

Occupational exposure to synthetic estrogens

Some methodological problems

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HARRINGTON JM. Occupational exposure to synthetic estrogens: Some methodological problems. *Scand j work environ health* 8 (1982): suppl 1, 167—171. Occupational exposure to synthetic estrogen is known to produce feminizing effects in males and menstrual disorders in females. Modern pharmaceutical practice has not eliminated this risk, though there have been few attempts to quantify it or establish safety standards. The main problems to be faced in undertaking an epidemiologic study of estrogen-exposed workers are outlined in the present study. They include the difficulty of confirming the clinical effects of exposure, the appropriate method for measuring exogenous estrogen, and the timing of the analytical samples. Some recent studies which used other measures of pharmacological effect are reviewed, and it is concluded that direct measurement of exogenous estrogen is the analysis of choice.

Key terms: epidemiology, feminization, menstrual disorders, pharmaceuticals, work hazards.

The so-called sex hormones can be divided into three broad groups (1), estrogens, progestins and androgens (fig 1). The naturally occurring sex hormones have important and often complementary roles to play in the development and maintenance of sexual function.

Estrogens are responsible for the development and maintenance of female sex organs and for the partial regulation of the menstrual cycle (11). Their action causes the proliferation of all Müllerian duct derivative tissue, as well as proliferation of mammary gland ducts. Other activities include increasing the size and pigmentation of the nipple, as well as exerting an influence on the pituitary hypothalamic axis in its release of follicular stimulating hormone (FSH), luteinizing hormone (LH), and prolactin. Their main therapeutic use is for postmenopausal replacement therapy, oral contraception, the treatment of prostatic and mammary cancer, and the alleviation of postpartum breast engorgement.

Progestins inhibit FSH and LH release and, following estrogenic priming, prepare the uterus for implantation, maintain pregnancy, and cause proliferation of mammary duct acini. An additional therapeutic use is as an ingredient of oral contraceptives.

Androgens, by contrast, maintain testicular germinal tissue, are responsible for the growth and maintenance of male secondary sexual organs, and exert a marked anabolic effect. Androgens are antagonists of estrogens, and the overt features of

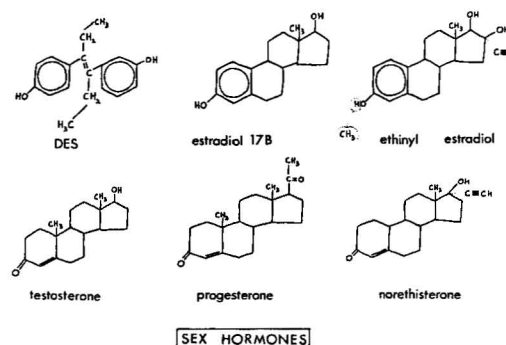


Fig 1. The three groups of sex hormones. (DES = diethylstilbestrol)

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masculinity or femininity are the result of the balance between the two groups of hormones.

The most modern estrogens are synthetic, and the first such compound was diethylstilbestrol (DES), which was produced in 1938 (5). Indeed, DES has two other remarkable features. Unlike most estrogens it is nonsteroidal, and it is produced in far larger quantities than the others — primarily for use as a growth promoter in animal food (7). Production figures for such hormones are difficult to ascertain, but it seems likely that in the United States over 1,000 workers are occupationally exposed to ethinyl estradiol (EE₂) alone (14). However, this figure is small compared with the estimated 80 million consumers of EE₂ worldwide, in oral contraceptive pills (16).

The significance of therapeutic and occupational exposure to sex hormones is heightened by the knowledge that, for some of these compounds, causative links with human cancer have been discovered (table 1). For others in the group, there is insufficient evidence for firm conclusions to be drawn, but it is likely that a number of these hormones produce a variety of human cancers (10).

Occupational exposure to estrogenic agents

The pharmaceutical industry has an enviable record for purity of product; yet few studies have been published of the occupational hazards despite the fact that all the end products are, by definition, biologically active. The first report of occupational ill health associated with estrogens appeared in 1942 when Scarff & Smith (15) reported gynecomastia and loss

of libido in two DES plant workers. A detailed series of case histories of similarly affected workers was published 2 a later; it included histological changes noted in breast biopsies (6). The clinical history of breast enlargement was noted to follow a set pattern starting with "nipple tingling," followed by soreness, impotence, thickened subareolar plaques or nodules, and extension of the enlargement into a breast quadrant. Removal from exposure was followed by a regression of symptoms and signs in the reverse order. This distinct clinical progression and regression has been noted elsewhere, and the earlier reports that the plaques of DES-induced gynecomastia are firmer than those induced by EE₂ have been confirmed (8). Further reports of occupational hyperestrogenism from DES have appeared, including one implicating dusty overalls as a cause of "pseudo pubertas praecox" in premenarchial children of such workers (11).

The realization that hormones can produce florid symptoms and signs has led to major control measures in the industry, though further reports of feminizing effects from working with DES in the United States were published in 1973 (2) and 1977 (12). Nevertheless, the majority of workers are exposed to steroidal estrogens such as EE₂ in the manufacture and formulation of oral contraceptives. Anecdotal evidence existed of occupationally induced hyperestrogenism. But it was not until 1978 that the first paper was published describing these effects (8), and attempts were made to estimate a safe workplace concentration for estrogen in air (9).

The 1978 study highlighted a number of methodological problems, which were inadequately addressed in the study. As a

Table 1. Sex hormones and carcinogenicity.

Compound	Exposure	Target organ
Diethylstilbestrol	Interuterine	Vagina and cervix, uteri (offspring)
Estrogens (including diethylstilbestrol)	Adult female	Endometrium, ? breast
Progestins	Adult	Inadequate data
Androgens	Adult female	? Liver adenoma
Oral contraceptives	Adult female	Liver adenoma, ? breast, ? cervix, uteri, ? endometrium, ? melanoma, pituitary

result, the US National Institute for Occupational Safety and Health proposed an industrywide investigation using an improved protocol. At the same time, similar proposals were promulgated in the United Kingdom, and both studies are currently awaiting industry approval before implementation. Reports of similar problems in Italy, France, and The Netherlands suggest that a wider discussion of these issues is warranted. Such is the purpose of this paper.

Methodological problems

The methodological problems can be broadly divided into a discussion of measures of outcome and measures of exposure.

Outcome measures

An epidemiologic investigation of a plant handling synthetic estrogens requires an accurate estimation of the clinical and biochemical effects of such exposure. Both aspects have inherent difficulties, not only for the exposed populations, but also for the selection of referent groups.

The *clinical effects* of estrogen exposure obviously vary depending on the sex of the exposed workers, but the main features are gynecomastia, loss of libido, and impotence in the male, and menstrual disorders in the female.

Epidemiologically, it is extremely difficult to quantify changes in sexual desire or performance; therefore most investigations have concentrated on breast enlargement as the clinical index of significant estrogen absorption. Nevertheless, minor degrees of subareolar glandular, ductal, and acinar enlargement can be difficult to ascertain, let alone quantify. Furthermore, changes in areolar pigmentation may be extremely subtle. Asymptomatic gynecomastia may affect up to two-thirds of pubertal boys and as much as 30% of the adult population (3). On the other hand, *symptomatic* breast enlargement is usually associated with consumption of one of a variety of drugs or a specific endocrinologic disorder. Such causes should obviously be excluded in any occupational survey. The remaining symptomatic suspects should then be examined

"blind" by a physician or physicians experienced in such examinations, and randomly included in the examination group should be a group of "referents."

Menstrual history is notoriously prone to error, loss of memory, and random biological variation sufficient to mask many occupationally induced changes. However, the most significant feature of occupational exposure to estrogen appears to be intermenstrual bleeding. Such information should be sought from both the exposed and nonexposed groups — preferably by the completion of a standard questionnaire or, ideally, by the prospective compilation of a menstrual diary. Workers who are at least 5 years beyond menopause could be subjected to vaginal cytology in an effort to determine estrogenic changes.

Biochemical effects include the measurement of exogenous estrogen, the estimation of other, secondary, hormonal effects, and estimation of liver, adrenal and thyroid function for exclusory causes of gynecomastia or menstrual disorders (table 2). Recent studies have included a bewildering array of such estimations, many of which are costly, complex, and of dubious value or significance.

The results of all these studies have been at least partially inconclusive. Experience gained from such work, combined with the views of recognized specialists in endocrinology and steroid biochemistry, suggests that a limited number of investigations should be carried out on plasma samples. While some tests, such as urea, electrolytes, and liver enzymes, are useful for excluding other causes of "hyperestrogenism," specific measures of estrogen absorption and effect could profitably be restricted to the following: (i) direct measurement of the exogenous estrogen(s) by radioimmunological assay, (ii) direct measurement of LH, FSH, testosterone, and estrone sulfate, (iii) possible measurement of sex hormone binding globulin (SHBG).

Unfortunately, it appears that the current lower limits of detectability for EE_2 and norethisterone (the most common constituents of oral contraceptives) are 10–15 pg/ml (37–55 pmol/l) and 30 pg/ml (90 pmol/l), respectively. These levels are close to the doses required to produce gynecomastia in the male and estrogenic changes in postmenopausal vaginal cytology. In

Table 2. Biochemical estimations and their value for sex hormone exposure.

Test	Biological matrix	Value ^a
Ethinyl estradiol	Blood	*****
Norethisterone	Blood	*****
Diethylstilbestrol (DES)	Blood	*****
Testosterone (bound and unbound)	Blood and saliva	***
Luteinizing hormone	Blood	***
Follicular stimulating hormone	Blood	***
Sex hormone binding globulin	Blood	***
Estrone sulfate	Blood	***
Prolactin	Blood	*
Estrone	Blood	*
Estradiol	Blood	*
Androgen (free and total)	Blood	*
Glucose, lipids, thyroxine, liver enzymes, electrolytes	Blood	For exclusory purposes
Gonadotrophins	Blood	?
Neurophysins	Blood	?
DES	Urine	*
Testosterone	Urine	*
"Estrogens"	Urine	*
Sodium	Urine	*
Aldosterone	Urine	*
Creatinine	Urine	*
Sperm count and motility	Semen	Unvalidated

^a Scale: * (poor) to ***** (good).

other words, according to Fotherby (personal communication), detectable plasma levels of EE₂ or norethisterone in occupationally exposed males or in females not taking oral contraceptives would be indicative of excess absorption.

Additional difficulties include the necessity of timing the venepuncture samples accurately in relation to oral contraceptive consumption and the work cycle. It is probable that blood samples should be taken at the start of the workweek, at the end of the first shift, and at the end of the last shift of the week. The pharmacokinetics of absorption are poorly understood (4), but it seems that most oral contraceptives have a half-time of about 8–12 h, though 25–30 % of consumers have EE₂ in excess of 30 pg/ml (90 pmol/l), 20 h after the last pill is taken (Fotherby, personal communication). Occupational exposures are intermittent, and absorption could be by the oral, dermal, or respiratory routes.

Exposure measures

Hormone-in-air levels also require considerable care in collection. Clearly, attention must be paid to the work cycle, and personal samplers should be used in pref-

erence to area or wipe sampling. Total dust samples should be analyzed for the appropriate hormone with radioimmuno-logical assay, as gas-liquid chromatographic methods have a limit of detection three times less precise. If the workers are wearing air-fed hoods or suits for all or part of the work cycle, the personal samplers should be worn inside the suits. Skin surface sampling has not been used to any extent but would be worth trying as a measure of possible skin absorption.

A suggested protocol

In summary, the following is a suggested protocol which might address some of the presented methodological issues and thereby improve the accuracy of the study and, in consequence, allow a better assessment of the dose-effect relationship. If this better assessment can be achieved, then a "safe" level of estrogen in air could be suggested with some measure of confidence.

Exposed group: 50–100 workers. (Larger numbers are unlikely to be feasible even in multiplant investigations.)

Reference group: similar numbers of workers, preferably in the same pharmaceutical company, matched for age, sex, and socioeconomic status.

Exposure measures: full hygienic survey of the work environment, including personal samples measured on a stratified random sample of workers and total dust estimated for the relevant hormones by radioimmunological assay.

Outcome measures (all study participants): (i) full clinical, occupational, and drug history (including details of oral contraceptive use and symptoms of breast enlargement); (ii) breast examination (men) and secondary "blind" assessment, menstrual history record (women) (at least 10 cycles); (iii) blood samples taken at start of workday, end of first shift, and end of workweek and analyzed for urea, electrolytes, liver enzymes, and exogenous hormones by radioimmunological assay. (? also measure testosterone, SHBG, LH and FSH).

Analysis: matched pair or group data.

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