this synthetic steroid to produce not only gynecomastia and menstrual disorders in the workers, but in the case of the Polish study, estrogenic effects in the children of the workers.

methods

The investigation of the plant included a walk-through survey, a clinical and epidemiological evaluation of plant employees, and an industrial

hygiene survey using environmental area and personal dust sampling procedures.

Fifty-five of 57 employees were available for study. These employees were interviewed by questionnare and 53 of them agreed to a clinical examination for signs of hyperestrogenism. Fifty-four agreed to have a venous sample drawn for determination of plasma ethinyl estradiol levels. Sixty matched non-factory controls completed the menstrual history of the questionnaire. Plasma ethinyl estradiol estimations were carried out using 6,7-^{3}H ethinyl estradiol and its antiserum in a radio-immunoassay procedure.[9]

A concurrent industrial hygiene evaluation involved observations of work practices as well as the collection of airborne and surface dust samples. Personal and area air samples were collected for 6-7 hours on glass fiber filters at a flow rate of 2 liters per minute using personal sampling pumps. Surface wipe samples were collected from a known area using glass fiber filters.

The steroids, mestranol and norethindrone, were extracted from the filters with ethyl ether, concentrated to a small volume, placed in conical reaction vials, a known amount of estriol internal standard in ethyl ether added, and the combined mixture evaporated to dryness under a stream of dry nitrogen. Dry pyridine and BSTFA [N, O-Bis (Trimethylsilyl)-Trifluoro-acetamide] + 1% TMCS (Trimethylchloro-silane) [Sigma Chemical Company] were added, the vials capped and derivatized at 90-100°C for 3 to 3.5 hours in a heating block. Excess reagents were removed by evaporation at 60°C under a stream of dry nitrogen, and the residue taken up in 50 μL of dry heptane. Estrogen standards were obtained from Sigma Chemical Company.

Two microliters of the resulting sample were injected into a Varian 2740 gas chromatograph equipped with flame ionization detectors and interfaced with a Perkin Elmer PEP-2 chromatography data system. The all-glass system was equipped with a 1.8 m (6 foot) by 0.64 cm (1/4 inch), (2 mm ID) column packed with 3% OV-1 on 80/100 mesh Supelcoport. Column conditions were 245°C isothermal for 12 minutes followed by rapid programming to 275°C to purge the column. Helium carrier flow rate was 40 ml/min. The silyl ethers of mestranol, norethindrone and estriol eluted in 6, 7, and 10 minutes respectively.

The identities of sample peaks were determined by use of relative retention times of standards compared to the internal standard reference. Filter samples contained many unidentified peaks. The probable identity of these samples was established by "spiking" filter extract derivatives with derivatized standards. Blank filters were also analyzed in an identical manner. The limits of detection by this method are about 2 nanograms (ng) derivative per injection.

results

the plant process

The plant began operations in 1974 and currently employs 57 workers in two shifts. The flow of materials through the plant begins at the warehouse, where the incoming raw materials are unloaded and stored. Quality assurance inspection is undertaken before the shipment is cleared for weighing. The inert fillers and lubricants are weighed by a technician wearing gloves and NIOSH approved respirators in an area where there is local exhaust ventilation.

The active ingredients, mestranol and norethindrone, are added to the inert mixture in the granulation room. After mixing, the batch is dried in an oven. The granulation room, in addition to having local exhaust ventilation in the weighing area and a changing room for employees, is also equipped with its own ceiling-to-floor forced air supply. When the active ingredients are handled, the workers wear air-supplied vinyl suits.

After granulation, the mixture is compressed into tablets in an adjacent room which is equipped with laminar air-flow ventilation. Three technicians work on compression /granulation on a rotational basis. After compression, the tablets are stored prior to packaging on an assembly line.

The line workers consist of two shifts of nine operators, one line tender, one line specialist, two mechanics, two quality control inspectors, and a supervisor. They wear personal protective clothing, finger cots, and disposable surgical masks.

TABLE I
Plasma Ethinyl Estradiol
(picograms per mL plasma, pg/mL)

Category	Job	Number of Employees with Elevated Levels*		Total Population	
1	Processing Technician	1	10	3	28
2	Quality Assurance	6		7	
3	Production Operatives	3		18	
4	Other Production Staff	3	8	13	26
5	Office Staff	5		13	

For Category 1 and 2 vs Category 3, 4, and 5, $X^2 = 2.99$ (with Yates Correction) $p = 0.08$.

*Elevated levels = > 30 pg/ml for women NOT currently using oral contraceptives and for all men.
> 150 pg/ml for women currently using low dose oral contraceptives.

the clinical epidemiological findings

The results of the clinical evaluation are described elsewhere.[10] In summary, five of the 25 male employees (20%) had clinical gynecomastia or gave a history of gynecomastia with or without decreased libido and increased areolar pigmentation. All affected males came into contact with the powdered product. Among the 30 female employees, 12 (40%) had had at least one episode of intermenstrual bleeding in the preceding 12 months. None of these cases occurred in office employees. The exposed females had a four-fold increased risk of this disorder compared with their matched controls. In short, there was clear clinical evidence of hyperestrogenism among the employees in closest contact with the product.

The venipunctures used to obtain blood samples for plasma ethinyl estradiol estimations were not accurately timed nor was timing obtained of the length of occupational exposure that day or when the last oral contraceptive was taken. In consequence, the ethinyl estradiol measurements are of limited value. Nevertheless, when the values obtained by risk category are grouped (Table I), Job Categories 1 and 2 had twice the prevalence of elevated levels as the lower risk Categories ($p = 0.08$).

industrial hygiene evaluation

Considerable care had been taken by the company to limit exposure of employees to the active ingredients of the formulation. Engineering controls appeared to be adequate, and personal protective equipment used in the granulation and compression rooms was appropriate. Nevertheless, it was theoretically possible for air to move from these areas into the changing room and shower facility, thereby exposing the unprotected workers to estrogen dust.

A second possible source of exposure was through contact with the finished product. Although line workers wore finger cots, the entire hand sometimes came into contact with the tablets. When tablets broke and fell the short distance from the hopper to the container, particulates were added to the air. Paper surgical masks were not invariably worn and probably provided inadequate respiratory protection; broken finger cots were noted to be replaced infrequently.

The third source of exposure involved the mechanics who periodically changed the filters in the ventilation system. These men seemed less aware of the potential risk than perhaps they should although disposable coveralls and appropriate respirators were provided for this work.

The area and personal sampling results are summarized in Table II. There are no recommended threshold limit values (TLVs) or Federal Standards for airborne estrogens, so no comparative analyses can be made with the data shown in Table II. The data represents a unique series of measurements not previously undertaken by NIOSH. The personal sampler values show wide variation within subjects and between subjects, ranging from below the

TABLE II
Environmental Measurements

Area Sampling (6-7 hour sampling time)	Mestranol (micrograms of steroid per cubic meter of air)	Norethindrone (micrograms of steroid per cubic meter of air)
Granulation Room	1.10	0.94
Compression Room	0.73	0.30
Line	2.19 - 6.47	1.80 - 11.74
Inspection	0.06	1.33
Packaging	0.59	N.D.*
Personal Sampling (6-7 hour sampling time)		
Technician in Compression Room	N.D. - 0.39	11.79 - 59.56
Line Operators	N.D. - 8.61	N.D. - 43.18
Wipe Samples	(μg/square cm)	
Packaging Room - Work Bench	0.003	0.019
- Opposite Tablet Re-inspection	0.94	4.90
Employees' Clothes	0.50 - 0.81	N.D. - 0.56
Changing Room	2.05	14.7

*N.D. - not detected

analytical limits of detection up to 8.61 micrograms of estrogen per cubic meter of air ($\mu g/m^3$) for mestranol and 43.18 $\mu g/m^3$ for norethindrone. Area and wipe samples also showed a wide range of values. The interpretation of such data is difficult in view of the experimental nature of the analysis and the absence of a dust standard. The only conclusions that can be drawn are that widely variable amounts of estrogen and progesterone dust are present in the work environment and that the area near the tablet reinspection and the changing room have relatively high concentrations of these hormones. This might imply the need for more stringent dust control measures in these areas.

discussion

This investigation documents clinical hyperestrogenism in some employees of a plant that formulates oral contraceptives. The clinical evidence for this is stronger in the males as gynecomastia is such a rare occurrence. In the female workers, the subjective nature of retrospective menstrual history data is somewhat offset by the finding of a four-fold increased risk of intermenstrual bleeding in the line workers compared with their matched controls. However, there is little corroboration of the clinical data by the environmental and biological concentrations of estrogen. There are several reasons for this. First, the clinical findings may be spurious, though this is difficult to envisage in the face of such clear cut evidence, especially in the males. The main reason for the discrepancy probably lies in the failure to time the samplings in relation to estrogen exposure. Exogenous estrogen has a short *in vivo* half life (3-8 hours after oral ingestion, personal communication Dr. Dale Collins), therefore, randomly timed venipunctures could not be expected to correlate with exposure — either occupational or therapeutic. In order to document such dose-response relationships, it would be necessary to determine employee exposures to airborne concentrations over a work shift, obtain a venipuncture for plasma levels at the end of that shift and accurate information on exactly when the last oral contraceptive tablet was ingested if the worker was on such medication. Under such circumstances, it may be possible to establish a dose-response relationship and thereby enable a better assessment to be made of what would be an appropriate level for an occupational air standard for synthetic estrogens in air.

It is known from the therapeutic uses of estrogens that 50 μg of ethinyl estradiol a day,

the usual oral dose of the average birth control pill, will inhibit pituitary follicular stimulating hormone in women and a similar dose in men will probably cause feminization in some individuals (200 μg is effective in producing gynecomastia).[5] Therefore, employing a safety factor of 100, it may be that a "no effect" dose of ethinyl estradiol is approximately 0.5 μg a day. Assuming that a person doing light work inhales 2.5 cubic meters of air in 8 hours, a "no effect" airborne concentration of ethinyl estradiol would be in the range of 0.2 $\mu g/m^3$ and a clinical effect noticeable at 20 $\mu g/m^3$. These assumptions are based on equal absorption of steroid by inhalation and by the oral route, an assumption for which no documentation is available. The estimates of estrogen in air concentrations for the plant under study lie between these values (personal sample mean value 1.94, standard deviation 2.46, area sample mean 1.43, standard deviation 1.93 $\mu g/m^3$ for mestranol).

Obviously further work is needed to delineate more clearly the relationship between airborne estrogens, body burdens, and clinical effect. The establishment of an estrogen dust standard would enable manufacturers to work toward engineering controls of dust evolution rather than rely on the present situation of assessing biological effect in the workers. The latter is inappropriate, inaccurate, and exposes workers to potent hormones whose long-term effects are unknown, especially in males.

It is hoped that the demonstration of a clinical occupational health problem in the oral contraceptive industry will lead to a marked decrease in worker exposure in that industry. Furthermore, consideration should be given to evaluating the occupational hazards of workers engaged in the manufacture and formulation of other potent biologics such as thyroid, pituitary, and adrenal hormones. There is no *a priori* reason for believing that they are not in a similar position to the oral contraceptive formulators and, as such, are worthy of further investigation.

acknowledgements

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