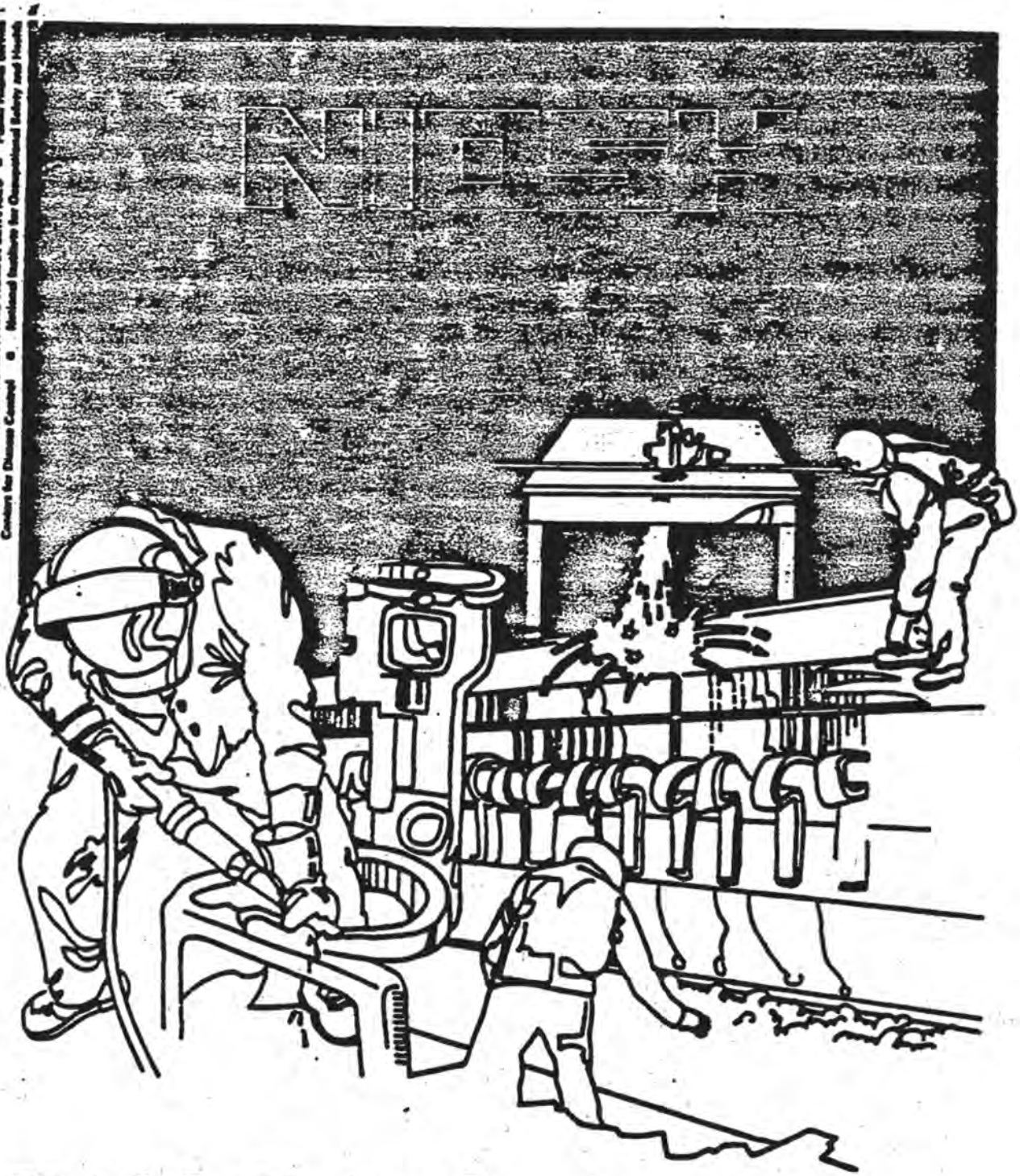
U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES • Public Health Service
Centers for Disease Control • National Institute for Occupational Safety and Health



Health Hazard Evaluation Report

HETA 82-257-1571
MANUFACTURING CHEMISTS INC.
INDIANAPOLIS, INDIANA

PREFACE

The Hazard Evaluations and Technical Assistance Branch of NIOSH conducts field investigations of possible health hazards in the workplace. These investigations are conducted under the authority of Section 20(a)(6) of the Occupational Safety and Health Act of 1970, 29 U.S.C. 669(a)(6) which authorizes the Secretary of Health and Human Services, following a written request from any employer or authorized representative of employees, to determine whether any substance normally found in the place of employment has potentially toxic effects in such concentrations as used or found.

The Hazard Evaluations and Technical Assistance Branch also provides, upon request, medical, nursing, and industrial hygiene technical and consultative assistance (TA) to Federal, state, and local agencies; labor; industry and other groups or individuals to control occupational health hazards and to prevent related trauma and disease.

HETA 82-257-1571
MARCH 1985
MANUFACTURING CHEMISTS INC.
INDIANAPOLIS, INDIANA

NIOSH INVESTIGATORS:
Tar-Ching Aw, MB
Richard L. Stephenson, IH
Alexander B. Smith, MD
Charles J. Glueck, M.D.

I. SUMMARY

In May 1982, the National Institute for Occupational Safety and Health (NIOSH) was requested to evaluate occupational exposure to an animal growth-promoter, zeranol. One female worker had developed breast symptoms and weight gain, and her male child had been found to have gynecomastia. Information from other workers from the same plant revealed three children of current workers and one child of another ex-worker with a history of gynecomastia. In one case, this was thought by the personal physician to be due to a possible exogenous cause.

Environmental sampling on May 15 - 17, 1984, found that personal (breathing zone) exposures to zeranol (the active ingredient in Ralgro®) ranged from between 4.1 ug/m^3 to 1554 ug/m^3. The highest exposures were found in the production area (median of 6 personal air samples, 547 ug/m^3). Median concentrations of airborne zeranol in the packaging area and laboratory were 46 ug/m^3 and 6.6 ug/m^3, respectively. Median concentrations of zeranol in dermal (gauze-patch) samples from the workers' palms were 90 mg/sample in the production department, 1.3 mg/sample in the packaging area, and 0.3 mg/sample in the laboratory. Surface wipe samples for zeranol showed contamination levels ranging from nondetectable (ND) to 14 mg. Measurements made in the lunch room showed levels of contamination ranging from ND-0.4 mg. A wipe sample taken in the interior of a ceiling supply-air duct in the production area revealed 5.1 mg. of zeranol. A sample of work clothing routinely laundered at home was contaminated with 16 mg. of zeranol. At present there are no criteria or standards for occupational exposure to zeranol. However, investigators have proposed occupational exposure levels for estrogens (ethinyl estradiol see Section V) and making comparable assumptions one could propose a zeranol exposure limit ranging from 0.05-0.3 mg/m^3.

Questionnaire interviews of the current workers and a comparison group of non-exposed volunteers showed more reporting of breast symptoms in the exposed group, though the difference was not statistically significant. There was no indication of a difference in weight change between the two groups over different periods of time. Clinical examination of male participants in both groups showed no evidence of distinct subareolar masses. One exposed worker had a serum estradiol level outside the laboratory normal range. Levels of serum prolactin, (FSH), and (LH) were normal in all exposed workers. Laboratory analysis of the blood by high performance liquid chromatography (HPLC) for zeranol, its precursor, and its main metabolites detected none of these compounds. (The limit of detection was 12.2 ng/ml for zeranol, 27.1 ng/ml for zearalenone, 18.8 ng/ml for zearalanone, and 11.5 ng/ml for taleranol.) Serum HDL-C levels, covariance adjusted for age and sex, were higher in exposed workers than in the comparison group (p=0.0539, t test).

On the basis of the data obtained during this investigation, NIOSH has established that workers at this plant have considerable exposure to zeranol via both airborne and skin contact routes, and this exposure poses a potential health hazard to the workers. Additionally, there is a risk of contamination of the home environment and resulting serious medical problems in exposed children. Due to the potency of zeranol, the potential health effects, and the lack of detailed information regarding human exposures, employee exposure to zeranol should be reduced to the lowest feasible level at this facility. Recommendations are included in Section IX of this report to reduce potential hazards and to provide a better work environment for the employees covered by this determination. Specifically, these recommendations are intended to help reduce direct skin contact with zeranol powder, to reduce exposure to zeranol dust, and to minimize the possibility of the agent being brought home on the workclothes or shoes to contaminate the home environment.

KEYWORDS: SIC 2879 (Agricultural Chemicals), 0219 (Livestock Products), Ralgro®, zeranol, gynecomastia, anabolic agent, estrogen, growth-promoter, zearalenone, estradiol.

II. INTRODUCTION

On May 17 1982, NIOSH received a request from the Indiana State Industrial Hygiene Compliance Section, Indiana Division of Labor (IOSHA), to assist in the investigation of breast symptoms and weight gain in an employee, and gynecomastia in a child of an employee, from Manufacturing Chemists, Inc. (MCI), Indianapolis, Indiana. Employees were reportedly exposed to zeranol, an animal growth promoter.

An initial walk-through survey was conducted at the plant on June 1-2, 1983. A follow-up environmental and medical evaluation was performed on May 14-18, 1984. Numerous delays were experienced because of problems related to access into the plant, access to records, and development of laboratory analytical methods for detection of zeranol in environmental and biological samples.

III. BACKGROUND

Manufacturing Chemists, Inc. is a small plant which formulates, compresses, and packages pharmaceutical products for human and veterinary use. Ingredients are not manufactured on the plant premises, but are bought from suppliers. Some products include aspirin, ferrous sulfate, phenobarbitol, antacids, and vitamin preparations. A major product is Ralgro®, a livestock growth promoter

The active ingredient in Ralgro® is zeranol, which is used to induce weight gain and feed efficiency in sheep and cattle. Ralgro® is made in the form of yellow, cylindrical pellets for subcutaneous implantation behind the ear of these animals by use of a device called the Ralogun®. This device implants three pellets of Ralgro® (containing a total of 36 mg of zeranol) in beef cattle, and one pellet (12 mg zeranol) in feedlot lambs. It is prohibited from use in dairy and breeding animals. One or more re-implants are sometimes done. A lag period of at least 65 days is required after the last implant before beef cattle are permitted to be killed for sale of meat for human consumption. For feedlot lambs the necessary lag period needed is 40 days [1]. MCI is the only plant in the United States producing a zeranol-based animal growth-promoter. Zeranol is made and supplied to MCI by International Minerals and Chemical Corp. (IMCC) of Terre Haute, Indiana. IMCC currently holds sole patent rights to this compound.

The main sections of the MCI plant are the production area, the packaging area, the quality control laboratory, and the administrative offices.

1. Production Department

Production materials brought into MCI are quarantined prior to their use. They are tested in the laboratory before being transferred to the production department where two, sometimes three, employees process the ingredients into compressed tablets and pellets.

Initially, zeranol and other ingredients are weighed and blended. A colored liquid is then added and blending is continued. The wet material is sized through stainless steel screens and then dried. The dried material is resized through stainless steel screens, blended with other ingredients and compressed on rotary presses into cylindrical or spherical pellets. Quality control checks are performed on the pellets for proper size, weight and hardness. Finished pellets are sent to the packaging area for inspection and packaging.

2. Packaging

Three to ten employees work in the packaging department inspecting and filling plastic cartridges with Ralgro® pellets and packaging these cartridges for shipment.

The pellets are first vacuumed. Empty, circular, plastic cartridges are placed into a jig which is then loaded with pellets. The jig is mechanically vibrated to assist in filling the cartridges with pellets. Following inspection for misfills and broken pellets, plastic backs for the cartridges (previously imprinted with specific lot numbers) are secured in place. Filled cartridges are heat-sealed in a plastic bag and finally packed in boxes for shipping.

3. Laboratory and offices

Three employees work in the laboratory performing various chemical, physical and microbiological analyses on all items entering the facility as well as all finished products before shipping. The administrative offices are in the same building as the packaging department, some distance away from the laboratory and production area. Administrative staff occasionally help out or supervise in the production area.

IV. EVALUATION DESIGN AND METHODS

A. Environmental

The initial environmental and medical survey was conducted at the plant on June 1 - 2, 1983. Information was obtained regarding work processes, number of employees, and background information concerning Ralgro® and zeranol. Additional activities accomplished included a walk-through tour of the facility, the taking of photographs, and completion of a few confidential employee interviews. Bulk samples of zeranol and Ralgro® were collected to aide in the development of air sampling and analytical methodology protocols.

Long term personal and area sampling were performed on May 15-17, 1984, throughout the plant to characterize employee exposure to zeranol. Wipe samples of work surfaces and other areas, and dermal (gauze-patch) samples from the hands were also collected as part of the environmental investigation. One bulk sample of an employee's work clothing was obtained.

A synopsis of the air, wipe, dermal, and bulk-clothing sampling and analytical methods[56] for evaluating exposure to zeranol is as follows:

The air samples were collected using SKC® Model 224 Universal sampling pumps calibrated at 2.5 liters per minute. The air sampling media consisted of Millipore Fluoropore® polytetrafluoroethylene type FHLP filters, 0.5 u pore size, 37 mm in diameter, with backup pads held in two-piece plastic cassette filter holders.

The back-up pads were discarded and the filters were placed in 20 ml scintillation vials and extracted with 2 ml of methanol aided by agitation in a sonic bath for one hour. One ml of water was added to make the samples compatible with the high performance liquid chromatograph HPLC mobile phase. After mixing, the sample was injected into an HPLC system.

Both the hand-dermal (gauze-patch) samples and the wipe samples were collected on 25 square centimeter cotton pads wetted with isopropanol. The wipe samples covered an area of 100 square centimeters.

For the gauze-patch samples, a wide-mesh piece of canvas was placed around the cotton gauze pad to protect the pad against abrasion. To help prevent contaminant breakthrough, aluminum foil cut to the size of the cotton pad was placed within the interweaving material and behind the pad. The assembled dermal sampler was attached to the palm of the worker's dominant hand (except in the packaging area, where the less dominant hand was used) using elastic straps.

The cotton gauze pads used as hand samples were placed in 20 ml scintillation vials. Twenty ml. of methanol was added to the vials and the samples were extracted for 1 hour aided by sonic bath agitation. Samples were then injected into an HPLC system. Samples with amounts of zeranol below the range of quantitation were concentrated and reanalyzed.

The cotton pads used to obtain wipe samples were put in a Micro-Soxhlet extractor. The two 5 ml aliquots of methanol used to rinse the vials were added to the extractor. After continuous extraction for 4 hours, the methanol was quantitatively transferred to a graduated tube and concentrated to 2 ml, and 1 ml of water was added. This solution was injected into an HPLC system.

The HPLC chromatographic conditions used for the analysis of the filter and the gauze pads were the same. The injection volumes and detector sensitivities, varied, depending on the sample concentration. Separation was achieved using an octadecylsiloxane (C_{18}) column and a mobile phase of 60% methanol and 40% water. Detection and quantitation was achieved by using a dual detection system. The system consisted of a Waters 450 Variable Ultra Violet detector set at 236 nm, and a Kratos Model FS9700 Spectrofluoro Monitor with an exitation wavelength set at 236 nm and an emission filter of 418 nm. Identification was achieved by retention time and absorbance ratioing of the two detectors.

The bulk sample of work clothing (half of an upper body garment) was cut into pieces, and each piece was extracted with methanol continuously for 8 hours in a Soxhlet extractor. After extraction, the methanol was concentrated to 50 ml. Prior to injection into the HPLC system, the samples were filtered. The mobile phase for the analysis of the garment was modified to achieve greater separation. A gradient program of 30% methanol/70% water to 100% methanol in 1 hour at 2 ml/min was used. Further confirmation, in addition to absorbance ratioing and retention time, was performed on a mass spectrometer. The presence of zeranol in the garment sample was confirmed by utilizing the desorption chemical ionization mode with a VG Micromass Model 7070H.

B. Medical

Medical evaluations were performed on all 11 workers at MCI and a referent group of 14 volunteers from the Indiana State Occupational Safety and Health Administration office (IOSHA). All participants were administered a questionnaire; nine MCI workers and 13 IOSHA workers provided blood samples during the work day for determination of serum levels of gonadotrophins, estradiol, zeranol and metabolites, lipid and lipoprotein cholesterol (total, HDL, and LDL cholesterol), and triglycerides. All male participants had an examination of the anterior chest wall to determine if gynecomastia was present.

1. Questionnaire

The questionnaire focussed on occupational and medical history, breast symptoms, and other symptoms relevant to estrogenic effects.

2. Physical examination

All participants had their heights and weights measured and recorded. Quetelet's index, which is used as an indicator of obesity, was calculated by use of the formula:

$$\text{Quetelet's Index} = \frac{\text{Weight} \times 1000}{\text{Height}^2}$$

Examination of male participants for gynecomastia was done by a physician who had not previously visited the plant or met any of the workers. Arrangements were made for the MCI male employees and the male participants from the comparison group to be seen by the physician at randomly allocated specified times. Because some of the MCI workers had sampling pumps for environmental monitoring attached to their belts, measures were taken to fit sampling pumps to all participants before they were seen by the physician. Participants from the comparison group were requested to dress informally. All of these procedures were intended to reduce the possibility of observer bias by the examining physician.

The protocol for the clinical assessments required the physician to make a determination of whether the person examined had gynecomastia. This determination was to be made solely by inspection and palpation of the anterior chest wall, without the knowledge of medical or occupational history. The

physician was also required to determine specifically if a subareolar nodule of more than 2 cm. diameter was present in either or both breasts of each man examined.

3. Laboratory method for detection of zeranol and its metabolites in blood.

Laboratory methods available for the detection of zeranol in blood include a radioimmunoassay (RIA) procedure developed by Thouvenot and Morfin,[2] and a high performance liquid chromatography (HPLC) method developed by Trenholm et al.[3] The RIA method requires the use of antibodies to zeranol. These antibodies are generated by immunizing pigs with a bovine albumin-bound antigen. The method detects all resorcylic acid lactones structurally similiar to zeranol, with a limit of detection of 5 ppb (5 ng/ml) in human serum. Because of the time and expense involved with this method, a modified HPLC method was used in this study.

The HPLC method requires a fluorescence detector for zeranol and its analogues. The NIOSH contract laboratory used a Waters Associates (Milford, MA) Model ALC 202 liquid chromatograph fitted with a Model 710B WISP automatic sample injector, a Model 730 Data Module, and a KRATOS Spectroflow 773 Absorbance Detector set at 263 nm. Initial efforts resulted in poor and non-reproducible recoveries from blood samples. Modification of the method by the addition of a second step to the extraction procedure was attempted to improve the detection limit. Even with the modification, the detection limit for zeranol was 12.2 ng/ml. This is still higher than the detection limit of 0.6 ng/ml reported by Trenholm[3] and co-workers.

4. Laboratory analysis for total cholesterol, triglycerides (TG), and high density lipoprotein cholesterol (HDL-C).

These determinations were performed by the Lipid Research Center, University of Cincinnati, following standardized Lipid Research Center methods, slightly modified for HDL-C determinations.[4,5,6] Low density lipoprotein cholesterol (LDL-C) levels were calculated in all subjects from total cholesterol and HDL-C readings. LDL-C/HDL-C ratios were calculated from these readings.

5. Laboratory analysis for serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin, and estradiol.

These analyses were performed by radioimmunoassay. Normal values for these parameters vary according to sex and phase of the menstrual cycle. Oral contraceptive use, endocrine disorders, and a medical history of a previous oophorectomy will also affect the serum levels of these hormones. These factors were determined in the questionnaire. For ascertaining the phase of the menstrual cycle, female subjects were asked, 8 weeks after collection of the blood samples, the dates of their last menstrual cycles . Because ovulation occurs two weeks before the beginning of a menstrual period (independent of the interval between periods), this procedure allows determination of whether each female subject was at the follicular, ovulatory, or luteal phase of the menstrual cycle at the time of blood collection. The serum FSH and LH were then compared with the laboratory's normal range for the appropriate phase of the menstrual cycle.

V. EVALUATION CRITERIA

A. Zeranol

Zeranol (also called zearalanol and 7-alpha-zearalanol) is a chemical derivative of a resorcylic acid lactone.[7,8] It is a non-steroidal, non-antimicrobial, anabolic agent with some estrogenic properties. The FDA approved the use of zeranol as an animal growth-promoter in 1969.[9] In this respect it is similiar to diethylstilbestrol, which was formerly used as an animal growth promoter. Zeranol is obtained by catalytic dehydrogenation from zearalenone.[2] This is an estrogenic[10] mycotoxin produced by numerous species of Fusarium, but especially by Fusarium graminearum (Gibberella zeae), a fungus found on corn and barley in storage and in the field.[11,12,13] The mode of action for zeranol includes stimulation of the pituitary gland to increase production of growth hormone (somatotrophin).[7] It can also alter blood levels of LH (luteotropic hormone), increase prolactin levels, and decrease serum FSH (follicular stimulating hormone).[7] Zeranol possesses some estrogenic properties, being more estrogenic than the precursor zearalenone[14,15,16]. Its estrogenic effects are used for the treatment of menopausal symptoms in humans [17,18,19,20,21]. It is sold as a pharmaceutical agent (trade name Frideron) for this purpose in some European countries, but not in the United States. Frideron is available in 50 mg and 75 mg tablets, and the doses

used for menopausal females range from 50-75 mg/day for three weeks, followed by one week without any treatment.[22,23] When used in this way, zeranol reverses vaginal atrophy and decreases hot flushes, sweating, nervousness, insomnia, tiredness, and depression. An overdose can lead to excessive uterine bleeding, breast tenderness, and water retention. Breast pain and tension have also been reported in postmenopausal women on long term treatment with zeranol[24].

Zeranol is metabolized to a less active major metabolite - zearalanone, and an even less active minor metabolite - taleranol (beta-zearalanol). Zearalanone is excreted in the urine and feces in the free form and conjugated as sulfates and glucuronides. The extent of conjugation varies from only 1% in dogs to 99% in humans.[25] Zeranol and zearalanone are excreted primarily via the bile in most animal species except in the rabbit and in man where excretion is mainly via the urine. Zeranol can be absorbed through the intact skin, and in animal experiments this route of exposure has been shown to produce systemic estrogenic effects.[26]

There are no NIOSH, OSHA, ACGIH or other criteria or standards for exposure to compounds with estrogenic activity. Harrington et al[27] in their study of workers exposed to oral contraceptives at a pharmaceutical plant proposed a "no effect" airborne concentration of 0.2 ug/m^3 for ethinyl estradiol. This was based on:

a. the therapeutic (contraceptive) dose of 50 ug of ethinyl estradiol a day;
b. the assumption that a person doing light work inhales 2.5 cubic meters of air in 8 hours;
c. a safety factor of 100; and
d. the assumption that estrogenic compounds are equally as well absorbed by inhalation as by the oral route.

Using similiar assumptions for zeranol (where the therapeutic dose for treatment of post-menopausal symptoms is 50 - 75 mg/day[22,23]) a similiar "no effect" airborne concentration of 0.2 mg - 0.3 mg/m^3 can be proposed. This airborne concentration may be relevant if the route of exposure is by inhalation alone. However, in the present case absorption is as likely to occur through the skin as well as by the oral route; zeranol has been reported to be readily absorbed through the skin [26]. Since multiple routes of absorption exist for zeranol, the "no effect" airborne concentration may be much lower than 0.2 mg/m^3. In a discussion of Harrington's

proposal of 0.2 ug/m^3 as an 8-hour time weighted average (TWA) exposure standard for ethinyl estradiol, Hochstrasser[28] suggested that this level may be too high. Using an 8-hour inhalation volume of 10 m^3 for a "Standard Man" at work, he proposed a 'permissible level' of 0.1 ug/m^3, and an 'action level' of 0.05 ug/m^3 for occupational exposure to ethinyl estradiol. Using similiar calculations, a 'permissible level' for zeranol would be 0.1 mg/m^3 and the 'action level' 0.05 mg/m^3.

B. Gynecomastia

Gynecomastia is defined as excessive development of the male mammary glands, even to the functional state.[29] In the extreme cases, the breasts become capable of secreting milk. Gynecomastia may take the form of a discrete, palpable mass easily distinguished from the surrounding adipose tissue, or it may produce a more diffuse mass resembling the surrounding fat. Gynecomastia may be physiological (as in gynecomastia of puberty), idiopathic, or secondary to a wide variety of conditions including genetic disorders (Klinefelter's syndrome), testicular tumors and primary testicular failure, liver disease, endocrine disorders, alcoholism and drugs.[30] Drugs associated with secondary gynecomastia include isoniazid, spironolactone, reserpine, digitalis, phenothiazines, amphetamines, marijuana, and steroids.[31] Gynecomastia is a common asymptomatic finding in two-thirds of prepubertal boys and 30% of adults [32]. Nuttall [33] observed gynecomastia in 36% of 306 military personnel and recruits, and Carlson [31] noted it in 32% of 100 male veterans. In both of those series there was an absence of symptoms of breast pain, tenderness, or perceived breast enlargement. Harrington [34] refers to the importance of symptomatic gynecomastia in cases secondary to drugs and endocrine disorders. The clinical assessment of whether gynecomastia exists can be very subjective. To standardize such evaluations, specific criteria have been developed to determine the presence and degree of gynecomastia. Nydick and co-workers [32] in their study on gynecomastia in adolescent boys defined gynecomastia as the presence of a firm, discoid, subareolar nodule with the following grades:

a. 1+: Limited to the subareolar area and not reaching the areolar margins (about 0.5 cm diameter)
b. 2+: Reaching up to the margins of the areola but not beyond (up to 1.5 cm)
c. 3+: 0.5 cm or less beyond the areola
d. 4+: More than 0.5 cm beyond the areola

Nuttall [33] defined gynecomastia as a palpable, discrete button of firm subareolar tissue measuring at least 2 cm in diameter.

C. Serum total cholesterol, triglycerides, HDL-C, and LDL-C levels.

Normal concentrations for these lipids and lipoproteins have been determined for American blacks and whites of both sexes in different age groups.[35] Concentrations of these lipids and lipoproteins are affected by a variety of factors, including a fatty meal prior to collection of the blood samples, oral contraceptives, genetic disorders of fat metabolism, exogenous anabolic steroids, hypothyroidism, obstructive liver disease, and diabetes. Alterations in the LDL-C/HDL-C ratio have been documented in patients taking anabolic agents.[36,37] High LDL-C and low HDL-C levels (and therefore a raised LDL-C/HDL-C ratio) are associated with an increased risk of coronary artery disease.[38,39] A reduction in serum total cholesterol has been reported in women given zeranol therapeutically.[21,23]

D. Serum FSH, LH, prolactin, and estradiol.

FSH, LH, and prolactin are hormones produced by the anterior pituitary gland; estradiol is produced by the ovaries and the adrenals. FSH and LH are gonadotrophins, acting directly on the gonads. They both increase estrogen production. A feedback mechanism exists whereby rising levels of estrogens inhibit gonadotrophin secretion. Prolactin is the lactogenic hormone. Its main action is to initiate and sustain milk secretion. Estradiol is one of the primary estrogens; it plays a role in initiating and maintaining the secondary sexual characteristics, and in the maintenance and control of menstruation.

Exogenously administered estrogens, such as oral contraceptives, suppress the formation and release of FSH and LH. Zeranol has been shown to reduce gonadotrophin levels when it is administered to women after physiological or surgical menopause[21,40]. In a reported epidemic of breast enlargement among pre-pubertal girls and boys in an Italian school[41] thought to be related to an uncontrolled supply of estrogen-fed poultry and beef, hormonal levels in those affected were within normal limits except for a slightly raised 17-beta estradiol level. Zeranol and its precursor zearalenone has also been shown to compete with estradiol for cytoplasmic receptor sites in experiments using calf or immature rat uterine cells.[42] In a study of workers exposed to synthetic hormones,

significantly lower natural total estrogen levels were noted in exposed men and women compared to non-exposed workers[45]. However, no consistent pattern of abnormalities in gonadotrophin levels were noted. The effects of absorption of small amounts of exogenous estrogens on gonadotrophin and estradiol levels are therefore varied.

Harrington has suggested that biochemical estimations of FSH and LH levels are of greater value than determination of prolactin and estradiol in evaluating occupational exposure to estrogens [34].

VI. RESULTS

A. Environmental

1. Exposure Levels

Results of the personal air and hand (gauze-patch) samples obtained on the May 1984 survey are presented in Tables II and III. Table I below is a synopsis of the data presented in Table II.

TABLE I: Summary of Results of Environmental Samples For Zeranol

Work Area (# of workers)	Air Samples (ug/m^3)			Gauze-patch samples (mg/Sample)		
	No. of (Samples)	Range	Median	No. of (Samples)	Range	Median
Production (2)	(6)	86-1554	547	(5)	1.1-410	90
Packaging (3)	(8)	30-145	46	(9)	0.04-2.3	1.3
Laboratory (3)	(9)	4-21	6.6	(9)	0.004-1.7	0.3

Air samples were obtained over a three-day period during production, packaging, and laboratory operations and on one day during routine janitorial clean-up procedures. Exposures were highest in the production area where the zeranol powder is made into pellets (median air concentration, 547 ug/m^3; median amount on the hand samples, 90 mg). Zeranol exposures decreased as the frequency and quantity of Ralgro® pellets and/or zeranol powder handled decreased. In the packaging area (the median air concentration was 46 ug/m^3 and the median amount on the hand samples, 1.3 mg/sample); in the laboratory (the median air concentrations was 6.6 ug/m^3 and the median amount on the hand samples, 0.3 mg/sample). During clean-up operations, at the end of the shift,the airborne zeranol exposure to the maintenance person was 215 ug/m^3 for the time period sampled. Area air samples obtained at the entrance of the plant, in the receptionist area, showed airborne zeranol levels of 1.6 - 4.8 ug/m^3.Surface wipe samples showed zeranol contamination in the lunchroom/breakroom 0.4 mg/sample, laboratory 0.2 mg/sample, packaging area 1.1 mg/sample and the production area (inside a ceiling supply air duct 5.1 mg/sample).

The sample of work clothing contained 16 mg of zeranol. Prior to the analysis, the worker (packager) had worn this garment for two consecutive days during routine packaging operations. If one assumes equal distribution of zeranol on the entire garment the amount of zeranol found would have been 32 mg, slightly less than three pellets (36 mg) of the finished product. This worker routinely laundered this garment at home.

2. Engineering Controls

Some of the production equipment/processes have exhaust ventilation systems. In the production area, the screening tables (wet and dry) and the pelletizing machines are equipped with local exhaust ventilation systems. In the packaging area an exhaust ventilation system is situated adjacent to the jigs used to fill the plastic cartridges with Ralgro®. In all three processes, (screening tables, pelletizing machines, and jigs) the ventilation systems have quite limited capture velocities and negligible contaminant collection efficiencies. This is due in part to the collection hood characteristics such as its orientation and distance from the emission sources.

3. Personal Protective Equipment

Packaging and production workers wear disposable half-face piece air-purifying dust respirators. The packaging workers do not wear gloves. The production employees wear protective gloves when wet screening Ralgro®, however, gloves are not used when dry screening, mixing or material transfers. The maintenance person performing clean-up operations in the production area does not wear a respirator or gloves.

B. Medical

1. Questionnaire

a. Demographic data

All 11 current workers at MCI were included in the study. The characteristics of this group and of the 14 IOSHA personnel forming the comparison group are as shown in Table IV. The IOSHA participants are younger, with a shorter duration of employment in their current jobs.

b. Breast symptoms

Participants were asked about their experience of nine specific symptoms relevant to the breasts. The number of participants from the zeranol-exposed group and the referent group who had these symptoms are as shown in Table V.

'Sensation of heaviness in the breasts' and 'nipple discharge' were not reported by any participant in either group. Each of the remaining seven symptoms was reported by at least one MCI worker compared to none of the IOSHA workers. None of the differences were statistically significant ($p > 0.05$; Fisher's exact test, one-tailed).

Similiar findings are obtained if the symptoms in males participants are analysed separately from the female participants. Six of the 11 MCI workers (55%) had at least one of the above symptoms compared to three of the 14 IOSHA (21%) participants. This difference is not statistically significant ($p=0.098$, Fisher's exact test, one-tailed).

c. Reproductive system symptoms

Except for 'spotting between periods', only MCI women workers reported reproductive system symptoms (Table VI)
Four of the six female MCI workers (67%), and two of the six female IOSHA workers (33%) had at least one of the above symptoms, but the difference is not statistically significant ($p=0.284$; Fishers exact test, one-tailed).

None of the male workers at MCI or at IOSHA had experienced any impotence, loss of interest in sex, or any perceived infertility.

d. Children with gynecomastia

From the questionnaire returns and other information obtained subsequent to the formal interviews, six MCI workers had a total of 15 children living at home. The children's ages ranged from 2 months to 16 years; they included 11 boys and 4 girls. Three of the boys have had breast enlargement and discomfort, and the mothers have sought medical advice for this problem. One boy was at a pre-pubertal age, but the other two were younger. None of the three children had any specific laboratory investigations done in regard to the breast symptoms. Four IOSHA employees had a total of 6 children living at home; 2 boys and 4 girls, aged 1 to 13 years. None of the them have any known breast or other health problems.

Two ex-workers from the plant were interviewed. They each have a male child with a history of breast enlargement. Both these children were independently seen by two different pediatricians and were noted to have definite gynecomastia. The children were aged 4 months and 2.5 years old at the time of diagnosis. Both developed breast enlargement at a time when the parents worked in the production department of the plant. One worker brought home the work uniform on many occasions, and the other used the same shoes at home and at work. Neither were aware that work clothes and shoes may be contaminated by chemicals at work, or that by using the same items of clothing at work and at home, contamination of the home environment by workplace chemicals could occur. One child had an extensive medical evaluation which found an increase in bone growth with an increased plasma estradiol level at the time the gynecomastia was present. In both children the gynecomastia reverted to near normal when the parents left employment at the MCI plant. At the same time the increased bone growth in the one child slowed down and his plasma estradiol level returned to normal.

2. Physical examination

a. Gynecomastia

One of the 5 MCI men was thought by the examining physician to have possible gynecomastia on inspection of the anterior chest wall. None of them nor any of the 8 IOSHA men had discrete subareolar breast masses.

b. Body Weight

Changes in body weight over a one year period just prior to the interviews according to the participants estimates ranged from -28 lbs. to +3 lbs. (mean = -6 lbs.; median = 0 lbs.) for the MCI workers. For the IOSHA referent group the range was -12 lbs. to +10 lbs. (mean = +1 lbs.; median = +1.5 lbs.) (Two pregnant subjects, one from each group, were excluded from the estimation of weight change.)

Quetelet's Index ranged from 25 to 47 (median = 34) for the MCI subjects, and 29 to 46 (median = 35) for the IOSHA subjects.

3. Laboratory results

a. Zeranol and analogues

No zeranol, zearalanone, zearalenone, nor taleranol were detected in any of the blood samples. The limits of detection and quantification for each of these substances are shown in Table VII.

b. Lipids and Lipoprotein Cholesterols

Mean total cholesterol, HDL-C, and LDL-C levels, and mean LDLC/HDLC ratios were higher in exposed than in non-exposed participants (Table VIII); mean triglyceride levels were lower. Only the differences in HDL-C levels between exposed and non-exposed individuals was close to statistical significance ($p=0.0539$, t test) after covariance adjustment for age and sex, suggesting that the other differences observed in Table VIII were due to differing age distributions between the groups (The MCI workers were older than the IOSHA participants - see Table IV).

c. Serum estradiol

All of the men had serum estradiol levels within the laboratory's normal range (10 - 60 ng/L), except for one MCI employee with an estradiol level of 69 ng/L. The mean ($\pm$ standard deviation) serum estradiol levels for the male MCI workers was 39 ± 20 ng/l (median = 30), compared to 41 ± 13 ng/L for the male IOSHA participants (median = 39). The difference was not statistically significant (t test; $p > 0.05$).

d. Serum prolactin

With the exclusion of female subjects who were pregnant or taking oral contraceptives, the serum prolactin levels for all participants were within the normal range. The mean (± standard deviation) serum prolactin levels for these MCI participants was 9.7±3.9, and for the IOSHA participants 8.9±5.2 accordingly. The difference is not statistically significant (t test; $p > 0.05$)

e. Serum FSH and LH

With the exclusion of pregnant women and those taking oral contraceptives, the LH levels were within the normal range for both exposed and non-exposed workers. The FSH levels were within normal limits for all MCI workers and marginally outside laboratory normal limits in three of the IOSHA workers. Two of these FSH levels were lower, and one was higher.

VII. DISCUSSION

A. Environmental

Prior to the NIOSH environmental hygiene survey, IMCC personnel conducted air sampling for zeranol on May 23 - 24, 1983 at MCI. The IMCC sampling data revealed employee exposures (personal air samples) to zeranol in the production department (683 ug/m^3, 2.13 mg/m^3, 2.94 mg/m^3, and 4.0 mg/m^3) and the packaging area (107 ug/m^3, 168 ug/m^3, and 251 ug/m^3). Two area air samples collected on consecutive days in the lunch/break room on top of the refrigerator showed airborne zeranol levels of 16.1 ug/m^3 and 25.8 ug/m^3. Detectable amounts of zeranol were found on dermal samples collected from some workers prior to and even after washing. Comparison of the IMCC data with NIOSH's suggests that employee exposures to zeranol have been reduced over the one year period from May 1983 to May 1984.

Zeranol contamination of an item of work clothing was documented by NIOSH's laboratory analysis of part of a worker's garment. Some employee interviews indicated that workclothes and workshoes were frequently worn home. Hence, there is a possibility of zeranol contaminating the workclothes and shoes and then the home environment, where children could be exposed to the compound. Contamination of the home environment with substances used in the workplace is well documented in the occupational health literature, in the cases of lead[43] and asbestos[44], for example.

The exposed plant workers had dermatologic contamination of zeranol powder on their skin, and there was probably exposure by inhalation and involuntary ingestion as well, especially prior to the regular use of respirators and among current workers (such as bearded employees) who cannot obtain a good respirator face-piece to face seal. Contamination of the lunch room and of the ventilation system (production area) was also documented.

There remains some uncertainty as to when respirators were provided; when their use was made mandatory, and when protective workclothes (caps, lab coats, slacks and shirts) were initially provided. The workers and management staff gave different times for the implementation of these.

B. Medical

The initial health hazard evaluation request concerned one zeranol exposed worker with breast symptoms, and a child with gynecomastia. Information gathered from the formal questionnaires, informal interviews, and other sources indicated that at least 4 other male children of current and previous workers have been noticed by their parents to have had transient breast enlargement during the time when the parents were working at the MCI plant. The extent of parental concern was such that all had independently consulted a physician or health care professional then. The children's ages at the time when the breast problems were noted ranged from 4 months to 16 years. Pre-pubertal gynecomastia is common among male children around the age of 16 years, and at younger ages a variety of other causes are possible, including exogenous estrogen exposure and absorption. At least one parent of each of these children worked with zeranol.

The current zeranol-exposed workers reported more breast and menstrual symptoms than the non-exposed comparison group. But the number of workers involved (even though it represented the total current population of workers in the U.S. pelletizing and packaging a zeranol-containing animal growth-promoter) is small, and statistically significant differences in symptoms reported between the two groups were not detected.

Examination of the men showed only one zeranol-exposed worker with apparent breast enlargement. Not one of the participants had any distinct palpable subareolar masses. Using Nuttall's definition for gynecomastia[33] then, none of the exposed or non-exposed workers had gynecomastia at the time of examination.

Blood tests showed no zeranol, zearalenone, zearalanone, or taleranol in all the blood samples. Since environmental sampling showed gross skin contamination of the exposed workers with zeranol, the absence of the compound and its precursor and metabolites from the blood samples may mean that it is not well absorbed through intact skin in humans, even though it is readily absorbed via this route in animal studies[10]. Or it may be absorbed and metabolized in amounts below the laboratory's limit of detection.

The serum estradiol, prolactin, and gonadotrophin levels showed no consistent pattern of abnormalities. Serum estradiol was elevated in one exposed worker, but was within the laboratory's normal range in all other blood samples. With the exception of pregnant women and those taking oral contraceptives, serum LH and prolactin were all within normal limits. Serum FSH was normal except for three non-exposed workers; one had a level marginally below the laboratory's normal range, and the other two were marginally above the upper limit of normal. There were no significant differences in the various hormone levels between the exposed and the unexposed workers. Mills and co-workers[45] have indicated that serum hormone levels may not be of much use to evaluate low-level occupational exposure to estrogenic agents.

Zeranol has in-vivo estrogenic action,[48] and the increased HDL-C levels in exposed subjects are consistent with epidemiologic and pharmacologic studies which point to the elevations of HDL cholesterol by endogenous and/or exogenous estrogens.[49,50]

Zeranol can be absorbed through the intact skin and has been shown to produce estrogenic effects by this route of exposure [26]. The cumulative exposures in these exposed MCI workers may be above those in persons who ingest zeranol residues in animal products. An epidemic of breast enlargement in an Italian school was suspected to be due to an uncontrolled supply of poultry and beef from animals which may have been fed estrogens to accelerate weight gain.[41] Recent reports from Puerto Rico have suggested that the finding of premature thelarche and precocious sexual development among children in Puerto Rico may be related to exogenous estrogen exposure.[51] Preliminary studies have shown significant quantities of estrogens in some poultry and some meats, and zearalenone has been isolated in blood samples from some of these children.[52] The source of exposure is not known however, and further studies are anticipated.

Schoental [53] in discussing the health hazards of secondary metabolites of Fusarium, refers to the induction of tumors in animals by estrogenic compounds, including natural or synthetic hormones, diethylstilbestrol, and zearalenone. Reference was also made to experiments on white Wistar rats exposed perinatally to large doses of zearalenone, which resulted in the development of benign and malignant tumors of the pituitary gland, uterus, and

testes. A carcinogenic bioassay on zearalenone conducted under the National Toxicology Program [54] concluded that it was not carcinogenic for F344/N rats of either sex, but was carcinogenic in $B6C3F_1$ mice. There are several similiarities between zearalenone and diethylstilbestrol, including estrogenic properties and being capable of inducing tumors in laboratory animals. Diethylstilbestrol was used for its anabolic effects on animals as a growth promoter for poultry and beef cattle [55]. It is used in human medicine for the treatment of menopausal disorders. Zeranol, the metabolite of zearalenone, is currently being used as a growth promoter for beef cattle and feedlot lambs, and is also used for the treatment of menopausal symptoms in humans. These similiarities suggest that it would be prudent to advice limiting exposure to zearalenone or zeranol, especially in the workplace. Pregnant women should avoid occupational contact with these compounds. The latter is in the light of the experience with diethylstilbestrol used for threatened abortions resulting in an increased risk of vaginal and cervical clear-cell adenocarcinoma in female offsprings [55], and abnormalities of male sex organs and decreased male fertility in male offsprings.[53]

Complete lists of workers who had previously worked at MCI were not available. Data that were available indicated that from January 1973 to March 1984, at least 34 permanent and 44 temporary employees worked in various jobs at the plant. The permanent workers stayed for a median duration of 10 months, and the temporary workers for a median duration of 1 month. The temporary jobs were almost all in the packaging department, and the permanent jobs included production, packaging, laboratory, and clean-up staff. Unavailability of phone numbers and forwarding addresses for these ex-workers made tracing difficult.

VIII. CONCLUSIONS

This study documented substantial exposures (both airborne and dermatologic) to zeranol, and contamination of the lunch room, ventilation ducts, and an item of worker's clothing. Exposed workers reported more breast symptoms than a non-exposed comparison group. Anecdotal and other information indicated a number of male children of different ages with transient breast symptoms. No definite cases of gynecomastia were observed among the current male workers, and no zeranol was detected in blood samples obtained. These negative physical and laboratory findings, however, do not negate the potential health hazard documented by the environmental findings, the symptoms among workers, and the reports of breast symptoms in workers' children.

IX. RECOMMENDATIONS

In view of the findings of this investigation, the following recommendations are made to ameliorate existing or potential hazards and to provide a better work environment. Specifically, these recommendations are intended to help reduce direct skin contact with zeranol powder, to reduce exposure to zeranol dust, and to minimize the possibility of the agent being brought home on workclothes or shoes.

1. Reduction of employee exposures can be accomplished by the implementation of improved engineering control of workplace contaminants by automation, redesign or replacement of existing mechanical ventilation systems, or a combination of these measures.

   a. Since Ralgro® is intended for animal use only, and since zeranol is a fairly potent substance capable of producing potential biological changes in relatively low concentrations, the manufacturing process of Ralgro® (including pelletizing and packaging) should be in one designated area, isolated from the production of pharmaceuticals intended for human consumption. Access to the Ralgro® processing area should ideally be controlled through the use of an air-lock system.

      Both doors of the air-lock should not be opened simultaneously. Workers would pass through the air-lock to a change room and remove their street clothing. They would then put on protective clothing and respirators and pass through a degown room before proceeding into the work area. Upon returning from the processing area they would degown (remove protective clothing) under a down-draft booth and then take a shower before putting on their street clothes in the change room. The degown room should be designed so that the shower room cannot be bypassed when going from the degown room to the change room.

   b. Effective engineering controls (local and general exhaust ventilation) are necessary for the production and packaging operations at MCI. Plant management should consider installing a glove box system for the weighing and screening of zeranol. Manometers should be installed as part of the ventilation system and periodically checked to assure effective filter collection of contaminants and that negative pressure is maintained in the Ralgro® processing area.

   c. Respirators as a means of controlling exposures should be used in the interim period while effective, feasible engineering controls are being implemented. Respirators are generally not recommended for use on a long-term basis. A proper respirator face-piece to face seal also cannot be established if there is facial hair (beard and/or sideburns) in the seal area. The importance of a good fit/seal of the respirator cannot be

overemphasized. Because of the potential adverse health effects resulting from exposure to zeranol, plant management should consider providing employees with respirators that have higher workplace protection factors than those currently in use.

The overall protection afforded by a given respirator design (and mode of operation) may be defined in terms of its protection factor (PF). The PF is a measure of the degree of protection afforded by a respirator, defined as the ratio of the concentration of contaminant in the ambient atmosphere to that inside the enclosure (usually inside the facepiece) under conditions of use. Respirators should be selected so that the concentration inhaled by the wearer will not exceed the appropriate limit. The maximum use concentration for a respirator is generally determined by multiplying a contaminant's permissible exposure limit by the protection factor (PF) assigned to the respirator. The maximum use concentration for the disposable half-face dust respirators used at the plant is 5 (PF) times 50 ug/m^3 or 250 ug/m^3. Using this logic, alternate types of respirators such as air purifying full-facepiece with high efficiency cartridges (50 PF) would provide maximum use concentrations of 2.5 mg/m^3.

The respiratory program at MCI should comply with those guidelines found in DHEW (NIOSH) Publication No. 76-189, "A Guide to Industrial Respiratory Protection," and to the General Industry Occupational Safety and Health Standards, OSHA (29 CFR 1910.134).

2. All workers in general, and production workers in particular, should be required to use the shower facilities provided at the plant before going home at the end of the workday.

3. Workclothes and shoes used at work and potentially contaminated with zeranol should not be worn outside the plant, and especially should not be worn home. A system of changing from street clothes and shoes to workclothes and work-shoes before work, and changing back after work, should be instituted. Disposable overshoes may be an alternative to changing shoes. Plant management should provide for the laundering of all workclothes.

4. Gloves should be provided and used in all areas of the plant where there is likely to be hand contact with the zeranol powder or Ralgro pellets. These gloves should cover the hands and forearms and should be thoroughly cleaned at the end of the workday with an appropriate solvent, such as isopropanol. Care should be taken to ensure that no contamination of the inside of the gloves occurs. Damaged gloves should not be used, and should be replaced immediately.

5. Plant employees should receive training on work practices that are effective in reducing or minimizing exposures.. Written procedures should be available regarding the proper handling of zeranol and other pharmacologically active agents. This should include instructions on decontamination and clean-up and on use and maintenance of protective clothing and equipment.

6. Levels of zeranol in the air in and around the production and packaging areas should be monitored regularly. Periodic checks on surface contamination, and contamination of lunch areas and ventilation ducts are also needed. In the event of detectable contamination, measures to thoroughly clean these areas (and recheck them to determine adequacy of cleanup) and investigations to determine and remedy the cause of the contamination should be instituted.

7. Workers should have pre-employment and periodic medical evaluation for gynecomastia, weight change, and reproductive system effects. The occurrence of breast and other symptoms related to estrogen exposure should be promptly evaluated by a physician familiar with the products and processes at the plant. Pregnant workers should be transferred to work in areas of the plant where there will be no direct contact with or exposure to zeranol.

X. REFERENCES

1. Federal Register, 35(168):13727-8;1970

2. Thouvenot D, Morfin RF. Radioimmunoassay for zearalenone and zearalanol in human serum: Production, properties, and use of porcine antibodies. Applied & Env. Microbiology 45(1):16-23, 1983.

3. Trenholm HL, Warner RM, Farnworth ER. High performance liquid chromatographic method using fluorescence detection for quantitative analysis of zearalenone and alpha-zearalenol in blood plasma. J. Assoc. Off. Anal. Chem. 64(2):302-310, 1981.

4. Lipid Research Clinics Program. Manual of laboratory operations. Vol 1. Lipid and lipoprotein analysis. DHEW publication NO. (NIH)75-628, Government Printing Office, Washington DC, 1974.

5. Ishikawa TT, Brazier JB, Steiner PM, Stewart LE, Gartside PS, Glueck CJ. A study of the heparin-manganese chloride method for determination of plasma alpha-lipoprotein cholesterol concentration. Lipids 11:628-33, 1976.

6. Friedewald WT, Levy EI, Fredrickson DS. Estimation of the concentrations of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin. Chem. 18:499-502, 1972.

7. Anderson CE, ed. Veterinary Pharmaceuticals & Biologicals 1982/1983. Kansas: Veterinary Medicine Publishing Co., 1983.

8. Ingerowski GH, Stan H-J. In vitro metabolism of the anabolic drug zeranol. Journal of Environmental Pathology and Toxicology 2:1173-1182, 1979.

9. Federal Register, 34:18243; 1969

10. Carcinogens and related substances: Analytical chemistry for toxicological research. Bowman MC. ed. New York and Basel: Marcel Dekker, Inc.

11. Shipchandler MT. Chemistry of zearalenone and some of its derivatives. Heterocycles: An international Journal for Reviews and Communications in Heterocyclic Chemistry. Heterocycles 3:471, 1975

12. Urry WH, Wehrmeister HL, Hodge EB, Hidy PH. The structure of zearalenone. Tetrahedron Lett. 27:3109-3114, 1966

13. Pathre SV, Mirocha CJ. Zearalenone and related compounds. Adv. Chem. Ser. 149:178-227, 1976

14. Martindale's Extra Pharmacopoeia. 27th ed. London: Pharmacy Press.

15. Baldwin RS, Williams RD, Terry MK. Zeranol: A review of the metabolism, toxicology, and analytical methods for detection of tissue residues. Regulatory Toxicology and Pharmacology 3:9-25, 1983.

16. Ueno Y, Tashiro F. # -Zearalenol, a major hepatic metabolite in rats of zearalenone, an estrogenic mycotoxin of Fusarium species. J. Biochem. 89:563-571, 1981.

17. Imparvio E, Marino L, Sallusto A: Controlled clinical evaluation of a new drug in the menopausal syndrome. Archivio di Ostretrucia e Ginecologia 77:203, 1972.

18. Giambanco V: Effectiveness of zeranol in the treatment of the climacteric syndrome. Patol. Clin. Ostet. Ginecol. (Italy) 5(1):37-48, 1977.

19. Menchini Fabris GF, Bianchi B: Evaluation of a new non steroid estrogen activity (Zeranol Ralone). Minerva Ginec. (Italy) 29(3):225-232, 1977.

20. Kullander S, Svanberg L: On climacteric symptoms and their treatment with a new nonsteroidal estrogen. Int. J. Gynaecol. Obstet. 13(6):277-81, 1975.

21. Ferraro R, Foglia G, Rossato P, Venturini PL: A non steroidal estrogen for treatment of surgically castrated women: hormonal and metabolic effects. Minerva Ginec (Italy) 28(12):977-984, 1976.

22. Package Insert for Frideron (Sandoz).

23. Utian WH. Comparative trial of P1496, a new, non-steroidal oestrogen analogue. Br. Med. J. 1:579-581, 1973.

24. Seppala M, Vara P: Zeranol (P1946), a nonsteroid estrogen analog, in long term treatment of climacteric syndrome. Ars Medici (Liestal) 66(11):467-70, 1976.

25. Migdalof BH, Dogger HA, Heider JG, Coombs RA, Terry M. Biotransformation of zeranol: Disposition and metabolism in the female rat, rabbit, dog, monkey, and human. Xenobiotica 13 (4):209-21, 1983.

26. Goisis C, Goisis F, Tammiso R. Estrogenic absorption through the skin: an experimental study. Acta Euro. Fertil. 11(1):61-85, 1980.

27. Harrington JM, Rivera RO, Lowry LK. Occupational exposure to synthetic estrogens - the need to establish safety standards. Amer. Ind. Hyg. Assoc. J. 39:139-43, 1978.

28. Hochstrasser JM: Exposure to synthetic estrogens. Am. Ind. Hyg. Ass. J. 39:675-7, 1978.

29. Dorland's Illustrated Medical Dictionary, 25th ed. W.B. Saunders: London, 1974.

30. Hall R, Anderson J, Smart GA, Besser M. Gynecomastia. In: Fundamentals of Clinical Endocrinology. 2nd ed. Bath: The Pitman Press, 1974.

31. Carlson HE. Current concepts: Gynecomastia. New Engl. J. Med. 303:795-9, 1980.

32. Nydick M, Bustos J, Dale JH Jr., Rawson RW. Gynecomastia in adolescent boys. JAMA 178:449-54, 1961.

33. Nuttall FQ. Gynecomastia as a physical finding in normal men. J. Clin. Endocrin. & Metab. 4:338-340, 1979.

34. Harrington JM. Occupational exposure to synthetic estrogens: Some methodological problems. Scand. J. Work Environ. Health. 8(1):167-71, 1982.

35. University of Cincinnati. Lipoprotein Research Division. 10th and 90th percentile distributions for plasma lipids and lipoprotein cholesterol.

36. Haffner SM, Kushwaha RS, Foster DM. Studies on the metabolic mechanism of reduced high-density lipoproteins during anabolic steroid therapy. Metabolism 32:413-20, 1983.

37. Taggart HM, Applebaum-Bowden D, Haffner S, et al. Reduction in high-density lipoproteins by anabolic steroid (stanozolol) therapy for post-menopausal osteoporosis. Metabolism 31:1147-52, 1982

38. Miller GJ, Miller NE. Plasma high-density lipoprotein concentration and development of ischaemic heart disease. Lancet 1:16-20, 1975.

39. Gordon T, Castelli WP, Hjotland MC, et al. High density lipoprotein as a protective factor against coronary heart disease: the Framingham study. Am. J. Med. 62:707, 1977.

40. Imparvio E, Marino L, Sallusto A. Controlled clinical evaluation of a new drug in the menopausal syndrome. Archivio di Ostretrucia e Ginecologia. 77:203, 1972

41. Fara GM, Del Corvo G, Bernuzzi S, Bigatello A, Di Pietro C, Scaglioni S, Chiumello G: Epidemic of breast enlargement in an Italian school. Lancet 2:295-7, 1979.

42. Kiang DT, Kennedy BJ, Pathre SV, Mirocha CJ: Binding characteristics of zearalenone analogues to estrogen receptors. Cancer Res. 38:3611-5, 1978.

43. Martin AE, Fairweather FA, Buxton R. St. J., Roots LM. Recent epidemiological studies on environmental lead of industrial origin. In: Recent advances in the assessment of the health effects of environmental pollution: proceedings, international symposium, Vol.2, June 1974; Paris, France. Luxembourg: Commision of the European Communities; 1113-22, 1975.

44. International Labour Office. Encyclopaedia of Occupational Health & Safety. 3rd. ed. Vol 1/A-K. Geneva: International Labour Office, 1983.

45. Mills JL, Jefferys JL, Stolley PD: Effects of occupational exposure to estrogens and progestogens and how to detect them. J. Occ. Med. 26(4):269-272, 1984

46. Webb, O.L., Laskarzewski, P.M., Glueck, C.J.: Severe depression of high density lipoprotein cholesterol levels in weight lifters andd body builders by self-administered exogenous testosterone and anabolic-androgenic steroids. Metabolism, in press, 1984.

47. Haffner SM, Kushwaha RS, Foster DM, Applebaum D, Hazzard WR: Studies on the metabolic mechanism of reduced high density lipoproteins during anabolic steroid therapy. Metabolism 32(4):413-420, 1983

48. Kitts WD, Newsome FE, Funeckles VC: The estrogenic and antiestrogenic effects of coumestrol and zearalanol on the immature rat uterus. Can J Anim Sci 1984; 63(4):823-34

49. Glueck, C.J., Fallat, R.W.: Gonadal hormones and triglycerides. Proc. Royal Society Medicine 67(7):667-669, 1974.

50. Heiss G, Johnson JN, Reiland S, Davis CE, Tyroler HA: The epidemiology of plasma high density lipoprotein cholesterol levels. Circulation 62(5):116-136, 1980.

51. Bongiovanni AM: An epidemic of premature thelarche in Puerto Rico. J. Paed. 103(2):245-6, 1983.

52. Saenz de Rodriguez CA: Environmental Hormone contamination in Puerto Rico. The New Engl. J. of Med. 310(26):1741-2, 1984.

53. Schoental R: Health hazards of secondary metabolites of Fusarium. Microbiologie - Aliments - Nutrition. 1:101-7, 1983.

54. National Toxicology Program: Carcinogenic Bioassay of Zearalenone in F344/N rats and B6C3F$_1$ mice (Feed Study). Technical Report Series No.235. 1982. National Institutes of Health, Public Health Service, U.S. Department of Health and Human Services.

55. International Agency for Research on Cancer: IARC Monographs on the evaluation of the carcinogenic risk of chemicals to humans: Sex Hormones (II). Vol. 21. 1979. World Health Organization.

56. National Institute for Occupational Safety and Health (NIOSH) air sampling and analytical methodology for zeranol, Charles E. Neumeister, NIOSH Cincinnati, Ohio. (unpublished method)

XI. DISTRIBUTION AND AVAILABILITY OF REPORT

Copies of this report are currently available on request from NIOSH, Division of Standards Development and Technology Transfer, 4676 Columbia Parkway, Cincinnati, Ohio 45226. After 90 days, the report will be available through the National Technical Information Service (NTIS), 5285 Port Royal, Springfield, Virginia 22161. Information regarding its availability through NTIS can be obtained from NIOSH Publications Office at the Cincinnati address. Copies of this report have been sent to:

1. Manufacturing Chemists Inc., Indianapolis, Indiana
2. International Minerals & Chemical Corporation, Terre Haute, Indiana
3. U.S. Food & Drug Administration, Indianapolis, Indiana
4. NIOSH, Region V
5. Federal OSHA, Region V
6. IOSHA, Indianapolis, Indiana

For the purpose of informing affected employees, copies of this report shall be posted by the employer in a prominent place accessible to the employees for a period of 30 calendar days.

XII. AUTHORSHIP AND ACKNOWLEDGMENTS

Report prepared by:

Tar-Ching Aw, MBBS, MSc, PhD.
Occupational Physician
Medical Section

Richard L. Stephenson, I.H.
Industrial Hygienist
Industrial Hygiene Section

Alexander Blair Smith MD, MS.
Chief
Medical Section

Originating Office:

Hazard Evaluations and Technical Assistance Branch
Division of Surveillance, Hazard Evaluations, and Field Studies

Field Assistance
Medical:

Abraham Osimbowale, MD, MSc.
Chief Resident (Occupational Medicine)
University of Cincinnati

	James Collins Health Technician Support Services Branch
Field Assistance Environmental:	James Boiano, I.H. Industrial Hygienist Industrial Hygiene Section
	Michael V. King Physical Science Technician Industrial Hygiene Section
Laboratory Analyses Medical:	Charles J. Glueck Director, General Clinical Research, Lipid Research, and CLINFO Centers University of Cincinnati
	Ken Menzies Arthur D. Little Inc. Cambridge, Massachusetts
	Frederick C. Phipps Chemist Clinical and Biochemical Support Section Technical Support Branch
	Barry R. Belinky Chemist Measurements Development Section
Laboratory Analyses Environmental:	Charles Neumeister Chemist Measurements Support Section
Report typed by:	Tar-Ching Aw Patty Johnson Tony Frey Lynette Jolliffe Kathy Conway

TABLE II
Results of Environmental Air Samples and Hand/Dermal Samples For Zeranol
Manufacturing Chemists Incorporated
Indianapolis, Indiana

HETA 82-257

Production Department

Sample Location	Personal Sample (P) or Area Sample (A)	Date/Time	Sample Volume (liters)	Airborne Zeranol[1] (ug/m^3)[2]	Zeranol Hand/Dermal[6] Samples mg/Sample[3]
On Dry Screening Table	A	5/15/84 0732-1527	1213	585	
Production Supervisor	P	5/15/84 0652-0930 1002-1223 1448-1518	823	680	160 [4]
Production Worker	P	5/15/84 0701-0930 0953-1224 1317-1502	1013	88.8	1.2
Weigh Table across from Pelletizer	A	5/15/84 0734-1527	1183	37.2	
In front of curved hood for local exhaust: Dry Screening Table	A	5/16/84 0658-1511	1233	389	
On top of and behind the curved hood for local exhaust: Dry Screening Table	A	5/16/84 0648-1511	1233	59.2	
Production Supervisor	P	5/16/84 0648-0837	273	659	410 [5]

Sample Location	Personal Sample (P) or Area Sample (A)	Date/Time	Sample Volume (liters)	Airborne Zeranol[1] (ug/m^3)[2]	Zeranol Hand/Dermal[6] Samples mg^3/Sample[3]
Production Worker	P	5/16/84 0702-1228 1310-1505	1103	435	
On table adjacent to an active pellitizer machine	A	5/16/84 0658-1511	1233	12.2	
Production Worker	P	5/17/84 0703-1233 1309-1459	1100	86.3	1.1
Production Supervisor	P	5/17/84 0750-1229 1312-1459	965	1,554	90
On work table counter top where dusting off of pellets occur	A	5/17/84 0742-1508	1115	39.5	

1. All concentrations are time-weighted averages for the period sampled.
2. ug/m^3= micrograms per cubic meter of air.
3. mg = milligrams of material
4. A separate hand/dermal sample obtained during the dry screening process which accounted for 150 of the 160 milligrams.
5. Wet and dry screening was performed while this sample was collected.
6. Hand/dermal samples were collected on 25 sq. centimeter (4 sq. in.) cotton pads on worker's dominant hand, except in the packaging area, where the sampling pad was positioned on the non-dominant hand.

Laboratory analytical limit of detection (LOD) in micrograms/sample	(0.02)	
Laboratory analytical limit of quantitation (LOQ) in micrograms/sample	(0.06)	
LOD in milligrams/sample		(6×10^{-5})
LOQ in milligrams/sample		(2×10^{-4})

TABLE II
Results of Environmental Air Samples and Hand/Dermal Samples For Zeranol
Manufacturing Chemists Incorporated
Indianapolis, Indiana

HETA 82-257

Packaging Area

Sample Location	Personal Sample (P) or Area Sample (A)	Date/Time	Sample Volume (liters)	Airborne Zeranol[1] (ug/m^3)[2]	Zeranol Hand/Dermal[4] Samples mg/Sample[3]
Sample on table in middle of inspecting area	A	5/15/84 0745-1530	1163	20.6	
Packager Worker A	P	5/15/84 0726-1228 1245-1528	1163	39.6	1.7
Packager Worker B	P	5/15/84 0730-1228 1246-1529	1153	86.7	1.0
Packager Worker C	P	5/15/84 0736-1128 1247-1530	1138	46.6	2.3
Sample on table adjacent to cartridge filling machines	A	5/15/84 0743-1530	1168	94.2	
On top of center table between two bagging/ sealing operations	A	5/16/854 0733-1515	1155	11.3	
On top of table in between two pellet, disk filling tables	A	5/16/84 0733-1515	1155	18.2	

Sample Location	Personal Sample (P) or Area Sample (A)	Date/Time	Sample Volume (liters)	Airborne Zeranol[1] (ug/m^3)[2]	Zeranol Hand/Dermal[4] Samples mg/Sample[3]
Packager Worker B	P	5/16/84 1704-1222	1100	109	1.2
Packager Worker A	P	5/16/84 0704-1249	863	37.1	0.04
Packager Worker C	P	5/16/84 0706-1222 1343-1515	1020	45.1	2.3
Sample on table top near worker C	A	5/17/84 0705-1206 1339-1504	963	21.8	0.9
Packager Worker A	P	5/17/84 0703-1214 1338-1503	990	30.3	1.9
Packager Worker B	P	5/17/84 0703-1205 1338-1503	968	145	1.3
Sample in center of work table	A	5/17/84 0712-1503	1178	14.4	

1. All concentrations are time weighted averages for the period sampled.
2. ug/m^3 = micrograms per cubic meter of air
3. mg = milligrams of material
4. Hand/dermal samples were collected on 25 sq. centimeter (4 sq. inch) cotton pads on worker's dominant hand, except in the packaging area, where the sampling pad was positioned on the non-dominant hand.

Laboratory analytical limit of detection (LOD) in micrograms/sample	(0.02)	
Laboratory analytical limit of quantitation (LOQ) in micrograms/sample	(0.06)	
LOD in milligrams/sample		(6×10^{-5})
LOQ in milligrams/sample		(2×10^{-4})

TABLE II
Results of Environmental Air Samples and Hand/Dermal Samples For Zeranol
Manufacturing Chemists Incorporated
Indianapolis, Indiana

HETA 82-257

Lab Area

Sample Location	Personal Sample (P) or Area Sample (A)	Date/Time	Sample Volume (liters)	Airborne Zeranol[1] (ug/m^3)[2]	Zeranol Hand/Dermal[4] Samples mg/Sample[3]
Laboratory Assistant	P	5/15/84 0701-1219 1320-1521	1098	5.8	0.004
On top of Refrigerator	A	5/15/84 0726-1522	1190	3.1	
On counter next to hardness tester	A	5/15/84 0722-1522	1200	5.7	
Quality control microbiologist	P	5/15/84 0705-1222 1307-1519	1123	8.9	1.
Director of quality control	P	5/15/84 0704-1219 1319-1530	1115	6.5	0.1
Quality control microbiologist	P	5/16/84 0700-1210 1307-1503	1065	10.3	1.7
Next to hardness tester	A	5/16/84 0717-1509	1180	5.8	
Laboratory Assistant	P	5/16/84 0701-1210 1315-1503	1043	5.6	0.2

Sample Location	Personal Sample (P) or Area Sample (A)	Date/Time	Sample Volume (liters)	Airborne Zeranol[1] (ug/m^3)[2]	Zeranol Hand/Dermal[4] Samples mg/Sample[3]
On top of refrigerator	A	5/16/84 0717-1509	1180	2.1	
Director of quality control	P	5/16/84 0651-1209 1305-1505	1095	14.6	0.6
Quality control microbiologist	P	5/17/84 0708-1202 1259-1459	1035	21.2	0.8
Director of Quality control	P	5/17/84 0706-1203	1038	4.1	0.3
Sample next to hardness tester	A	5/17/84 0742-1509	1118	9.8	
Laboratory Assistant	P	5/17/84 07121203 1305-1501	1018	6.6	0.1

1. All concentrations are time-weighted averages for the period sampled.
2. ug/m^3 = micrograms per cubic meter of air.
3. mg = milligrams of material
4. Hand/dermal samples were collected on 25 sq. centimeter (4 sq. inch) cotton pads on the worker's dominant hand, except in the packaging area, where the sampling pad was positioned on the non-dominant hand.

Laboratory analytical limit of detection (LOD) in micrograms/sample	(0.02)	
Laboratory analytical limit of quantitation (LOQ) in micrograms/sample	(0.06)	
LOD in milligrams/sample		(6×10^{-5})
LOQ in milligrams/sample		(2×10^{-4})

TABLE II
Results of Environmental Air Samples and Hand/Dermal Samples For Zeranol
Manufacturing Chemists Incorporated
Indianapolis, Indiana

HETA 82-257

Miscellaneous Area Air Samples

Sample Location	Personal Sample (P) or Area Sample (A)	Date/Time	Sample Volume (liters)	Airborne Zeranol[1] (ug/m^3)[2]	Zeranol Hand/Dermal[4] Samples mg/Sample[3]
New building Receptionist area	A	5/16/84 0730-1537	1218	1.6	
New building on top of filing cabinet near the entrance to the restricted area	A	5/17/84 0710-1507	1193	4.8	
Receptionist area on table behind secretary's desk	A	5/17/84	1190	4.8	
Maintenance Worker	P	5/17/84 1805-1127	205	215	0.003[4]

1. All concentrations are time-weighted averages for the period sampled.
2. ug/m^3= micrograms per cubic meter of air.
3. mg = milligrams of material
4. This sample was attached to the worker's lapel next to the cassette filter: 25 sq. centimeter (4 sq. inch cotton pad)

Laboratory analytical limit of detection (LOD) in micrograms/sample	(0.02)	
Laboratory analytical limit of quantitation (LOQ) in micrograms/sample	(0.06)	
LOD in milligrams/sample		($6x10^{-5}$)
LOQ in milligrams/sample		($2x10^{-4}$)

Table III
RESULTS OF WIPE SAMPLES FOR ZERANOL

Manufacturing Chemists, Incorporated
Indianapolis, Indiana
HETA 82-257

Sample Location	Date	Zeranol Wipe Samples[1] mg/sample
Breakroom/Lunchroom On top of refrigerator	5/15/84	0.3
Breakroom/Lunchroom On top of lunch table	5/15/84	0.4
Breakroom/Lunchroom On top of coffee pot	5/16/84	ND
Breakroom/Lunchroom Inside ceiling supply air duct	5/17/84	ND
Laboratory - On top of work table adjacent to refrigerator	5/15/84	0.2
Laboratory - Computer Room On top of file cabinet	5/16/84	ND[2]
Laboratory - Micro-Lab On table near window	5/16/84	0.2
Packaging Area On tope of Heat Sealer A	5/15/84	0.4
Packaging Area On top of Toledo Scales	5/15/84	0.4
Packaging Area Work table between 2 cartidge filling operations	5/15/84	0.7

(Continued)

Table III
(Continued)

Sample Location	Date	Zeranol Wipe Samples[1] mg/sample
Packaging Area On top of Heat Sealer B	5/16/84	1.1
Box Storage Area just outside of Packaging Area. Inside ceiling supply air duct	5/17/84	ND
Inside air handler upstream of filter (see sample location below)	5/17/84	0.6
In ventilation duct just downstream of air handler filter HVAC system for new building	5/17/84	ND
Production Supervisor's Office On top of coffee pot	5/16/84	1.7
Production Area Top of cabinet across from Pelletizer	5/15/84	14
Production Area on the back of the exhaust hood where dry screening is performed	5/15/84	1.8
Production Area Behind left screening table (wet) on wall	5/16/84	0.6
Production Area Behind right (dry) screening table on wall	5/16/84	2.0

(Continued)

Table III
(Continued)

Sample Location	Date	Zeranol Wipe Samples[1] mg/sample
Production Area On top of cabinet in South-East Corner	5/16/84	0.6
Production Area (North West Corner) Opening of exhaust duct in ceiling vent	5/17/84	9.5
Production Area (North West Corner) Inside supply duct in ceiling vent	5/17/84	5.1

Laboratory analytical limit of detection (mg/sample) 6×10^{-5}
Laboratory analytical limit of quantitation (mg/sample) 2×10^{-4}

1. The area wiped was 100 square centimeters (16 square inches).
2. ND: Non-Detectable concentration

TABLE IV. THE STUDY POPULATION AND THE COMPARISON GROUP

		MCI	IOSHA
1.	No. of workers	11	14
2.	Age (years) Range:	21 - 49 years	21 - 39 years
	Mean:	33 years	27 years
	Median:	31 years	25 years
3.	Sex	5 males; 6 females	8 males; 6 females
4.	Race	9 whites; 2 non-whites	13 whites; 1 non-white
5.	Current smokers (%)	2 current smokers (18%)	3 current smokers (21%)
6.	Alcohol consumption of more than 5 drinks/wk (%)	1 (9%)	4 (29%)
7.	Duration of current employment	Range : 2 mths. - 15 years	2 mths. - 5 years
		Median : 5 years	5.5 mths.

TABLE V. BREAST SYMPTOMS

SYMPTOM	MCI (11 persons)		IOSHA (14 persons)	
	MALES	FEMALES	MALES	FEMALES
1. Increase in size of one or both breasts, not related to the menstrual cycle or pregnancy	1/5	0/6	1/8	0/6
2. Sensation of 'heaviness' of the breasts	0/5	0/6	0/8	0/6
3. Sharp pain in the breasts	1/5	1/6	0/8	0/6
4. Tingling sensation in the breasts	1/5	0/6	0/8	0/6
5. Burning sensation in the breasts	1/5	0/6	0/8	0/6
6. Aching of the breasts	2/5	1/6	0/8	0/6
7. Irritation of the breasts	3/5	0/6	0/8	0/6
8. Increased sensitivity of the nipples or breasts.	3/5	2/6	0/8	2/6
9. Nipple discharge	0/5	0/6	0/8	0/6
10. One or more of the above symptoms		6/11 (55%)		3/14 (21%)

TABLE VI. REPRODUCTIVE SYSTEM SYMPTOMS

SYMPTOM	MCI (6 females)	IOSHA (6 females)
1. Irregular periods since starting present job	1 (17%)	0
2. 'Spotting' between periods since starting present job	2 (34%)	2 (34%)
3. Infertility	1 (17%)	0
4. Loss of interest in sex	1 (17%)	0
5. Miscarriage since starting present job	1 (9%)	0
6. Stillbirth	0	0
7. Baby born with a birth defect	0	0
8. One or more symptoms	4 (67%)	2 (33%)

TABLE VII. LIMITS OF DETECTION & QUANTIFICATION FOR ZERANOL AND ANALOGUES

Compound	Limit of detection (ng/ml)	Limit of quantification (ng/ml)
1. Zeranol	12.2	25
2. Zearalenone	27.1	55
3. Zearalanone	18.8	40
4. Taleranol	11.5	25

TABLE VIII: LIPIDS AND LIPOPROTEIN CHOLESTEROLS (mg/dl) IN ZERANOL EXPOSED AND NON-EXPOSED SUBJECTS

MEAN LEVELS ± S.E.

	n*	TC	TG	HDLC	LDLC	LDLC/HDLC
EXPOSED	8*	214±13	133±22	57±8	130±13	2.69±0.52
NON-EXPOSED	12*	178±11	175±21	43±3	100±9	2.42±0.23

* excluding one pregnant female from each group

TABLE IX: COVARIANCE ADJUSTED (for age, sex) MEANS FOR LIPIDS AND LIPOPROTEIN CHOLESTEROLS (mg/dl) IN ZERANOL EXPOSED AND NON-EXPOSED SUBJECTS

GROUP	n*	AGE	TC	TG	HDLC	LDLC
Exposed	8	35±3	199	130	58	115
Non-exposed	12	27±2	188	177	42	110
p-value**			0.5787	0.2080	0.0539	0.7704

* excluding one pregnant female from each group
**significance of effect of exposure, covariance adjusted for age and sex