This is to certify that the

thesis entitled

**THE INFLUENCE OF AN ANTIESTROGENIC COMPOUND ON POSSIBLE ALTERED SEXUAL DEVELOPMENT AND REPRODUCTIVE PERFORMANCE OF SWINE INDUCED BY IN UTERO AND LACTATIONAL EXPOSURE TO ZEARALENONE**

presented by

**HAN-HUA YANG**

has been accepted towards fulfillment
of the requirements for

**M.S.** degree in **ANIMAL SCIENCE**

Major professor

Date **July 22, 1993**

# THE INFLUENCE OF AN ANTIESTROGENIC COMPOUND ON POSSIBLE ALTERED SEXUAL DEVELOPMENT AND REPRODUCTIVE PERFORMANCE OF SWINE INDUCED BY *IN UTERO* AND LACTATIONAL EXPOSURE TO ZEARALENONE

By

Han-Hua Yang

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Animal Science

1993

## ABSTRACT

### THE INFLUENCE OF AN ANTIESTROGENIC COMPOUND ON POSSIBLE ALTERED SEXUAL DEVELOPMENT AND REPRODUCTIVE PERFORMANCE OF SWINE INDUCED BY *IN UTERO* AND LACTATIONAL EXPOSURE TO ZEARALENONE

By

Han-Hua Yang

Tamoxifen was administered to gilts to determine whether it could counteract the detrimental effects of zearalenone (ZEN). Four groups of twelve gilts were individually fed diets containing 2 ppm ZEN, 10 ppm tamoxifen, or 2 ppm ZEN plus 10 ppm tamoxifen or a control diet starting on day 30 of gestation through lactation. Reproductive performance of sows was not affected by ingestion of ZEN and/or tamoxifen. However, there was a tendency for smaller ovaries and larger uteri in both the sows and the female offspring when compared to controls with the effect being more pronounced in the combination group. Puberty was delayed in the male offspring of gilts exposed to ZEN during gestation and lactation but there was no significant effect on subsequent reproductive performance.

The relative estrogenic effect of ZEN and tamoxifen were investigated by determining their binding affinities to porcine uterine estrogen receptor. The data indicated that the relative binding affinity of ZEN was greater than tamoxifen.

## ACKNOWLEDGMENTS

Many people contributed to this work and I would like to take this opportunity to acknowledge their efforts. I wish to express my sincere appreciation to my major advisor, Dr. Steve Bursian, for his patience, guidance and support throughout this research and my graduate program. I want to thank Dr. William Helferich for the knowledge and training that I received from him while working in his lab. I also want to thank the rest of my committee: Drs. Robert Cook, Elwyn Miller, and James Pestka for each of their contributions throughout my program. A special thanks to the Swine Teaching and Research Center and Paultry Farm for their assistance. Finally, I would like to thank my family for their encouragement and support.

TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

# INTRODUCTION

In recent years, the occurrence of estrogenic substances in food products has received increasing attention because of speculations concerning their possible etiological role in outbreaks of certain toxicoses. The presence of mycotoxins such as zearalenone (ZEN) in edible plants (Stob, 1983; Price and Fenwick, 1985) could constitute potential sources of estrogenic exposure for both man and animals. The contamination of feed by ZEN resulting in hyperestrogenism has long been a problem in animal husbandry (Mirocha and Christensen, 1974).

The pig is one of the most sensitive species to ZEN toxicity. Typical signs of hyperestrogenism are prolonged estrus, anestrus, changes in libido, infertility, increased incidence of pseudopregnancy, increased udder or mammary gland development, and abnormal lactation (Mirocha and Christensen, 1974; Etienne and Jemmali, 1982). Stillbirths, abortions, mastitis, vulvovaginitis, and rectal or vaginal prolapses are secondary complications associated with ZEN ingestion (Sundlof and Strickland, 1986). The reproductive disfunction in pigs has been estimated to cost £25 million annually in the United Kingdom (Anon, 1975). Therefore, problems of infertility

caused by ZEN in livestock are a serious economic problem in many parts of the world.

Some previous studies had addressed the possible effects of *in utero* and lactational exposure to this estrogenic compound on subsequent reproductive development. It has been shown that newborn female mice injected with ZEN developed ovary-dependent reproductive tract alterations (Williams *et al.*, 1989) while rats displayed persistent anovulatory estrus in adulthood (Kumagai and Shimizu, 1982). Gray *et al.* (1985) demonstrated that neonatal female hamsters injected with ZEN were masculinized but not defeminized, indicating an alteration of brain differentiation by early exposure to an estrogenic compound. When male mink were exposed to low levels of ZEN *in utero* and during lactation, they were less likely to mate than control males. Also, there is evidence of morphological alterations in a sexually dimorphic nucleus of the preoptic anterior hypothalamus in these male mink (Aulerich *et al.*, 1991). Such effects, however, have not been examined in the species most sensitive to ZEN, the pig. It is possible that low level *in utero* and lactational exposure to ZEN influences subsequent sexual development in the pig and could have a significant economic impact on the swine producer.

Since ZEN exerts its effects by the binding of it and its metabolites to estrogen receptors, it is possible that the adverse effects of this mycotoxin on sexual development could

be prevented by the addition of an antiestrogen to the diet. Tamoxifen, a triphenylethylene derivative, has been shown to be a complete estrogen antagonist in male humans and chickens (Patterson, 1981) and as such improved sperm count in humans (Willis *et al.*, 1977) and stimulated testes growth and precocious spermatogenesis in cockerels (Rozenboim *et al.*, 1986).

Therefore, the objectives of this study were (1) to determine if *in utero* and lactational exposure to the estrogenic mycotoxin ZEN influence sexual development of the pig, (2) to determine if inclusion of an antiestrogenic compound (tamoxifen) in the diet would prevent or ameliorate the effects of dietary ZEN in swine, and (3) to determine the relative binding affinities of ZEN and tamoxifen to porcine uterine estrogen receptors.

# LITERATURE REVIEW

## I. REPRODUCTION AND SEXUAL BEHAVIOR IN SWINE

### A. The Female

#### Anatomy of The Reproductive Tract

The reproductive tract of the female pig includes the ovaries, the oviducts, the uterus consisting of the body and two uterine horns, the cervix, the vagina, and the vulva. The ovaries are lobular, owing to follicles in varying stages of development. They vary in weight from 3 to 7 g and in diameter from 2 to 4 cm (Boda, 1959). There may be 10-25 individual mature follicles, each 8-12 mm in diameter. The uterine horns are long and tortuous to accommodate numerous developing fetuses.

#### Puberty and Estrus Cycle

Puberty (onset of the estrous cycle) may occur at 6 or 7 months of age. Breed is an important factor in the onset of first estrus. The estrus cycle of the gilt is normally 21 days in duration with 18 to 24 days considered within the normal range. The day of heat is usually counted as day 0. Estrus cycles occur throughout the year with no obvious seasonality.

The cycle can be divided into proestrus, estrus, and diestrus. During proestrus (1-3 days), females are alert to the approach of the boar, will mount other females, and accept mounting by diestrus females. However, the female will not tolerate mounting by a boar during this period. During

estrus, the swollen vulva and vaginal discharge first observed during late proestrus are accompanied by restlessness, mounting of other animals, frequent mounting by other females, and frequent urination. This swollen and redden vulva is apparently due to the secretion of estrogen by developing follicles. When pressure is applied to the back or rump, the animal takes on a rigid stance and has a characteristic ear carriage. The external signs of estrus may last for a period of 3 to 4 days or longer, but the female will mate only for a period of 2 to 3 days (Webel, 1978).

## Ovulation

Ovulation usually occurs on the second day after the onset of estrus. The mean number of eggs ovulated increases from 8 to 10 at the first postpubertal heat to 12 to 14 at the third heat. Sows will ovulate 15 to 20 eggs. Although the mechanism of ovulation is not completely understood, it is closely governed by delicate interactions among hormones of the pituitary gland, ovary, and uterus. Plasma progesterone concentration rises gradually for the first 10 days after ovulation and the peak lasts for 4 to 5 days. By day 16, progesterone concentration declines precipitously in the course of a 48-hour period and remains low for 4 or 5 days until the next ovulation (Stabenfeldt *et al.*, 1969). Estrogen rises during the follicular phase, triggering a peak of luteinizing hormone (LH) at the onset of estrus (Hansel *et al.*, 1973). Ovulation occurs about 40 hours after the LH

surge. Corpora lutea regress and follicles begin development near day 15 post-ovulation in a nonpregnant gilt. During the luteal phase, estrogens are very luteotropic. Administration of estrogen will prolong the life of the corpora lutea for several weeks (Long *et al.*, 1988).

After ovulation, the epithelial cells of the oviduct and uterus rapidly hypertrophy and become high columnar-type cells by the end of the first week following ovulation (Steinbach and Smidt, 1970). About 10 days after ovulation, the columnar epithelium persists and the invasion of eosinophilic leukocytes is at its peak. If conception does not occur, the surface epithelium again reverts to the low columnar cell-type after day 10 and the eosinophilic leukocytes disappear.

In approximately 21 days, the cycle repeats itself. The vaginal epithelium increases in height at estrus (Steinbach and Smidt, 1970) and then progressively decreases to a minimum at day 12 to 16 of the cycle. The superficial layers begin to slough at about the fourth day and this process continues to day 16. Leukocytes invade the epithelium during late diestrus and reach a maximum just after estrus.

## Gestation and Parturition

The gestation period of swine averages about 114 days with a range of 109 to 120 days. Embryos at the four-cell stage are moved through the oviduct into the uterus at about 48 hours after ovulation. At about day 9, embryos reach the uterine body, where they continue to move into the horn

opposite from the one of origin and finally implant (Dziuk *et al.*, 1964). It is common to have a 30-40% loss of developing young between the first and 114th day of gestation. More than half of these losses occur during the first 25 days (before or shortly after implantation). Mummified fetuses also account for a surprisingly large total. Most mummified fetuses are at least 50 mm long and often nearly full term. Mummified fetuses are more prevalent in large than in small litters.

About 5 to 10% of fully formed fetuses are stillborn (Sprecher *et al.*, 1974). This may be because their lungs have not inflated during the birth process. Stillborn piglets are not the small runts, but often the larger fetuses. A greater percentage of fetuses are stillborn in litters larger than 14 and smaller than 5. Most stillbirths in large litters occur when the interval between births is extended, and when the fetuses are located in the tip of the uterus (Dziuk, 1991). Thus, the litter size is limited by the number of ova produced, by the percentage of fertilization, by the uterine space for embryos, and by the number of prenatal deaths and stillbirths.

## B. The Male

### Anatomy of The Reproductive Tract

The reproductive tract of the boar is comprised of the testes, epididymides, and accessory organs. The testes produce the spermatozoa and the male sex hormone testosterone. The spermatozoa pass from the testis into the epididymis where

they acquire the capacity for fertilization and motility and are then stored there. The main accessory sex glands in the male are the seminal vesicles, the prostate, and Cowper's glands (also known as the bulbourethral glands), all of which produce secretions which contribute to ejaculated semen.

## Puberty

Sexual maturity in the boar is a gradual process in which sperm production and sexual desire begin concurrently in increasing intensity at about 4 months of age. Under the continual influence of androgens, the various components of male sexual behavior develop with the production of seminal fluids and sperm. In general, boars reach puberty at about 125 days of age. Spermatozoa are found in the testes, however, there may be a further delay before they are capable of fertilizing ova. Mounting activity occurs as early as 4 months of age, but sequential patterns of sexual behavior culminate after 5 months. The first ejaculation occurs at 5 to 8 months of age, but the volume and percentage of normal sperm are less than in ejaculates from mature boars. The boar does not reach mature size until more than 1 year of age. From 40 to 250 days of age, the paired testes weight increases markedly. The number of spermatozoa and the semen volume continue to increase during the first 18 months of life (Anderson, 1987). Therefore, the size of the testis tends to increase proportionately with total body size and is directly related to total sperm output (Pond and Maner, 1984).

Sexual behavior of males has been described by Houpt and Wolski (1982). It includes phases of courtship, mounting, intromission and ejaculation. When in contact with an estrous gilt, the boar will pursue the female and attempt to nose her sides, flanks, and vulva. Tactile stimulation of the female continues and increases in intensity as the boar's sexual excitement increases. The boar usually emits urine rhythmically and pheromones in the urine may further increase the female's willingness to stand. Several mounting attempts may be made until the female becomes immobile, after which mounting and intromission follow rapidly. The duration of copulation is 3 to 20 minutes or longer (Signoret *et al.*, 1975).

## Composition of Semen

The total composition of semen is a reflection of the secretions from the accessory sex glands as well as from the testes. The composition of a single ejaculate is conveniently divided into three components: (1) the pre-sperm fraction which is often high in bacterial contamination and contains primarily secretions of the accessory sex glands; (2) the sperm-rich fraction which contains the largest concentration of sperm and contributes the largest volume; (3) the post-sperm fraction which consists mainly of gelatinous material. The total volume of semen obtained in a single ejaculate is affected by the size of the boar and the frequency of semen collection. There is a sizable increase in total semen volume

between initial semen collection at puberty and at 1 year of age.

Total volume of the ejaculate and the total number of sperm produced may be influenced by many factors, e.g., age, season, social environment, nutrition, breed, testis size, and frequency of collection (Cameron, 1985; Colenbrander and Kemp, 1990). In general, however, the normal ejaculate of the mature boar will average 200 to 250 ml and will average 40 to 50 X $10^9$ spermatozoa which amounts to 200 to 250 X $10^6$ cells per ml of whole strained semen (Herrick and Self, 1962).

## II. THE GENERAL CONCEPTS OF ESTROGEN ACTION

### Estrogen Biosynthesis

Estrogens are regarded as the primary female hormones. It is believed that the thecal cells in the ovary are the major sites of estrogen production which predominates during the follicular phase of the estrus cycle. During pregnancy, the feto-placenta is an additional source of estrogens (Diczfalusy and Troen, 1961; Villee, 1969). The adrenal cortex in both males and females (Baird *et al.*, 1969; Saez *et al.*, 1972) and the male gonads (Longcope *et al.*, 1972) also produce considerable quantities of estrogens from steroid precursors. Furthermore, the ability of the brain to aromatize androgens is a major source of estrogens in the male and postmenopausal female (Reddy *et al.*, 1974). The pathways shown in Figure 1 have been established as the principal routes of estrogen biosynthesis (Ryan and Smith, 1965).

### Metabolism

Estrogens are secreted and distributed very rapidly in the circulation from their sites of production throughout the body. An important step in estrogen metabolism is hydroxylation to more polar molecules. Estradiol is readily oxidized to estrone within the liver and estrone may be further hydrated to estriol (Breuer, 1962). Estriol makes up the largest fraction of estrogens which appear in the urine. Hydroxylation of the estrogens at the C-2 position gives rise to 2-hydroxyestrogens. These hydroxylation products, also

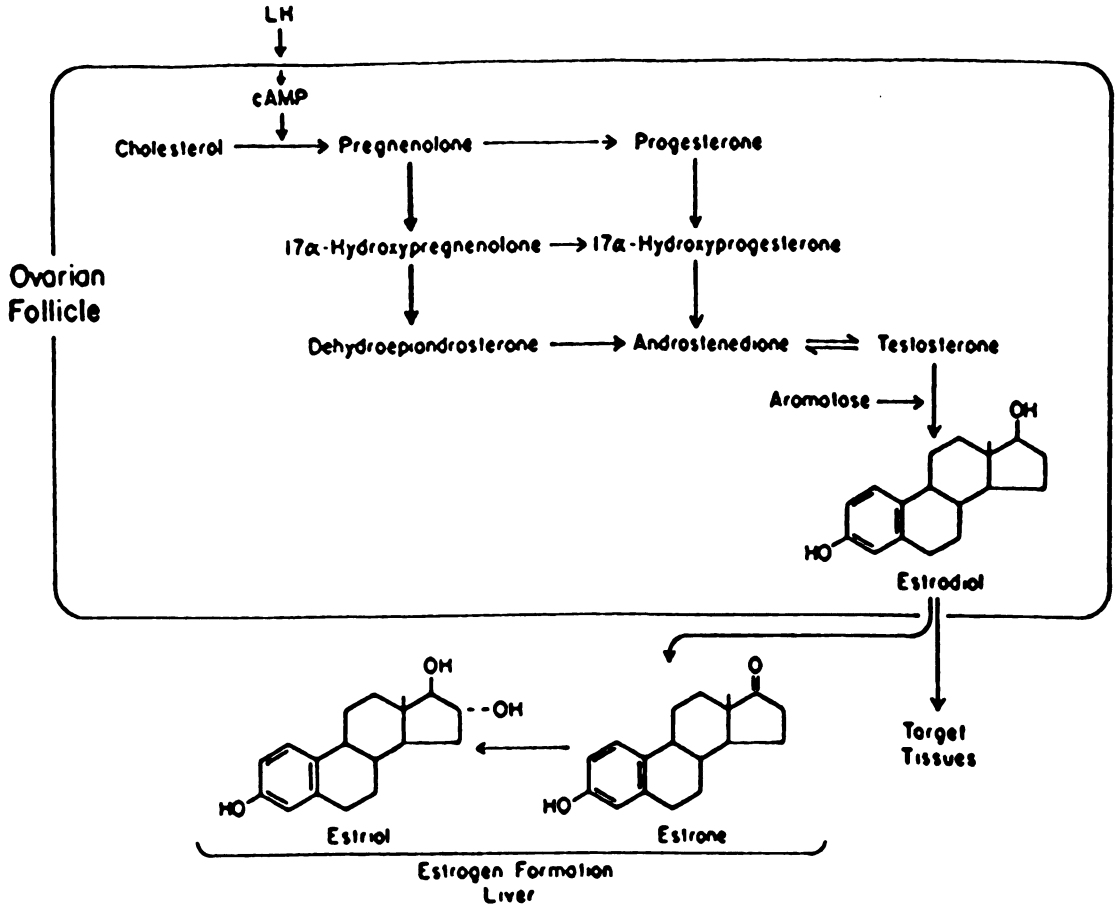



Figure 1. Biosynthetic pathways of estradiol (Ryan and Smith, 1965 as cited by Hadley, 1988).

known as catechol estrogens, form a significant component of the urinary estrogens (Ball *et al.*, 1975). The brain, particularly the hypothalamus, is also able to metabolize estrone and estradiol to catechol estrogens (Fishman and Norton, 1975). The decisive metabolic step for estrogen elimination is esterification with glucuronic or sulfuric acid to water-soluble conjugates. The liver plays the most significant role not only in the hydroxylation but also in the conjugation of estrogens. The estrogen conjugates are excreted for the most part as glucuronides in the urine (Breuer *et al.*, 1969).

## Mechanism of Action

Only a small percentage of the circulating estrogen is present in the free form with the majority being bound to a specific plasma protein, a ß-globulin (Rosenbaum *et al.*, 1966). This protein binds both androgens and estrogens with different affinities and is designated sex hormone binding globulin (SHBG) (Mercier-Bodard *et al.*, 1970). The binding is reversible, but it is strong enough to prevent the bound hormone molecule from passing into the cells of the target organ and there exerting an effect. Therefore, only the free, non-protein-bound estrogen fraction is biologically active. On the other hand, the protein binding protects the hormone molecule from rapid metabolism (Vermeulen *et al.*, 1969).

The unoccupied estrogen receptor (no estrogen ligand) is thought to be a nuclear protein bound to nuclear components by

low affinity interactions. Estrogens are lipophilic and therefore can diffuse through cell membranes, cytoplasm, and nuclear envelope to interact with the nuclear receptor. As a result of this interaction, rapid changes occur in the conformation of the receptor protein. These conformational changes result in new physical properties, including a higher affinity for nuclear components which prevents low salt extraction of the transformed estrogen-receptor complex. The nature of the interaction between the estrogen-receptor complex and the nucleus is still unknown but nuclear components involved could include chromatin proteins, the nuclear matrix, DNA, or various combinations of these. Two potential pathways have been proposed (Figure 2): (1) the receptor complex is eventually destroyed within the nuclear compartment and the sensitivity of the tissue is maintained by re-synthesis of receptor in the cytoplasm (Sarff and Gorski, 1971) and (2) the ligand (estrogen) dissociates from the complex in the nucleus and the free receptor re-partitions to the cytoplasmic compartment to bind more ligand (Kassiss and Gorski, 1981). Currently, the estrogen receptor is believed to be located in the nucleus of cell as demonstrated by King and Greene (1984) and McClellan *et al.* (1984) by immunocytochemistry in a variety of target tissues and cells. Welshons and coworkers (1984) investigated the subcellular distribution of unfilled estrogen receptor using cytochalasin B-induced enucleation also indicating that the unoccupied

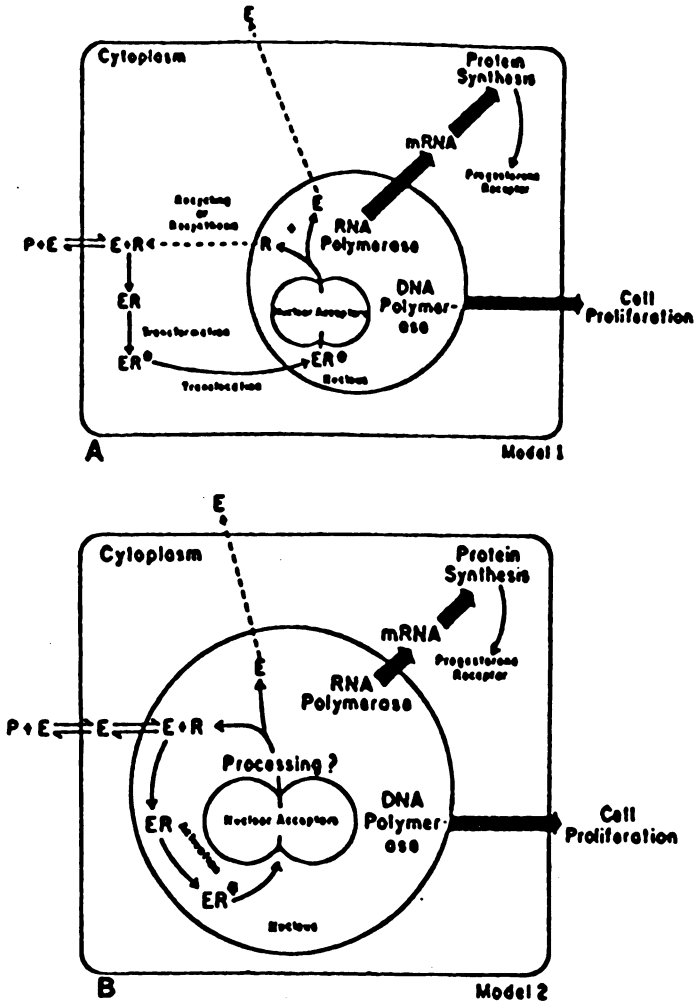



Figure 2. A and B, models for the subcellular mechanism of estrogen (E) action. E dissociates from plasma proteins (P) and diffuses into target tissue cells. E binds to estrogen receptors (R) in the cytoplasm (model 1) or the nucleus (model 2). The estrogen receptor complex (ER) is activated (*) (transformation) before initiating the events associated with estrogen action: activation of RNA polymerase and subsequently protein synthesis and DNA polymerase and cell division (Jordan, 1984). Model 2 is currently more accepted as a description of estrogen mechanism of action (King and Greene, 1984; McClellan *et al.*, 1984; Welshons *et al.*, 1984).

estrogen receptor is predominantly nuclear in the intact cell. They further proposed that in the intact cell, there is no nuclear translocation of receptor as part of the steroid response, but rather an increase in receptor affinity for nuclear element. These issues still require further investigation. However, it is clear that estrogen binding to the receptor causes increased rates of transcription of a variety of genes, depending upon the respective target cell (Gorski *et al.*, 1986). The initiation of these nuclear events by estrogen results in an increase in whole uterine lipid, RNA (via activation of RNA polymerase) (Gorski, 1964) and proteins (Aizawa and Mueller, 1961), and finally triggers DNA synthesis and cell division (Kaye *et al.*, 1972).

## Physiological Roles of Estrogen

Estrogens are found in both females and males and can be demonstrated in an active form in both sexes. However, they exert their main effects on the primary and secondary female sex organs. Estrogens produced during pubertal maturation in the female are responsible for growth and development of the vagina, uterus, and oviducts, organs essential to ovum transport and zygote maturation and implantation of the conceptus. Mammary growth and development is also initiated by the actions of estrogens in concert with other hormones. Estrogens acting on specific nuclear sites within the brain affect the libido which is usually first evidence in the adolescent female. Other physiological actions of estrogens

are summarized in Table 1 (Hadley, 1988).

## Nonsteroidal Estrogens and Antiestrogens

A number of nonsteroidal compounds possess estrogenic activity such as diethylstilbestrol (DES), plant estrogens, and some estrogenic pesticides. The major plant estrogens include: (1) coumestans (coumestrol), (2) isoflavones (genistein and daidzein), and (3) resorcylic acid lactones (zearalenone) which are more correctly classified as fungal estrogens (Price and Fenwick, 1985). Nelson *et al.* (1978) showed that chlorinated pesticides, such as the o,p isomer of DDT, methoxychlor and kepone have estrogenic activity as determined by the rat uterine weight assays. Thus, these compounds can now be classified experimentally as estrogen agonists or partial agonists.

Some compounds possess antiestrogenic activity. Triphenylethylene derivatives such as tamoxifen and estrogens with a rapid dissociation rate from the estrogen receptors like dimethylstibestrol (DMS) function as such (Jordan, 1984). Antiestrogens, through competition for estrogen receptors, may prevent endogenous estrogens from initiating full expression of their effects in their target tissues. By this action they antagonize a variety of estrogen-dependent processes, such as uterine growth and negative feedback to the hypothalamus.

Nonsteroidal estrogens may exert their biological effect through an interaction with receptor proteins in target tissues, with the subsequent induction of protein synthesis

TABLE 1. THE PHYSIOLOGICAL EFFECTS OF ESTROGENS IN MAMMALS

| Primary site of action | Physiological action |
|---|---|
| CNS | Maintains libido and sexual behavior |
| Pituitary | Has negative and positive feedback effects on gonadotropin secretion<br>Increases pituitary TRH receptors<br>Increases pituitary GnRH receptors<br>Increases oxytocin production |
| Ovary | Required for ovum maturation |
| Vagina | Causes proliferation and cornification of the mucosa |
| Oviducts | Causes growth and development in preparation for gamete transport |
| Uterus | |
| Cervix | Increases mucus secretion |
| Endometrium | Increases blood flow<br>Increases number of oxytocin receptors at term<br>Causes decidualization response |
| Myometrium | Synthesizes contractile proteins of smooth muscle cells<br>Increases membrane excitability<br>Increases sensitivity to oxytocin |
| Mammary glands | Causes ductule and stromal growth and development and fat accretion |
| Skin | Induces sebaceous gland secretion (thinner fluid)<br>Stimulates axillary and pubic hair growth |
| General body effects | Causes $H_2O$ and $Na^+$ retention, weight gain, and female type of fat distribution<br>Maintains bone mineral deposition |
| Liver | Causes hepatic angiotensinogen production<br>Causes hepatic production of thyroid binding globulin |
| Blood | Decreases plasma cholesterol formation |

Adapted from Hadley (1988).

(Kawabata *et al.*, 1982). The interaction of ZEN and tamoxifen with estradiol binding sites on the calf uterus has been described (Kiang *et al.*, 1978). Kiang and coworkers (1978) demonstrated that ZEN and tamoxifen can compete with estradiol for binding with cytosol estrogen receptors, in which the relative binding affinity of 17β-estradiol, ZEN, and tamoxifen to receptors was approximately 10:1:0.3, respectively. As the result of differences between binding affinity and biological activity, and a longer retention in the nucleus (Koseki *et al.*, 1977), tamoxifen is used as antiestrogen in the treatment of advanced breast cancer (Kiang and Kennedy, 1977).

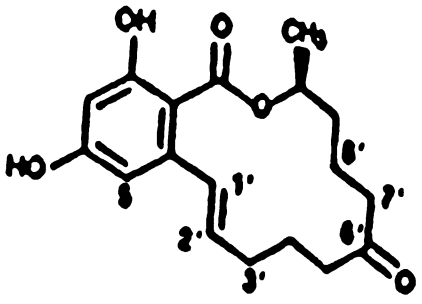
## III. THE MYCOTOXIN ZEARALENONE AND ITS EFFECTS ON SWINE

### Chemical Properties

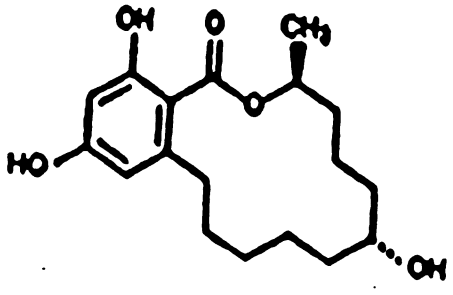
Zearalenone (ZEN), a non-steroidal estrogenic compound, is chemically described as 6-(10-hydroxy-6-oxo-*trans*-1-undecenyl)-ß-resorcylic acid lactone with the molecular formula $C_{18}H_{22}O_5$. ZEN is a white, crystalline compound with a melting point of 164-165°C (Cole and Cox, 1981). ZEN is insoluble in water (0.002 g/100 ml), carbon disulfide, and carbon tetrachloride, but it is slightly soluble in n-hexane and progressively more soluble in benzene, acetonitrile, methylene chloride, methanol, ethanol, and acetone (Hidy *et al.*, 1977). The chemistry of ZEN and its derivatives (Figure 3) has been reviewed by Shipchandler (1975) and by Pathre and Mirocha (1976).

### Occurrence and Potentiation Factors

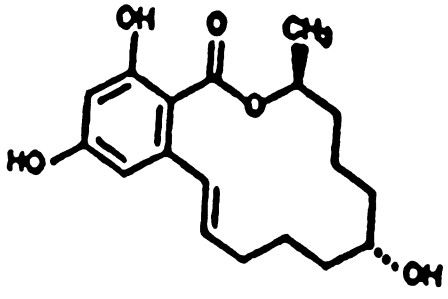
ZEN is not an intrinsic component of food plants but it is a secondary mold metabolite of fungal species, principally *Fusarium roseum (graminearum)* which is a common field organism (Caldwell *et al.*, 1970; Eugenio *et al.*, 1970; Abbas *et al.*, 1984). *Fusarium roseum* is the most important corn ear-rot fungus, and it also causes kernel rot in storage. ZEN production does not seem to occur in significant amounts prior to harvest, but under proper environmental conditions, it is readily produced on corn and small grains in storage. During storage, alternating low and moderate temperatures are necessary for production of this toxin. Temperatures between



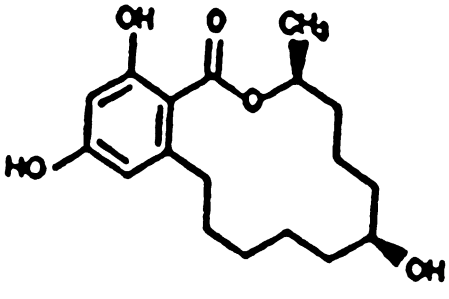
(I)



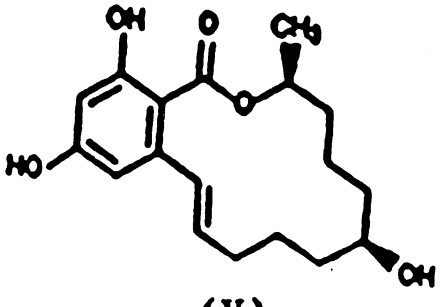
(II)



(III)



(IV)



(V)

Figure 3. Chemical structures of zearalenone (I), α-zearalanol (II), α-zearalenol (III), ß-zearalanol (IV), and ß-zearalenol (V).

53-57°F and moisture content of 23% or above induce the enzymes involved in biosynthesis of this toxin and optimum production occurs at 81°F (L'vova *et al.*, 1981).

ZEN production is correlated with plant density, soil fertility, insect damage, cool and damp weather conditions, mechanical damage during harvest, and improper storage conditions (Mirocha *et al.*, 1977; Sutton *et al.*, 1980; Montani *et al.*, 1988). The *Fusarium* fungi may die or be destroyed and be absent in the toxic feed sample. However, since ZEN is fairly stable to heat and other detrimental factors, the mycotoxin may remain in the feed unaltered for long periods of time (Mirocha *et al.*, 1971). Therefore, the presence or absence of *Fusarium* mold in a sample of feed at a given time has little or no relationship to the presence of ZEN in that sample (Nelson *et al.*, 1970). Presence of ZEN in a variety of grains and feeds has been reviewed by Bennet and Shotwell (1979) and Kuiper-Goodman *et al.* (1987).

## Metabolism

ZEN is ingested by swine and appears to be fairly rapidly absorbed through the gut into the circulatory system following oral administration (Dailey *et al.*, 1980; Olsen *et al.*, 1985a). ZEN can then be transported to both insensitive tissues and sensitive target tissues where it exerts estrogenic effects, or it can be transported to the liver where it is reduced and/or converted to a more soluble glucuronide form. The main metabolites of ZEN are the

epimeric α- and ß-zearalenols (Shipchandler, 1975). ZEN and its metabolites are excreted in the urine mainly as glucuronide conjugates (Mirocha *et al.*, 1981; Olsen *et al.*, 1985a). According to Hidy *et al.* (1977), the major route of excretion for most species is via the feces and urine directly, and the feces indirectly via the bile. On the basis of work with 3 sows, Vanyi *et al.* (1983) found that the major route of excretion of ZEN and metabolites (as glucuronide conjugates) is the feces.

When rats ingested diets containing 250 ppm ZEN, about 50% of the compound was absorbed from the intestine into the systemic circulation (Smith, 1980). Once absorbed and in the systemic circulation, ZEN becomes associated with hydrophobic regions of serum albumin and sex hormone-binding globulins (Martin *et al.*, 1978) as ZEN is essentially insoluble in aqueous solution. When ZEN is spontaneously dissociated from carrier proteins and diffuses through capillary cells into the liver, it is metabolized along 2 major pathways (Kiessling and Pettersson, 1978; Mirocha *et al.*, 1981; Olsen *et al.*, 1981; Bock *et al.*, 1987):

1. zearalenone + NADH or NADPH —(3 α-hydroxysteroid dehydrogenase)→ α-zearalenol + $NAD^+$ or $NADP^+$

2. zearalenone (α-zearalenol) + glucuronic acid —(UDP-glucuronosyl-transferase isoenzymes)→ zearalenone-glucuronic acid conjugate

Absorption and metabolism of ZEN are rapid processes.

Plasma concentrations of ZEN metabolites were measured in a gilt following oral administration of ZEN at a dose of 192 μg/kg b.w./day for 4 days (Olsen *et al.*, 1985a). Detectable concentrations of ZEN, present as a glucuronide conjugate, were found as early as 30 min after the start of feeding and traces were found until the fifth day after feeding had been discontinued. A reduced metabolite, α-zearalenol, was also conjugated to glucuronic acid and was found in the plasma in amounts exceeding ZEN by 3-4 times. ß-Zearalenol was also formed but in much lesser amounts (Olsen *et al.*, 1985a). Considerable differences between species exist in the ability to reduce ZEN (Olsen and Kiessling, 1983; Pompa *et al.*, 1986). Of note is that of five species examined, swine have one of the greatest abilities to form the more highly estrogenic α-zearalenol (Olsen *et al.*, 1985b).

## Mechanism of Action

The exact mechanism by which ZEN adversely affects reproduction has not been determined. However, zearalenone, zearalenol and zearalanol bind to mammalian estrogen receptor sites because of their chemical similarity to estradiol (Hurd, 1977). It is known that free ZEN diffuses through plasma membranes and binds to estrogen receptors (ER) in reproductive and other sensitive tissues. The ER is associated with the nuclear membrane and contains hormone- and DNA-binding domains (Koike *et al.*, 1987). Subsequent translocation of the ZEN-ER complex into cell nuclei initiates expression of specific

genes which cause an "estrogenic response". ZEN and its metabolites compete with 17ß-estradiol and bind specifically to hepatic (Powell-Jones *et al.*, 1981), uterine (Kiang *et al.*, 1978; Katzenellenbogen *et al.*, 1979; Greenman *et al.*, 1979), mammary (Boyd and Wittliff, 1978) and hypothalamic ER (Kitagawa *et al.*, 1982).

ZEN and its metabolites have different affinities for the ER. When measured by competitive or direct binding assays using rat uterine cytosol receptors, the relative binding affinities are 17ß-estradiol > α-zearalanol > α-zearalenol > ß-zearalanol > zearalenone > ß-zearalenol (Tashiro *et al.*, 1980). ZEN and zearalenol are weak estrogens and like estradiol cause inhibition of the hypothalamus and anterior pituitary resulting in atrophy of the ovaries, testes, prostate and seminal vesicle. It has been suggested (Ueno and Tashiro, 1981) that the estrogenic effect of ZEN is due to its metabolism to zearalenol, and may be partly and secondarily related to inhibition of steroid metabolism (Olsen, 1985). The relative binding affinity of α-zearalenol was greater in the pig than in the rat and significantly greater than in the chicken. Thus, interspecies differences in ZEN sensitivity may be due to the binding affinity of α-zearalenol for estrogen receptors and differences in ZEN metabolites formed (Fitzpatrick *et al.*, 1989).

## Clinical Effects

Estrogenic mycotoxicosis was first reported in the United States by Buxton in 1927 and Legenhausen and McNutt *et al.* in 1928. The appearance of clinical signs is dependent on the amount of toxin consumed, and signs are generally expressed in 4 days to 7 days (McNutt *et al.*, 1928; Eriksen, 1968). Recovery after discontinuing ZEN-contaminated rations usually occurs within 3 to 4 weeks (McNutt *et al.*, 1928). In terms of species and age susceptibility, prepubertal gilts are the most sensitive to ZEN and most frequently affected (Bristol and Djurickovic, 1971; Shreeve *et al.*, 1978; Ruhr *et al.*, 1978; Berger *et al.*, 1981). Normally, feed concentrations between 1 and 5 ppm ZEN are sufficient to produce clinical signs of vulva edema in young gilts. In experimental studies with young pigs, doses as low as 0.02 mg/kg b.w./day caused vulvar swelling, and higher doses caused a variety of signs characteristic of hyperestrogenism (Mirocha and Christensen, 1974). The prominent signs of hyperestrogenism in prepubertal gilts are edematous swelling of the vulva, mammary gland enlargement, hypertrophy of the nipples, uterine hyperplasia with some ovarian atrophy (Stob *et al.*, 1962; Kurtz *et al.*, 1969; Bristol and Djurikovic, 1971; Nelson *et al.*, 1973; Vanyi *et al.*, 1973; Young *et al.*, 1981). In severe cases, these symptoms are accompanied by rectal and/or vaginal prolapse (Bristol and Djurikovic, 1971).

One of the effects of ZEN in mature cycling swine is

prolongation of the estrous cycle (Cantley *et al.*, 1982; Edwards *et al.*, 1984). In adult sows fed a ZEN-containing diet, infertility, pseudopregnancy, nymphomania, and constant estrus were observed (Chang *et al.*, 1979). The no-observed-adverse-effect-level (NOAEL) for the pubertal pigs was 0.06 mg/kg b.w./day (Kuiper-Goodman *et al.*, 1987). Long *et al.* (1982) fed a diet containing unpurified ZEN derived from a *Fusarium roseum* culture to early pregnant sows. The characteristic changes in reproductive parameters were accompanied by changes in serum concentration of progesterone and estradiol. The NOAEL for most of these effects was 0.13 mg/kg b.w./day.

Reproductive problems as a result of consumption of ZEN-contaminated feeds by pregnant sows are abortion, reduction in litter size, smaller offspring, weak piglets, juvenile hyperestrogenism and stillbirths (Miller *et al.*, 1973; Sharma *et al.*, 1974; Shreeve *et al.*, 1978; Chang *et al.*, 1979). The most likely reasons for the detrimental effects of ZEN on pregnancy include alteration of the endometrial environment and direct effects on developing embryos (Long and Turek, 1989). Long *et al.* (1988) indicated that the period from day 7 to day 10 postmating is the "critical" period for ZEN to exert its detrimental effects on early pregnancy in swine due to altered migration of blastocysts.

Very few reports on the effects of ZEN ingestion during lactation are available in the literature. Feeding 25 to 100

ppm ZEN during lactation and after weaning resulted in ovarian atrophy and tortuous uterine horns, particularly at the higher dose levels (Chang *et al.*, 1979), whereas 2.1 to 4.8 ppm, when fed continuously throughout pregnancy and lactation, had no consistent effect on postweaning rebreeding performance (Young *et al.*, 1982). Neither ZEN nor its metabolites were detected in milk obtained 1 or 14 days postpartum (Young *et al.*, 1982). Edwards *et al.* (1987b) reported that feeding 10 ppm purified ZEN diet during lactation delayed the return to estrous after weaning, but it did not affect subsequent fertility when the animals were removed from the ration at weaning.

There are conflicting field reports concerning the effects of ZEN on male swine. It seems apparent that in some situations the male has signs of estrogenic stimulation. In young barrows, there is hypertrophy of the mammae and a few reports indicate atrophic changes in the gross appearance of the boars' testicles along with edematous swelling of the prepuce. These animals have been noted to void small amounts of urine intermittently. Some reports have indicated ZEN may cause a feminizing effect with preputial and mammary enlargement, decreased testicular size, reduced epididymis and vesicular gland weights, and loss of libido in boars (Legenhausen, 1928; Koen and Smith, 1945; Stob *et al.*, 1962; Christensen *et al.*, 1972; Palyusik, 1973; Berger *et al.*, 1981). Young and King (1986b) found that the percent motility of sperm was significantly decreased and a trend was observed

for lower total and gel-free volumes of semen.

The mortality in an outbreak of ZEN mycotoxicosis is usually low. However, if rectovaginal prolapse is a prominent feature, secondary complications such as bacterial infections of the exposed mucosa may lead to death (Buxton, 1927; McNutt *et al.*, 1928).

## Pathology

The primary pathologic changes in the estrogenic syndrome are physiologic alterations of the genital tract. These physiologic changes are principally characterized as an accumulation of interstitial edema and cellular proliferation and metaplasia of the mucosal epithelium into squamous-type epithelium in the vagina and cervix. The vulva, vagina, cervix, and myometrium are all thickened due to edema and a combination of cellular hypertrophy and hyperplasia of the elements composing the wall of these structures (Kurtz *et al.*, 1969).

The uterotropic effects of ZEN consist of grossly enlarged uterine horns. The endometrium is grossly thickened by edematous fluid. Histologically, the endometrium is characterized by an interstitial edema of the submucosa and hyperplasia of the submucosal endometrial glands. No inflammatory cells comprise the cellular components of this area but occasionally a few eosinophils are present.

The ovaries of prepubertal gilts appear grossly hypoplastic with numerous, small follicles and no evidence of

corpora luteal formation. Histologically, there is evidence of follicular atresia (Kurtz *et al.*, 1969; Palyusik, 1973) and oocyte degeneration (Voluntir *et al.*, 1971). Older gilts seem to have considerable secondary follicular development and concurrent follicular atresia. However, the ovaries have no evidence of ovulation because there is a complete absence of corpora lutea in the ovarian tissue of these animals. Perhaps the larger follicles observed in older gilts are caused by the influence of endogenous estrogens produced in these older pigs. Voluntir *et al.* (1971) report some ovarian fibrosis, but this change was not present in the ovaries studied by Kurtz *et al.* (1969).

The mammary glands and nipples are enlarged grossly. Some reports have suggested massive enlargement of these glands but usually mammary enlargement is moderate. Histologic study reveals epithelial hyperplasia and squamous metaplasia along with an increased mitotic index principally of the ductal elements in the mammary tissue. There is edematous infiltration in the interstitium of the parenchyma in the mammary tissue of prepubertal gilts. These histologic alterations produced by ZEN are mimicked when estradiol (17ß-cyclopentylpropionate (ECP)) is injected intramuscularly into similar size gilts (Kurtz *et al.*, 1969).

In testes, the germinal epithelium is damaged and there is a regressive alteration in testicular substance. The interstitium around the seminiferous tubules tends to enlarge

showing a serous infiltration and a marked eosinophilia which is followed by an increasing proliferation of the interstitial elements (Vanyi and Szeky, 1980).

## Method of Control

When reproductive problems or a high concentration of ZEN in feed are observed, the contaminated feed should be withdrawn from the swine diet. However, prevention of ZEN production is probably the best long-term solution to ZEN mycotoxicosis. Some insecticides inhibit production of ZEN in grain or liquid cultures (Wolf and Mirocha, 1973; Berisford and Ayres, 1976) and applications of fonofos, maneb, and carbaryl to corn inoculated in the field with *Fusarium roseum* significantly reduced ZEN concentrations in grain (Draughon and Churchville, 1985). In addition, plant geneticists are developing corn and cereal varieties that are resistant to invasion and proliferation of *Fusarium* species. This appears to be a practical and economic long term method to control ZEN contamination of feedstuffs.

## IV. THE PHARMACOLOGY AND ANTIESTROGEN ACTIONS OF TAMOXIFEN

### Chemical Properties

Tamoxifen, code number ICI 46,474, is the trans(Z) isomer of a triphenylethylene derivative. The chemical structure is (Z)-2-[4-(1,2-diphenylbut-1-enyl)phenoxy]-*N*,*N*-dimethylethanamine, with the molecular formula $C_{26}H_{29}NO \cdot C_6H_8O_7$ and a molecular weight of 563.6. This chemical is sold as the monocitrate salt using the proprietary name "Nolvadex" in most countries. The corresponding cis(E) isomer is weakly estrogenic and less than 1% is present in Nolvadex. Tamoxifen citrate is a fine, white, odorless, crystalline powder, slightly soluble in water and soluble in ethanol, methanol and acetone. The physical, chemical, biochemical and animal pharmacological properties of tamoxifen are described in detail by Furr *et al.* (1979).

### Metabolism

Tamoxifen is well absorbed and extensively distributed. After i.v. injection of female mice with [$^{14}$C]tamoxifen, the concentrations were higher after 5 minutes and 4 hours in lung, liver, adrenal, kidney, pancreas, uterus, salivary glands and mammary tissue than in blood (Wilking, 1981; Wilking *et al.*, 1981; 1982). Major *et al.* (1976), using [$^{3}$H]tamoxifen, showed that radioactivity was concentrated and retained by the reproductive tract and pituitary gland of female rats.

Using [$^{14}$C]tamoxifen, Fromson *et al.* (1973a) were the

first to examine the route of excretion of radioactivity in the female rat, mouse, rhesus monkey and dog. In all species examined, the metabolites (Figure 4) were excreted chiefly as conjugates in the bile and little or no tamoxifen was eliminated as unchanged drug. In this study it was also shown that tamoxifen has a long half-life and the excretion of radioactivity is apparently biphasic. In rats, the initial half-life is 53 hours with a terminal half-life of 10 days. Kemp and coworkers (1983) have suggested that tamoxifen is first converted to N-desmethyltamoxifen, then to didesmethyltamoxifen (primary amine), and finally to metabolite Y (primary alcohol). The catechol metabolite D is present as a glucuronide in the feces and metabolite E is found as a minor metabolite in dog bile. In the rat uterus and chicken oviduct, Borgna and Rochefort (1979) identified the metabolite 4-hydroxytamoxifen. It has been shown that 4-hydroxytamoxifen is an antiestrogen and that its ability to inhibit [$^3$H]estradiol binding to estrogen receptors *in vitro* is greater than that of the parent drug and is equivalent to that of estradiol (Jordan *et al.*, 1977). Like estradiol, and in contrast to tamoxifen, 4-hydroxytamoxifen shows a very slow dissociation rate from the estrogen receptor (ER) (Rochefort *et al.*, 1979). The duration of action of 4-hydroxytamoxifen is much shorter than tamoxifen (Jordan and Allen, 1980), probably because 4-hydroxytamoxifen can be more readily conjugated and excreted.

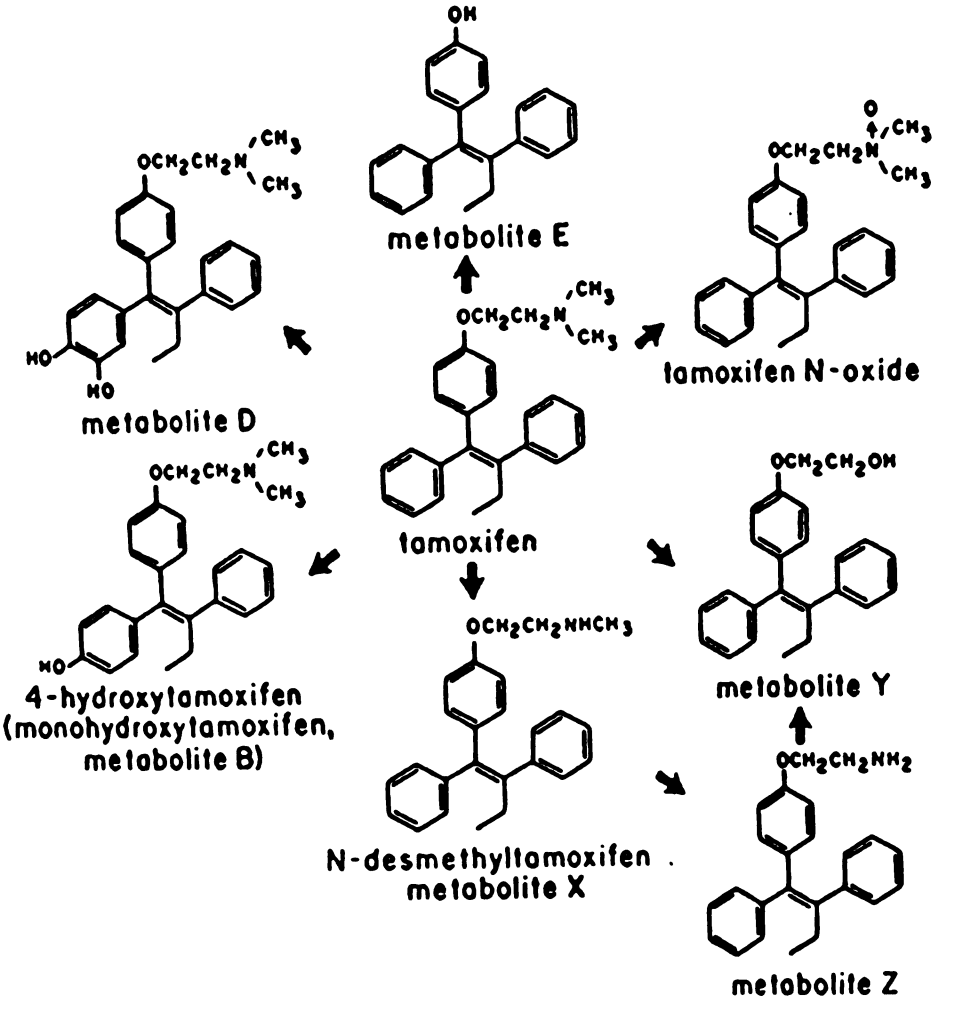



Figure 4. The metabolites of tamoxifen in animals and man (Jordan, 1984).

## Biological Effects

Tamoxifen is generally considered as a nonsteroidal antiestrogen. However, the biological effect of tamoxifen on estrogen target tissues varies greatly according to the species and tissues examined, the parameter measured, and the endocrine status prevailing at the time the response is measured (Patterson, 1981). Sakai *et al.* (1978) reported that tamoxifen has a direct estrogen-like action by increasing sex hormone binding globulin. Tamoxifen also has been shown to posses antiestrogenic properties towards a number of mammary cell lines and carcinogen-induced mammary tumors (Legha and Carter, 1976; Furr and Jordan, 1984). Tamoxifen is used in some countries for the induction of ovulation (Klopper and Hall, 1971; Williamson and Ellis, 1973; Gerhard and Runnerbaum, 1979). However, its major clinical application is in the treatment of breast cancer (Legha and Carter, 1976; Heel *et al.*, 1978).

In the immature mouse uterine weight test, tamoxifen is a full agonist (Terenius, 1971; Campen *et al.*, 1985) with no detectable antiestrogenic activity (Terenius, 1970). Similarly, in ovariectomized mice and guinea pigs, the administration of tamoxifen results in full vaginal cornification (Harper and Walpole, 1966; Furr *et al.*, 1979; Pasqualini *et al.*, 1986). In contrast to its effect in the mouse, tamoxifen behaves as a partial agonist in the rat based on vagina histology and uterine weight (Harper and Walpole,

1967a; Marois and Marois, 1977). The biological effects of tamoxifen in the rabbit, like those in the rat, are of a weak agonist as determined by its ability to prime the uterus to respond to progesterone (Pasqualini *et al.*, 1988). In the chick oviduct, tamoxifen was uniformly antiestrogenic with virtually no detectable agonist actions (Sutherland *et al.*, 1977; Binart *et al.*, 1979; Sutherland, 1981). In the immature pig, Lin and Buttle (1991b) concluded that tamoxifen acts as an estrogen agonist in the uterus as well as stimulating mammary duct growth. However, concurrent administration of tamoxifen with estradiol showed antagonism between the two compounds in the mammary gland. Treatment of pregnant gilts with tamoxifen did not affect the development of mammary structures or the ability to lactate at parturition. However, mammary progesterone receptor content in tamoxifen-treated animals tended to be lower when compared to controls at day 90 of pregnancy (Lin and Buttle, 1991a).

Some histopathological changes were observed in the reproductive tract of rats treated with tamoxifen (Furr *et al.*, 1979). Ovarian weights were reduced, corpora lutea were either absent or less numerous, and follicular cysts were found. Another feature was the disappearance of endometrial glands in the uterus. The uterine epithelium consisted of a single layer of columnar cells with small areas of flattening and occasional squamous metaplasia. One important qualitative difference between the uterine response to estrogen and

tamoxifen was that estrogen caused a massive accumulation of uterine fluid whereas tamoxifen did not. Co-administration of tamoxifen inhibited this estrogen-stimulated fluid accumulation (Marois and Marois, 1977). It was also found that estradiol stimulated endometrial hyperplasia with an increase in total uterine DNA content, whereas tamoxifen stimulated endometrial hypertrophy with only a slight increase in uterine DNA content (Jordan *et al.*, 1978).

In male rats, reduction of testes and accessory sex organ weights and interruption of spermatogenesis were observed (Harper and Walpole, 1967). However, in contrast to these observations, Rohr *et al.* (1979) showed that tamoxifen caused a stimulation of prostate glandular epithelium. These workers concluded that tamoxifen was acting as an antiestrogen at the hypothalamic-pituitary level, thereby causing an increase in LH and testosterone secretion, which in turn led to stimulation of the prostate epithelium. This is supported by Donaldson *et al.* (1981a, b) who indicated that tamoxifen did cause an acute stimulation of LH and testosterone secretion in male rats. A clear antiestrogenic effect of tamoxifen was seen in the dog prostate where the drug prevented the estradiol-induced squamous metaplasia of the epithelium and decreased the induced stromal proliferation in castrated animals (Funke *et al.*, 1980; 1981).

## Potential Mechanisms of Action of Antiestrogens

An antiestrogen is usually identified and classified as a compound that will inhibit the vaginal cornification produced by estradiol in ovariectomized rats or will inhibit the increase in uterine weight produced by estradiol in immature rats. The effect of tamoxifen may be either additive or antagonistic to estradiol, depending on the dose ratio. Macnab *et al.* (1984) suggested that the effects of tamoxifen are additive at low doses of estradiol and antagonistic at higher estradiol doses. It is possible that this dualism of agonism and antagonism seen in other target organs and species is a function of these basic characteristics of a partial agonist. However, this property of tamoxifen does not completely explain antiestrogen action. The mechanism of action of antiestrogens is believed to be related to their structure whereas their potency is related to their relative affinity for the ER (Lieberman *et al.*, 1983).

In general, antiestrogens regulate estrogen-stimulated prolactin synthesis, progesterone receptor production, protein synthesis and [$^3$H]thymidine incorporation, DNA increases, and the cell cycle of estrogen-sensitive cells (Jordan, 1984). In an estrogen target tissue, an antiestrogen can bind to ER, antiestrogen binding sites (Sutherland *et al.*, 1980; Sudo *et al.*, 1983) or calmodulin (Lam, 1984). The interaction equilibria are dependent upon the relative binding affinities (RBA) of the antiestrogen from the proteins. Tamoxifen has a

high binding affinity for the antiestrogen binding sites (RBA = 100, $E_2$ RBA = 0), but a low binding affinity for the estrogen receptor (RBA = 5, $E_2$ RBA = 100). Tamoxifen is a potent inhibitor of calmodulin-mediated phosphodiesterase (Lam, 1984). Calmodulin is believed to be intimately involved in cell division (Chafouleas *et al.*, 1984) and inhibitors of calmodulin will produce a block in the $G_1$ phase of the cell cycle (Ito and Hidaka, 1983).

Most studies at present have focused upon the interaction of antiestrogens with the ER. The following observations have been made: (1) antiestrogens inhibit the binding of [$^3$H]estradiol to the ER (Skidmore *et al.*, 1972); (2) [$^3$H]antiestrogens bind directly to the ER (Jordan and Prestwich, 1977); (3) estrogens and antiestrogens may have a different method of "activating" receptors (Rochefort and Borgna, 1981); (4) differences in the size of nuclear estrogen and antiestrogen receptor complexes have been noted (Eckert and Katzenellenbogen, 1982); (5) there are differences in the interaction of estrogen and antiestrogen receptor complexes with DNA (Evans *et al.*, 1982); (6) ER re-synthesis was believed to be impaired by antiestrogen (Clark *et al.*, 1974).

Structural alterations of the tamoxifen molecule can be divided into two main categories: substitution in the phenyl portion of the molecule (equivalent to the A ring of the steroid nucleus), and alteration or removal of the dimethylaminoethoxy side-chain (Furr and Jordan, 1984). The

importance of the side chain for the antiestrogen activity of tamoxifen has been studied. Removal of the side chain to produce the phenol (metabolite E) destroys antiestrogen activity and increases estrogenic activity (Jordan and Gosden, 1982). The substitution of a 4-phenolic hydroxyl in tamoxifen confers potent antiestrogen activity and very high binding affinity for the ER (Jordan *et al.*, 1977). Overall, Jordan and Lieberman (1983) concluded that compounds can be classified into three categories based upon their structure. Antiestrogens have a side chain extending away from the binding site, partial agonists have a *bis* phenolic structure and agonists are unsubstituted.

Tamoxifen has been widely used to probe estrogen activity and to provide valuable comparative information to establish a molecular mechanism for both estrogens and antiestrogens. However, there is still no satisfactory molecular mechanism to explain either estrogen or antiestrogen action. This is, in part, due to the complex, perplexing and often contradictory pharmacology of this drug.

## MATERIALS AND METHODS

### Animals and Procedures

Twelve crossbred gilts were bred and randomly assigned to one of four dietary treatment groups. The pigs were housed in an environmentally controlled confinement facility at the Michigan State University Swine Research and Teaching Center. The treatments consisted of (1) corn-soybean meal basal diet (Control), (2) basal diet containing 2 ppm ZEN, (3) basal diet containing 10 ppm tamoxifen, and (4) basal diet containing 2 ppm ZEN plus 10 ppm tamoxifen. The treatment diets were individually fed to the gilts *ad libitum* beginning on day 30 of gestation through lactation. At farrowing, the number of offspring produced, their body weights, and sex were determined. Gilts then were killed shortly after the piglets were weaned and their reproductive tracts were weighed and prepared for histological examination.

At 21 days of age, all piglets were weaned and weighed. Three males and three females from each treatment were kept for subsequent breeding. The remaining animals were killed and gross necropsies performed. Reproductive tracts from these animals were removed, weighed, and processed for subsequent histological examination. The six animals per group kept for breeding were provided a control diet for the duration of the trial. Beginning at five months of age, the 12 F1 females were checked for estrus daily with the aid of a mature boar to determine the age of first observed estrus.

Estrus was presumed if a gilt exhibited a positive back-pressure test and allowed a boar to mount. Once a female had gone through one estrus period, it was killed and the reproductive tract was processed for histological examination.

Beginning at five months of age, the 12 F1 males were exposed twice per week to a gilt in estrus and the day of first attempt to mount was recorded. After showing a willingness to copulate, males were electroejaculated for semen collection. Semen evaluation included sperm concentration and sperm motility. At eight months of age, each boar was given an opportunity to mate with two females which were allowed to farrow. Boars then were killed and their reproductive tracts were removed and processed for histological examination. Sperm from the cauda epididymides was also evaluated. At the time of farrowing, the number of piglets born, their sex, and their body weights were determined.

The interaction of ZEN and tamoxifen with estradiol binding sites on the calf uterus has been described (Kiang *et al.*, 1978). For a better understanding of the mechanism of action of ZEN and tamoxifen in producing estrogenic effects, we further investigated the relative binding abilities of ZEN and tamoxifen to porcine cytoplasmic estrogen receptors. The binding ability of the unlabeled ZEN and tamoxifen relative to 17ß-estradiol was determined by competitive protein binding to cell-free uterine cytosol *in vitro* (Kiang *et al.*, 1978;

Fitzpatrick *et al.*, 1989).

**Experimental Diet**

Purified crystalline ZEN (P-1492; Pitman-Moore, Inc., Terre Haute, IN) and/or tamoxifen citrate (Stuart Pharmaceuticals, Division of ICI Americas, Inc., Wilmington, Del) were dissolved in 100% absolute ethanol and added to an appropriate amount of standard corn-soybean swine diet to give the final concentration of 2 ppm ZEN, 10 ppm tamoxifen and 2 ppm ZEN plus 10 ppm tamoxifen.

**Analytical Methods**

**Feedstuff analysis:** Samples from each treatment diets were taken randomly. The concentration of ZEN in each treatment diet was determined by Dr. W. E. Braselton of the Animal Health Diagnostic Laboratory, Michigan State University.

**Tissue examination:** The portions of the reproductive tract prepared for histopathologic examination were the ovaries and uterus from the female and testes, epididymides, Cowper's glands and seminal vesicles from the male. Tissues were fixed in Bouin's solution and then examined by Dr. B. Yamini of the Animal Health Diagnostic Laboratory, Michigan State University.

**Sperm analysis:** Semen collection by electroejaculation was performed by Dr. P. H. Coe of Large Animal Clinic Sciences, Michigan State University. Sperm from caudal epididymides was collected at autopsy. After collection,

semen or sperm was incubated in a boar semen extender (Modena, Swine Genetics International, Ltd., Cambridge, Iowa) for 2 hours at 37°C. Sperm concentration and motility were analyzed by using a CellSoft computer automated sperm analyzer (Cryo Resources, Ltd., Montgomery, NY).

**Estrogen receptor binding assay:**

A. Preparation of soluble cellular protein: All the experiments described in this study were performed at 0-4°C unless specified differently. The frozen uteri from immature gilts (5-6 weeks old) were pulverized and then homogenized in TEDG buffer (10 mM Tris, 1.5 mM EDTA, 1.0 mM dithiothreitol, 10% glycerol, pH 7.4) with a PCU-2-110 Polytron (Brinckmann Instruments, Inc., Westbury, NY) for 10-second intervals twice. Tissue to buffer ratio was 1 g uterus/ 8 ml TEDG buffer. The homogenate was centrifuged at 800 x *g* (Beckman GPR Centrifuge) for 5 minutes. Soluble cellular protein was obtained by subjecting the supernatant to ultracentrifugation at 100,000 x *g* (Beckman L7 Ultracentrifuge) for 60 minutes. The protein concentration was determined by coomassie blue binding (Reisner *et al.*, 1975) and was approximately 3 mg/ml.

B. Competition binding assay: The competitors used against estradiol at the receptor binding sites were ZEN and tamoxifen. The 17β-[2,4,6,7-$^{3}$H(N)]estradiol with a specific activity of approximately 100 Ci/mmol was from New England Nuclear (Boston, MA). A mixture of 1 nM [2,4,6,7-$^{3}$H(N)]estradiol, one of the cold competitors (0.1 nM to 100

μM), and pig uterine estrogen receptor were incubated at 0°C for 2 hours in order to reach the equilibrium of a maximal binding. After incubation, 0.2 ml aliquot of the incubation mixture was added to Eppendorf tubes containing 0.25 ml of hydroxylapatite (HAP; Biorad, Rockville, NY) mixture. The mixture was gently resuspended with vortexing, incubated for 5-10 minutes, and washed with 2.5 ml TEG buffer (10 mM Tris, 1.5 mM EDTA, 10% glycerol, pH 7.4) 3 times. The washed HAP pellet was then added to the scintillation vial with 5 ml scintillation cocktail and counted for radioactivity (LS 100C, Beckman Instruments, Fullerton, CA). The binding of 17ß-[2,4,6,7-$^{3}$H(N)]estradiol to estrogen receptors in the absence of competitor served as maximal binding. The binding of 17ß-[2,4,6,7-$^{3}$H(N)]estradiol in the presence of a competitor was expressed as percentage of the maximal binding.

**Statistical Analysis**

Data were analyzed using the Statistical Analysis System (SAS Institute, Inc., Cary, NC). The effects of treatment were determined by one-way analysis of variance (ANOVA). Significant differences between treatment groups were determined by least significant differences (LSD) for the comparisons of multiple means. All statements regarding significance are based on $p < 0.05$ unless otherwise indicated.

## RESULTS

### Feedstuffs analysis

Samples of each diet were analyzed and the mean concentration of ZEN in the ZEN and combination diets was 2.12 ppm. No ZEN was detected in the control and 10 ppm tamoxifen feed (Tables 2, 3).

### Clinical observations and post-mortem examination

F0 sows:

Table 4 summarizes the results of breeding and farrowing performances of F0 sows. During the period of administration of treatment diets (approximately 105 days), no clinical abnormalities were observed with the exception of one sow fed the 2 ppm ZEN diet which showed evidence of vulva enlargement. There was no significant effect on the reproductive performance of the 12 sows fed ZEN and/or tamoxifen throughout pregnancy. All of the sows farrowed successfully. The ZEN and combination sows farrowed numerically larger litters (16 and 32%, respectively) in terms of live pigs as well as total pigs per litter (30 and 26%, respectively). However, ingestion of ZEN appeared to lead to an increase in litters farrowed with more dead and weak pigs. Differences in fetal mortality were not significant although mortality was numerically less for sows fed the control diet than those fed the ZEN diet. The addition of ZEN and/or tamoxifen to the diet of pregnant and lactating sows had no effect on combined male and female piglet body weights at birth and at 21 days of

TABLE 2. COMPOSITION OF GESTATION DIETS USED IN THE FEEDING STUDY

| Ingredient[a] | Control | ZEN[b] | TAM[b] | ZEN+TAM[b] |
|---|---|---|---|---|
| Shelled Corn | 70.45 | 70.45 | 70.45 | 70.45 |
| Soybean Meal | 14.50 | 14.50 | 14.50 | 14.50 |
| Wheat Bran | 10.00 | 10.00 | 10.00 | 10.00 |
| Calcium Carbonate | 1.30 | 1.30 | 1.30 | 1.30 |
| Mono-Dical-Phos 21% | 2.00 | 2.00 | 2.00 | 2.00 |
| MSU Swine VIT-TM Premix | 0.60 | 0.60 | 0.60 | 0.60 |
| Selenium & E Premix | 0.50 | 0.50 | 0.50 | 0.50 |
| Choline Chloride 60% | 0.15 | 0.15 | 0.15 | 0.15 |
| Salt | 0.50 | 0.50 | 0.50 | 0.50 |
| Tamoxifen, ppm | 0.00 | 0.00 | 10.00 | 10.00 |
| Zearalenone, ppm (analyzed) | < MDL[c] | 2.12[d] ±0.20 | < MDL[c] | 2.12[d] ±0.26 |

[a] Data expressed as percent in ration.
[b] ZEN refers to 2 ppm zearalenone, TAM refers to 10 ppm tamoxifen, and ZEN+TAM refers to 2 ppm zearalenone plus 10 ppm tamoxifen.
[c] Limit of detection for zearalenone is 0.2 ppm.
< MDL: concentration is less than method detection limit.
[d] Data presented as mean ± standard deviation.

TABLE 3. COMPOSITION OF LACTATION DIETS USED IN THE FEEDING STUDY

| Ingredient[a] | Control | ZEN[b] | TAM[b] | ZEN+TAM[b] |
|---|---|---|---|---|
| Shelled Corn | 71.85 | 71.85 | 71.85 | 71.85 |
| Soybean Meal | 23.50 | 23.50 | 23.50 | 23.50 |
| Calcium Carbonate | 1.35 | 1.35 | 1.35 | 1.35 |
| Mono-Dical-Phos 21% | 1.90 | 1.90 | 1.90 | 1.90 |
| MSU Swine VIT-TM Premix | 0.60 | 0.60 | 0.60 | 0.60 |
| Selenium & E Premix | 0.50 | 0.50 | 0.50 | 0.50 |
| Salt | 0.30 | 0.30 | 0.30 | 0.30 |
| Tamoxifen ppm | 0.00 | 0.00 | 10.00 | 10.00 |
| Zearalenone ppm (analyzed) | < MDL[c] | 2.12[d] ±0.20 | < MDL[c] | 2.12[d] ±0.26 |

[a] Data expressed as percent in ration.
[b] ZEN refers to 2 ppm zearalenone, TAM refers to 10 ppm tamoxifen, and ZEN+TAM refers to 2 ppm zearalenone plus 10 ppm tamoxifen.
[c] Limit of detection for zearalenone is 0.2 ppm.
< MDL: concentration is less than method detection limit.
[d] Data presented as mean ± standard deviation.

TABLE 4. THE EFFECT OF ZEARALENONE AND/OR TAMOXIFEN ON BREEDING AND FARROWING PERFORMANCE OF SOWS[a]

| Parameter | Control | ZEN[b] | TAM[b] | ZEN+TAM[b] |
|---|---|---|---|---|
| No. of Sows | 3 | 3 | 3 | 3 |
| Sow Body Weight (kg) | 129.39 ±12.81 | 132.73 ±13.39 (103) | 126.06 ± 6.58 ( 97) | 130.30 ± 4.64 (101) |
| Litter Size | 7.7 ± 0.58 | 10.0 ± 2.65 (130) | 7.7 ± 4.16 (100) | 9.7 ± 3.51 (126) |
| Live Births/Litter | 6.3 ± 1.53 | 7.3 ± 4.73 (116) | 6.3 ± 4.51 (100) | 8.3 ± 2.52 (132) |
| % Mortality | 17.86 ±15.57 | 32.14 ±36.25 (180) | 22.22 ±19.24 (124) | 11.79 ±10.47 ( 66) |
| % Female/Male | 34/66 | 71/29 | 58/42 | 42/58 |
| Birth Weight (kg) | 1.56 ± 0.40 | 1.57 ± 0.18 (101) | 1.53 ± 0.20 ( 98) | 1.64 ± 0.59 (105) |
| Weaning Weight (kg) | 6.52 ± 1.41 | 6.68 ± 1.20 (102) | 5.89 ± 0.76 ( 90) | 6.22 ± 1.58 ( 95) |

[a] Data presented as mean ± standard deviation.
Number in parentheses refers to percent of control value.
[b] ZEN refers to 2 ppm zearalenone, TAM refers to 10 ppm tamoxifen, and ZEN+TAM refers to 2 ppm zearalenone plus 10 ppm tamoxifen.

age when compared to control weights.

A summary of the effects of ZEN and/or tamoxifen on the reproductive tract of the sows which were necropsied after 21 days of lactation is presented in Table 5. The dietary treatments had no effect on body weight at this point in the trial. A trend of ovarian atrophy and uterine enlargement was observed in sows ingesting ZEN and/or tamoxifen. The percent reduction in the relative ovarian weights for sows fed ZEN, tamoxifen, or ZEN plus tamoxifen was 26, 33 and 46%, respectively. Relative uterine weights were numerically elevated in all three treatment groups with tamoxifen resulting in the smallest increase (6%) and the combination causing the greatest increase (18%). However, using analysis of variance, there were no significant differences ($p > 0.05$) between treatment or treatment by period interactions for any of the measured parameters.

Table 6 summarizes the results of the histopathologic examination of the reproductive tracts of sows. All sows had evidence of corpora albicans (CA) but not of corpora lutea (CL). There were numerous large follicles observed in all control ovaries, while in the sows fed ZEN and/or tamoxifen, the ovaries contained predominantly small and atretic follicles. The histologic appearance of the uterus and cervix in the treatment sows was similar to controls except that some pustules and suppurative exudate were present in the ZEN and tamoxifen sows.

TABLE 5. THE EFFECT OF ZEARALENONE AND/OR TAMOXIFEN ON OVARIAN AND UTERINE WEIGHTS OF SOWS[a]

| Parameter | Control | ZEN[b] | TAM[b] | ZEN+TAM[b] |
|---|---|---|---|---|
| No. of Sows | 3 | 3 | 3 | 3 |
| Body Weight (kg) | 150.91 ±16.52 | 144.55 ±17.91 ( 96) | 161.21 ±13.31 (107) | 146.82 ±13.28 ( 97) |
| Ovarian Weight (g) | 8.60 ± 2.52 | 6.43 ± 4.96 ( 75) | 6.05 ± 1.08 ( 70) | 4.55 ± 1.09 ( 53) |
| Ovarian Weight/Body Weight (X 1,000) | 0.057 ±0.015 | 0.042 ±0.028 ( 74) | 0.038 ±0.006 ( 67) | 0.031 ±0.006 ( 54) |
| Uterine Weight (g) | 653.53 ±367.22 | 691.53 ± 26.73 (106) | 750.37 ± 90.68 (115) | 762.67 ±129.31 (117) |
| Uterine Weight/Body Weight (X 1,000) | 4.37 ±2.55 | 4.83 ±0.60 (111) | 4.66 ±0.55 (107) | 5.17 ±0.51 (118) |

[a] Data presented as mean ± standard deviation.
Number in parentheses refers to percent of control value.
[b] ZEN refers to 2 ppm zearalenone, TAM refers to 10 ppm tamoxifen, and ZEN+TAM refers to 2 ppm zearalenone plus 10 ppm tamoxifen.

TABLE 6. SUMMARY OF HISTOPATHOLOGICAL EXAMINATION OF THE REPRODUCTIVE TRACTS OF SOWS WHICH WERE EXPOSED TO ZEARALENONE AND/OR TAMOXIFEN DURING GESTATION AND LACTATION

| Dietary treatment | No. | Histopathologic observations[b] |
|---|---|---|
| Control | 3 | Normal active ovaries and uterus.<br>Ovary: numerous large follicles (4-7 mm in diameter); evidence of CA but no CL; numerous primordial, primary, secondary and tertiary follicles.<br>Uterus: lumen clear, LE columnar; SC diffuse, mild infiltration of neutrophils; endometrium moderately populated with medium sized glandular structures; LP distended and edematous.<br>Cervix: lumen contained eosinophilic granular materials; SC, multifocal, minimal mononuclear inflammatory cell infiltration. |
| ZEN[a] | 3 | Ovary: numerous tertiary follicles, 2 mm in diameter, many in degenerating process with neutrophils in the theca cell layer; evidence of CA but no CL; numerous primordial, primary, secondary follicles.<br>Uterus: lumen contained neutrophils and eosinophilic materials expended to LP; LE contained multiple pustules.<br>Cervix: lumen filled with neutrophils and suppurative exudate; LE contained many pustules. |
| TAM[a] | 3 | Ovary: similar to those in ZEN group.<br>Uterus: SC diffuse, mild mixed inflammatory cell infiltration.<br>Cervix: submucosa contained diffuse, mild inflammatory cell infiltration. |
| ZEN+TAM[a] | 3 | Ovaries, uterine, and cervix were similar to those in TAM group. |

[a] ZEN refers to 2 ppm zearalenone, TAM refers to 10 ppm tamoxifen, and ZEN+TAM refers to 2 ppm zearalenone plus 10 ppm tamoxifen.
[b] CA refers to corpora albicans, CL refers to corpora lutea, LE refers to luminal epithelium, SC refers to stratum compactum, and LP refers to lamina propria.

**F1 female piglets:**

**Results of the examination of the reproductive tracts of the 21-day-old female piglets are given in Table 7. None of the suckling piglets in the litters of treated sows showed vulva swelling or reddening. Consumption of ZEN and/or tamoxifen by sows during gestation had no significant effect on female piglet birth weights while piglets from sows in the tamoxifen group weighed significantly less than piglets from the combination sows at weaning. Piglets in the tamoxifen treatment group had numerically higher birth weights (1.49 ± 0.27 kg vs 1.33 ± 0.13 kg) but significantly lower weaning weight (5.40 ± 0.51 kg vs 6.10 ± 0.16 kg) than those piglets in the control group. The effect of ZEN and/or tamoxifen on the reproductive tract of female piglets at 21 days of age was similar to the effects observed in the sows. There was a strong trend towards smaller ovaries and an enlarged uterus as a result of *in utero* and lactational exposure to ZEN and/or tamoxifen. The combination of ZEN and tamoxifen resulted in uterine weights which were 31% higher than control weights ($p < 0.05$). In female piglets exposed to tamoxifen, there was a significant decrease in the ovarian weights and increase in relative uterine weights ($p < 0.05$) in comparison with those of control piglets. Although there were differences in the weight of the reproductive tracts among groups, the gross histology of the ovaries and uterus was normal in all female piglets.**

TABLE 7. THE EFFECT OF *IN UTERO* AND LACTATIONAL EXPOSURE TO ZEARALENONE AND/OR TAMOXIFEN ON BODY WEIGHT AND OVARIAN AND UTERINE WEIGHTS OF 21-DAY-OLD FEMALE PIGLETS[a]

| Parameter | Control | ZEN[b] | TAM[b] | ZEN+TAM[b] |
|---|---|---|---|---|
| No. of Piglets | 4 | 10 | 9 | 6 |
| Birth Weight (kg) | 1.33<br>±0.13 | 1.55<br>±0.20<br>(116) | 1.49<br>±0.27<br>(112) | 1.70<br>±0.59<br>(128) |
| Weaning Weight (kg) | 6.10<br>±0.16 | 6.60<br>±1.19<br>(108) | 5.40[c]<br>±0.51<br>( 89) | 7.31<br>±1.57<br>(120) |
| Ovarian Weight (g) | 0.147<br>±0.053 | 0.121<br>±0.026<br>( 82) | 0.110[d]<br>±0.021<br>( 75) | 0.127<br>±0.023<br>( 86) |
| Ovarian Weight/Body Weight (X 1,000) | 0.024<br>±0.009 | 0.019<br>±0.004<br>( 79) | 0.021<br>±0.005<br>( 88) | 0.018<br>±0.003<br>( 75) |
| Uterine Weight (g) | 2.032<br>±0.258 | 2.479<br>±0.451<br>(122) | 2.269<br>±0.478<br>(112) | 2.662[d]<br>±0.549<br>(131) |
| Uterine Weight/Body Weight (X 1,000) | 0.334<br>±0.047 | 0.382<br>±0.069<br>(114) | 0.419[d]<br>±0.068<br>(125) | 0.367<br>±0.044<br>(110) |

[a] Data presented as mean ± standard deviation.
Number in parentheses refers to percent of control value.
[b] ZEN refers to 2 ppm zearalenone, TAM refers to 10 ppm tamoxifen, and ZEN+TAM refers to 2 ppm zearalenone plus 10 ppm tamoxifen.
[c] Significantly different from ZEN+TAM ($p < 0.05$).
[d] Significantly different from Control ($p < 0.05$).

**F1 male piglets:**

There were no differences in the male piglet birth weights and weaning weights due to feeding ZEN and/or tamoxifen to the sows (Table 8). The effects of *in utero* and lactational exposure to ZEN and/or tamoxifen on the reproductive tracts of 21-day-old male piglets are presented in Table 8. Piglets from sows fed treatment diets had lighter testes than did those from sows fed the control diet. The data suggest that ZEN and tamoxifen alone caused an equal decrease in testes weight (15%) while the combination resulted in a further decrease (26%) ($p < 0.05$). When piglet reproductive organ weights are expressed as a percent of body weight, a trend toward increased epididymides and seminal vesicle weights were associated with the combination of ZEN and tamoxifen, which caused a 30% increase when compared to control values. However, these effects were not induced by *in utero* and lactational exposure to ZEN or tamoxifen alone. Also, no difference was found in mean Cowper's gland weights or in the histology of the reproductive tracts among the pigs in the four treatment groups.

**F1 gilts:**

None of the 12 F1 gilts exposed to ZEN and/or tamoxifen *in utero* and during lactation and raised to reproductive age exhibited signs of "hyperestrogenism" such as a red, swollen vulva or mucus discharge. Clinical defeminization and masculinization of sexual behavior were also not observed in

TABLE 8. THE EFFECT OF *IN UTERO* AND LACTATIONAL EXPOSURE TO ZEARALENONE AND/OR TAMOXIFEN ON BODY WEIGHT AND WEIGHT OF THE COMPONENTS OF THE REPRODUCTIVE TRACTS OF 21-DAY-OLD MALE PIGLETS[a]

| Parameter | Control | ZEN[b] | TAM[b] | ZEN+TAM[b] |
|---|---|---|---|---|
| No. of Piglets | 9 | 6 | 4 | 12 |
| Birth Weight (kg) | 1.55 ±0.35 | 1.72 ±0.24 (111) | 1.52 ±0.25 ( 98) | 1.42 ±0.34 ( 92) |
| Weaning Weight (kg) | 6.09 ±1.54 | 5.98 ±1.10 ( 98) | 5.85 ±0.56 ( 96) | 5.27 ±0.98 ( 86) |
| Testes Weight (g) | 5.84 ±1.77 | 4.82 ±1.01 ( 83) | 4.99 ±2.55 ( 85) | 4.30[c] ±1.03 ( 74) |
| Testes Weight/Body Weight (X 1,000) | 0.996 ±0.251 | 0.811 ±0.103 ( 84) | 0.886 ±0.505 ( 92) | 0.830 ±0.221 ( 86) |
| Epididymides Weight (g) | 1.58 ±0.45 | 1.61 ±0.29 (102) | 1.48 ±0.25 ( 94) | 1.75 ±0.44 (111) |
| Epididymides Weight/ Body Weight (X 1,000) | 0.259 ±0.041 | 0.270 ±0.023 (104) | 0.256 ±0.059 ( 99) | 0.342[d] ±0.100 (132) |
| Cowper's Glands Weight (g) | 2.04 ±0.53 | 2.17 ±0.79 (107) | 2.27 ±0.73 (111) | 1.73 ±0.49 ( 85) |
| Cowper's Glands Weight/ Body Weight (X 1,000) | 0.349 ±0.106 | 0.371 ±0.142 (106) | 0.383 ±0.102 (110) | 0.332 ±0.084 ( 95) |
| Seminal Vesicles Weight (g) | 0.824 ±0.203 | 0.836 ±0.206 (101) | 0.924 ±0.181 (112) | 0.921 ±0.359 (112) |
| Seminal Vesicles Weight/ Body Weight (X 1,000) | 0.139 ±0.029 | 0.142 ±0.034 (102) | 0.159 ±0.036 (114) | 0.182 ±0.080 (131) |

[a] Data presented as mean ± standard deviation.
Number in parentheses refers to percent of control value.
[b] ZEN refers to 2 ppm zearalenone, TAM refers to 10 ppm tamoxifen, and ZEN+TAM refers to 2 ppm zearalenone plus 10 ppm tamoxifen.
[c] Significantly different from Control ($p < 0.05$).
[d] Significantly different from Control and TAM ($p < 0.05$).

these gilts. In comparison to the controls, the length of time to the first observed estrus of F1 gilts in the ZEN group was 16 days longer but the difference was not significant (Table 9). The dietary treatments did not appear to have a consistent influence on the reproductive tracts (Table 9). Gilts in the ZEN and tamoxifen groups had similar ovarian weights which were about 10% lower than control ovarian weights, whereas combination gilts had 48% larger ovaries than control animals. Enlargement of the uterus attributable to ZEN was still observed (15%) but F1 gilts in the tamoxifen group had an average uterine weight which was 51% lower than controls while the F1 gilts in the combination group had an average uterine weight which was only 9% lower when compared to controls.

Results of the histopathologic examination of the reproductive tracts of F1 gilts are summarized in Table 10. There was evidence of CA, CL, and numerous large follicles up to 5 mm in diameter in the control and combination groups. However, although there were large follicles observed in the ZEN and tamoxifen groups, no CA and CL could be found. The histological appearance of the uterus did not vary significantly across treatments including the control.

F1 boars:

One boar in the 2 ppm ZEN group died early in the experiment. *In utero* and lactational exposure to ZEN and/or tamoxifen did not affect body weight (Table 11). The mean age

TABLE 9. THE EFFECT OF *IN UTERO* AND LACTATIONAL EXPOSURE TO ZEARALENONE AND/OR TAMOXIFEN ON BODY WEIGHT AND OVARIAN AND UTERINE WEIGHTS OF F1 GILTS[a]

| Parameter | Control | ZEN[b] | TAM[b] | ZEN+TAM[b] |
|---|---|---|---|---|
| No. of Gilts | 3 | 3 | 3 | 3 |
| Body Weight (kg) | 120.91 ± 7.44 | 128.49[c] ± 8.82 (106) | 110.91 ±12.05 ( 92) | 118.94 ± 5.23 ( 98) |
| Age at First Observed Estrus (day) | 216.0 ± 10.4 | 232.7 ± 12.7 (108) | 207.7 ± 25.3 ( 96) | 202.3 ± 22.1 ( 94) |
| Ovarian Weight (g) | 9.00 ±1.05 | 8.17 ±3.30 ( 91) | 8.09 ±6.07 ( 90) | 13.33 ±6.35 (148) |
| Ovarian Weight/Body Weight (X 1,000) | 0.074 ±0.006 | 0.064 ±0.027 ( 86) | 0.071 ±0.047 ( 96) | 0.111 ±0.049 (150) |
| Uterine Weight (g) | 556.40 ±326.28 | 640.97 ± 55.23 (115) | 284.35 ±132.74 ( 51) | 507.47 ±228.01 ( 91) |
| Uterine Weight/Body Weight (X 1,000) | 4.724 ±3.079 | 5.010 ±0.629 (106) | 2.531 ±0.985 ( 54) | 4.218 ±1.775 ( 89) |

[a] Data presented as mean ± standard deviation. Number in parentheses refers to percent of control value.
[b] ZEN refers to 2 ppm zearalenone, TAM refers to 10 ppm tamoxifen, and ZEN+TAM refers to 2 ppm zearalenone plus 10 ppm tamoxifen.
[c] Significantly different from TAM ($p < 0.05$).

TABLE 10. SUMMARY OF HISTOPATHOLOGICAL EXAMINATION OF THE REPRODUCTIVE TRACTS OF F1 GILTS WHICH WERE EXPOSED *IN UTERO* AND DURING LACTATION TO ZEARALENONE AND/OR TAMOXIFEN

| Dietary treatment | No. | Histopathologic observations |
|---|---|---|
| Control | 3 | Normal active ovaries and uterus.<br>Ovary: evidence of CA and CL; numerous large tertiary follicles up to 4-5 mm in diameter; numerous primordial, primary, and tertiary follicles.<br>Uterus: lumen clear; SC diffuse, mild neutrophil infiltration; endometrium moderately populated with medium to large sized glandular structures.<br>Cervix: lumen eosinophilic materials and mild neutrophil infiltration. |
| ZEN[a] | 3 | Ovary: no CA and CL; numerous large tertiary follicles up to 5 mm in diameter; numerous primordial, primary and tertiary follicles.<br>Uterus: similar to those in control group.<br>Cervix: submucosa multifocal, mild mononuclear infiltration. |
| TAM[a] | 3 | Ovary: no CA and CL; many tertiary follicles up to 3-4 mm in diameter; many primordial, primary and secondary follicles.<br>Uterus: similar to those in control group.<br>Cervix: submucosa minimal mononuclear infiltration. |
| ZEN+TAM[a] | 3 | Ovary: similar to those in control group.<br>Uterus: lumen clear; endometrium well populated with well development large glands; multifocal, mild-moderate mononuclear infiltrate in LP.<br>Cervix: lumen clear; submucosa minimal mononuclear infiltration. |

[a] ZEN refers to 2 ppm zearalenone, TAM refers to 10 ppm tamoxifen, and ZEN+TAM refers to 2 ppm zearalenone plus 10 ppm tamoxifen.
[b] CA refers to corpora albicans, CL refers to corpora lutea, LE refers to luminal epithelium, SC refers to stratum compactum, and LP refers to lamina propria.

TABLE 11. THE EFFECT OF *IN UTERO* AND LACTATIONAL EXPOSURE TO ZEARALENONE AND/OR TAMOXIFEN ON BODY WEIGHT AND TESTICULAR AND EPIDIDYMAL CHARACTERISTICS OF F1 BOARS[a]

| Parameter | Control | ZEN[b] | TAM[b] | ZEN+TAM[b] |
|---|---|---|---|---|
| No. of Boars | 3 | 2 | 3 | 3 |
| Body Weight (kg) | 149.39 ±15.94 | 160.91 ± 3.86 (108) | 151.67 ± 2.74 (102) | 146.52 ± 6.37 ( 98) |
| Age of First Mount (day) | 151.7 ±12.5 | 188.0[c] ±32.5 (124) | 153.7 ± 5.7 (101) | 151.0 ± 9.2 (100) |
| Testes Weight (g) | 786.03 ±88.65 | 789.40 ±232.92 (100) | 674.83 ± 60.59 ( 86) | 719.83 ±146.73 ( 92) |
| Testes Weight/Body Weight (X 1,000) | 5.27 ±0.48 | 4.92 ±1.57 ( 93) | 4.45 ±0.32 ( 84) | 4.92 ±1.03 ( 93) |
| Epididymides Weight (g) | 268.17 ±27.84 | 307.35[d] ±99.21 (115) | 237.23 ±19.98 ( 88) | 201.87 ±43.10 ( 75) |
| Epididymides Weight/ Body Weight (X 1,000) | 1.80 ±0.08 | 1.92 ±0.66 (107) | 1.56 ±0.11 ( 87) | 1.37 ±0.24 ( 76) |
| Sperm Concentration[e] (number/ml x $10^6$) | 64.44 ±86.50 | 1056.2 ±1370.7 (1639) | 436.22 ±336.77 (677) | 413.93 ±334.92 (642) |
| % Motility[e] | 58.17 ±25.94 | 68.69 ± 7.25 (118) | 69.10 ±10.57 (119) | 52.48 ±11.54 ( 90) |
| Sperm Concentration[f] (number/ml x $10^6$) | 3495.37 ±416.52 | 4361.25 ±510.81 (125) | 4293.8 ±1166.5 (123) | 2028.0 ±1781.4 ( 58) |
| % Motility[f] | 52.86 ±24.62 | 42.93 ±58.07 ( 81) | 77.12 ± 8.55 (146) | 50.73 ±37.31 ( 96) |

[a] Data presented as mean ± standard deviation. Number in parentheses refers to percent of control value.
[b] ZEN refers to 2 ppm zearalenone, TAM refers to 10 ppm tamoxifen, and ZEN+TAM refers to 2 ppm zearalenone plus 10 ppm tamoxifen.
[c] Significantly different from Control, TAM, and ZEN+TAM ($p < 0.05$).
[d] Significantly different from ZEN+TAM ($p < 0.05$).
[e] Samples were collected using electroejaculation at about 6 months of age.
[f] Samples were collected from cauda epididymides at about 10 months of age.

at first mounting an estrus gilt was approximately 152 days for boars in the control, tamoxifen, and the combination groups. However, there was a significant delay in ZEN boars with a mean value of 188 days ($p < 0.05$). Numerical reductions in the relative testes weights were found among treatments with the effect being most pronounced in the tamoxifen group (16%). The weight of epididymides in animals in the combination group was 25% less than the weight of epididymides of control animals and 24% less when weights were expressed as a percent of body weight. Although the sperm concentrations produced by the boars at 6 months of age in all 3 treatment groups were much higher than control means, the differences were not significant. The percentage of motile sperm was numerically different among diets at both collection times, with a tendency towards higher motility in the tamoxifen group. The basic histological changes in most of the F1 boars were mild degeneration of seminiferous tubular and mild vasculopathy of the pampiniform plexus.

The results of the effects of *in utero* and lactational exposure to ZEN and/or tamoxifen on the reproductive capacity of F1 boars are presented in Table 12. All boars in the present study displayed normal precopulatory and copulatory behavior. None of the boars would be classified as unsuitable for breeding. All of the sows bred by these boars farrowed normally at term. Abnormalities were not observed in the piglets or their development. There were no differences in

TABLE 12. BREEDING AND FARROWING PERFORMANCE OF GILTS WHICH WERE MATED WITH BOARS EXPOSED *IN UTERO* AND DURING LACTATION TO ZEARALENONE AND/OR TAMOXIFEN[a]

| Parameter | Control | ZEN[b] | TAM[b] | ZEN+TAM[b] |
|---|---|---|---|---|
| Litter Size | 9.5<br>± 4.12 | 8.3<br>±3.06<br>( 88) | 9.3<br>±3.20<br>( 98) | 9.7<br>± 1.51<br>(102) |
| Live Births/Litter | 8.6<br>± 2.87 | 8.3<br>± 3.06<br>( 95) | 9.0<br>± 2.83<br>(103) | 9.5<br>± 1.52<br>(109) |
| No. of Mummies | 0.0 | 0.0 | 0.83<br>±0.98 | 0.0 |
| % Mortality | 5.0 | 0.0 | 2.6 | 1.7 |
| % Female/Male | 57/43 | 44/56 | 44/56 | 43/57 |
| Birth Weight (kg) | 1.35<br>± 0.12 | 1.30<br>± 0.27<br>( 96) | 1.24<br>± 0.15<br>( 92) | 1.34<br>± 0.22<br>( 99) |

[a] Data presented as mean ± standard deviation.
Number in parentheses refers to percent of control value.
[b] ZEN refers to 2 ppm zearalenone, TAM refers to 10 ppm tamoxifen, and ZEN+TAM refers to 2 ppm zearalenone plus 10 ppm tamoxifen.

litter size, fetal mortality, and birth weight among the groups, although some mummified fetuses were observed in the tamoxifen group.

**Estrogen receptor binding assay:**

ZEN and tamoxifen were tested for their potency in competition with estradiol for binding at pig uterine receptor sites (Figure 5). Both ZEN and tamoxifen showed significant competitive binding compared to estradiol. The relative binding affinities of 17ß-estradiol, ZEN, and tamoxifen to receptors, calculated by the concentrations able to produce 50% inhibition of [$^3$H]estradiol binding, were approximately 100:5:1, respectively.

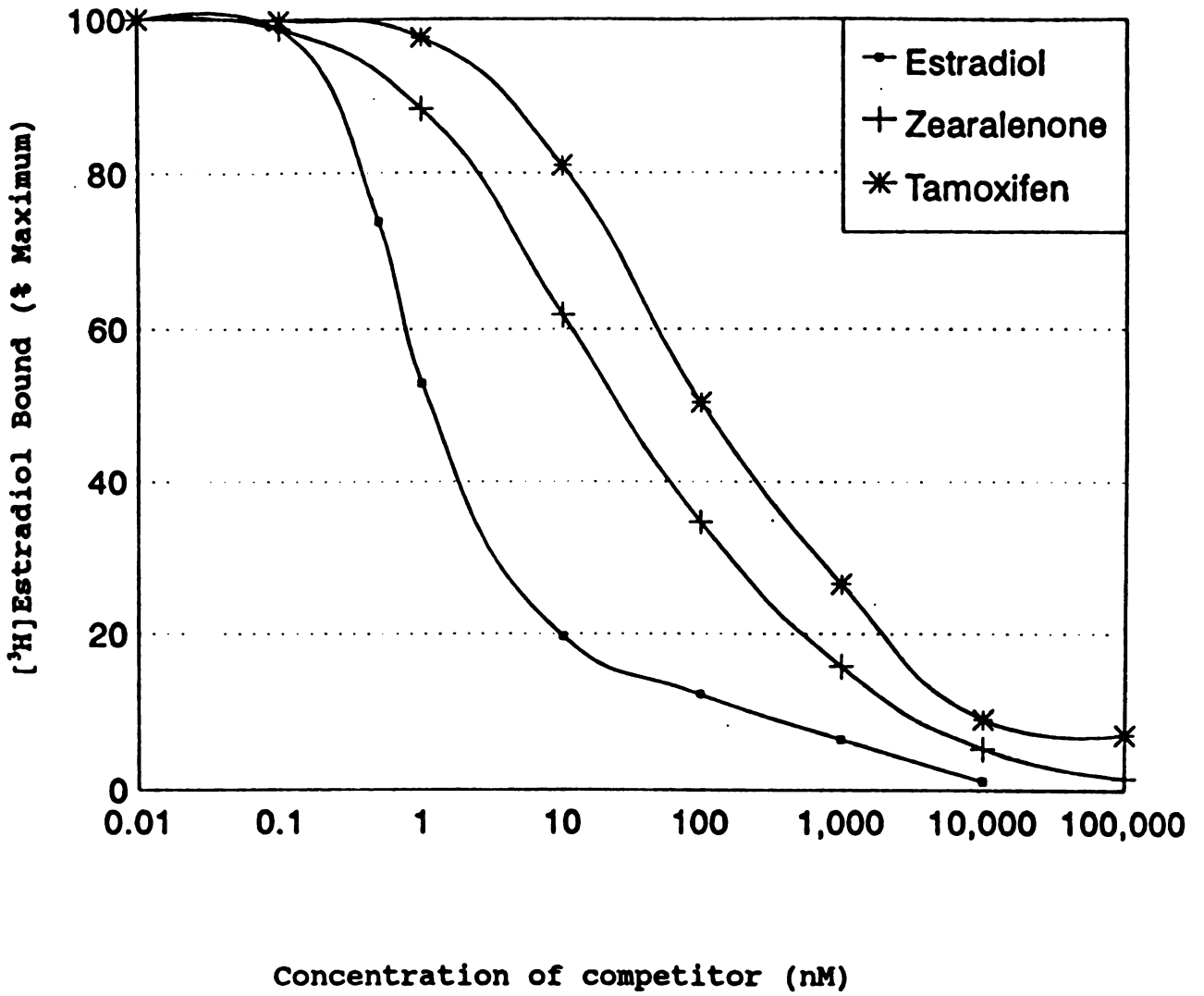



Figure 5. Competition of estradiol, zearalenone and tamoxifen, with [$^3$H]estradiol (1 nM) at the receptor binding sites of the porcine uterus.

## DISCUSSION

The concentration of ZEN selected for this study was 2 ppm of complete ration. This concentration was selected because mature gilts have been reported to be affected at this level (Young *et al.*, 1982) and it approximates levels of contamination reported in the field. The concentration of tamoxifen used in this work was chosen to mimic a dose found to be effective in chickens in terms of antiestrogenic effects (Rozenboim *et al.*, 1986).

The differences in the body weights of F0 sows at the termination of the feeding trial were not significant (Table 4), indicating that ZEN and/or tamoxifen had no effect on feed intake. In the present experiment, most of the sows fed feed containing ZEN and/or tamoxifen showed no hyperestrogenic signs during gestation and lactation. Signs of hyperestrogenism in prepubertal gilts have been indicated with as little as 1 ppm ZEN in the diet (Kurtz *et al.*, 1969; Mirocha *et al.*, 1977). However, dietary concentrations required to produce problems in mature sows were generally higher than those causing hyperestrogenism in prepubertal gilts (Chang *et al.*, 1979; Long *et al.*, 1982).

Moldy feed in the diet of pregnant sows has been associated with abortion, weak pigs, stillbirths, decreased litter size, and fetal mummification (Sharma *et al.*, 1974; Shreeve *et al.*, 1975; Mirocha *et al.*, 1977; Christensen, 1979). It is difficult to assess the overall effects of

consuming ZEN-contaminated feed after mating, based on the literature, due to variation in time of initiation of feeding, the concentration in the diet, duration of feeding and time of assessment after withdrawal of contaminated feed. However, the feeding of dietary concentrations of 2.2 or 3.6 ppm ZEN to pregnant sows did not affect pregnancy (Shreeve *et al.*, 1978; Etienne and Jemmali, 1979). These findings agree with those of Long *et al.* (1982) and Long and Diekman (1984) who reported that feeding up to 15 ppm ZEN for various periods of time after mating did not appear to affect litter development.

In the present study, all parturitions were clinically normal but numerically more stillbirths were found in the ZEN sows (Table 4). This is similar to the work of Miller *et al.* (1973) who observed a high stillbirth rate when sows were allowed to consume ZEN up to farrowing time. A reduction in litter size due to ingestion of *Fusarium*-contaminated feedstuffs has been reported by Miller *et al.* (1973) and Sharma *et al.* (1974). Shreeve *et al.* (1978) observed no significant effects on reproductive performance when sows were fed moldy wheat containing 2.2 ppm ZEN. Our results tend to agree with those of Shreeve *et al.* (1978) in that a concentration of 2.0 ppm ZEN had little effect on overall reproductive performance. Etienne and Jemmali (1979) did not observe a decrease in litter size at 80 days of pregnancy when diets contained 3.61 ppm ZEN were fed. Our data on the total number of piglets born and the number born alive support their

observation. However, the results of the present study suggest that there was a considerable death loss in the ZEN group during farrowing and shortly thereafter. Even though mortality was decreased in the combination group, no clear relationship between ZEN and tamoxifen on fetal mortality could be ascertained.

The data regarding the influence of dietary ZEN and/or tamoxifen on the birth weight and weaning weight of piglets are similar to those previously reported (Table 4). Young *et al.* (1982) found that piglet weight at birth was not affected by levels of ZEN up to 4.8 ppm feed offered to first parity gilts throughout pregnancy. Sharma *et al.* (1974) observed that ingestion of moldy feed did not influence birth weight or the weaning weight of pigs indicating that the subsequent lactation performance was not affected.

Thus, our data suggest that the feeding of diets containing 2 ppm pure ZEN and/or 10 ppm tamoxifen from day 30 of gestation through lactation is unlikely to cause appreciable reproductive problems in pregnant or lactating sows. However, the timing of ZEN administration during early pregnancy is important. Long and Diekman (1986) indicated that the period from day 7 to day 10 postmating is the critical period for ZEN to exert its detrimental effects on early pregnancy on swine. Long and Diekman (1986) found that embryonic mortality occurred when sows were fed 108 mg purified ZEN daily on days 7 to 10 post-breeding but not when

similarly dosed 2 to 6 or 11 to 15 days post-breeding. In the sow, the intrauterine process of blastocyst migration and spacing begins day 7 to 10 postmating (Perry *et al.*, 1973; Anderson, 1978; Geisert *et al.*, 1982). Therefore, it seems likely that ZEN on day 7 to 10 might alter migration of blastocytes. This finding may explain why we did not observe adverse effects of ZEN on the breeding performance of the sows in this study.

It appears that a concentration of 2.0 ppm ZEN and/or 10 ppm tamoxifen in the diet of sows that were fed during gestation through lactation resulted in gross alterations of the reproductive tract. This effect was manifest as obvious changes in the weights of the ovaries and uterus (Table 5). In the present study, ovarian atrophy and uterine enlargement were observed with more pronounced effects in the combination sows than in the ZEN and tamoxifen sows. However, there were too few animals to have any statistical meaning in view of the great variability of the affected characteristics.

The results of the present experiment are in agreement with previous reports associating the consumption of *Fusarium roseum* contaminated feed or ZEN with gross changes in the reproductive tract. Ovarian atrophy and uterine enlargement were observed in gilts ingesting ZEN (Mirocha *et al.*, 1968; Kurtz *et al.*, 1969; Christensen, 1979; Chang *et al.*, 1979; Friend *et al.*, 1986; 1990). The reasons for these effects are not clear. They could be mediated by hormonal imbalance

during pregnancy in relation to a continuous intake of estrogenic compounds. Perhaps the detrimental effects of ZEN on the development of the reproductive tract are mediated locally through the ovary. All necropsied sows treated with estrogenic chemicals had atrophied ovaries. Similar to the activity of estrogen (Amoroso, 1969), ZEN acts to inhibit the release and secretion of follicular stimulating hormone and progesterone but increases estrogenic activity. Therefore, the maturation of ovarian follicles during the preovulation stage is depressed (Chang *et al.*, 1979). The results of histopathological examination of ovaries from sows in the present experiment support the above hypothesis (Table 6). The control sows seemed to be under the influence of endogenous estrogen because of the obvious follicular development in the ovaries when the animals were slaughtered after weaning, while in sows fed treatment diets, the ovaries contained only small follicles. This follicular maturation arrest caused by ZEN and/or tamoxifen may cause changes in the secretory activity of the ovary and hence the environment in the uterus. Moreover, ZEN may impair development of the uterus and uterine contents when fed during particular stages of pregnancy (Etienne and Jemmali, 1982). In the present study, obvious histopathologic differences in the endometrium and uterine mucosa among the control and treatment sows were not observed. Chang *et al.* (1979) reported that squamous metaplasia seemed to be the ultimate characteristic effect of

ZEN on the epithelium of genital organs. However, changes of the uterus and cervical epithelium did not occur in all female pigs. This is in contrast to other studies (Kurtz *et al.*, 1969; Vanyi *et al.*, 1976; Chang *et al.*, 1979; Long *et al.*, 1982; Long and Diekman, 1984) in which morphologic changes in the endometrium persisted for an extended period after ZEN treatment. Seemingly, the ZEN treatment was of a sufficiently low dose that it did not cause histological changes in the present study.

Based on the data presented in Table 5, the hypertrophic uterine response and ovarian atrophy observed in the tamoxifen-treated females strongly suggest that tamoxifen acts as an estrogen agonist in the sow. Although considerable information is available about the biological effects of tamoxifen in a number of animal species and in variety of tissues, very few reports pertain to pigs. Lin and Buttle (1991b) concluded that tamoxifen is an estrogen agonist in the uterus of immature pigs and administering tamoxifen to gilts during late gestation did not affect the development of mammary structures or the ability to lactate at parturition. In the present study, the stimulation of uterine growth by tamoxifen is similar to the results obtained with the uterus of the guinea pigs (Sumida and Pasqualini, 1985), ovariectomized mice (Terenius, 1971; Campen *et al.*, 1985) and immature pigs (Lin and Buttle, 1991b).

The failure of 10 ppm tamoxifen to counteract the adverse

effects of 2 ppm ZEN on sows reflects the known variability in the biological effects of tamoxifen between species and even between tissues of the same species (Furr and Jordan, 1984). Tamoxifen acts as an estradiol receptor blocker in human and rat mammary tissue, but in other species such as mice and guinea pigs, it acts as a weak estrogen. Although a significant interaction was not evident between ZEN and tamoxifen, our data suggest that the effect of tamoxifen was additive to that of ZEN on the reproductive tract of sows when both compounds were given together. This is in agreement with the findings in immature gilts which indicated that tamoxifen was additive to estradiol in terms of the synthesis of total uterine protein (Lin and Buttle, 1991b). The concurrent administration of tamoxifen with ZEN showed agonism between the two compounds in the ovary as well as in the uterus. In the present experiment, it was not possible to distinguish the effects of tamoxifen from those caused by ZEN. Both of these compounds seem to act on genital tissue in a manner similar to a known estrogenic substance. Therefore, administration of tamoxifen to sows consuming ZEN during pregnancy was not effective as a therapeutic agent in treating ZEN toxicoses.

The absence of a ZEN effect on the swelling of vulva in our piglets is in contrast to a previous report indicating swollen vulvas in 21-day-old piglets nursing sows fed 40 ppm ZEN (Palyusik *et al.*, 1980). The apparent difference between these two studies could be due to the low concentration of ZEN

fed in our study. However, similar alterations in the reproductive tracts of female piglets were obtained. The most consistent findings in this trial were increased uterine weights and decreased ovarian weights in piglets (Table 7). Furthermore, the data showed that there was a tendency for reduced testes weight when piglets were exposed *in utero* and during lactation to estrogenic compounds (Table 8).

The collective estrogenic syndrome, observed in the genital organs of both male and female offspring of mothers fed the contaminated feed during pregnancy and lactation, lead to the conclusion that the chemicals may pass through the placenta affecting the embryos or fetuses and/or are present in the milk thus affecting the developing piglets. A report of juvenile hyperestrogenism in neonatal pigs suggested that placental transmission of the toxin may be possible (Doman, 1970). Furthermore, the excretion of ZEN in the milk of sows could explain the alteration observed in the newborn piglets. Clinical observations indicated that the vulva of 1- to 2-week-old piglets became swollen when the lactating sow was fed ZEN (Rozsas *et al.*, 1978). Since piglets of this age generally do not consume compact feed, it seems reasonable to suppose that the source of the estrogenic toxin was the ingested milk. Miller *et al.* (1973) reported ZEN metabolites to be present in sows' milk 12 hours after injection with ZEN. They detected α- and ß-zearalenol and negligible amounts of ZEN in the milk. Palyusik *et al.* (1980) demonstrated that

when the feed of lactating sows contained 40 ppm ZEN, the primary form found in the milk was zearalenol. ß-zearalenol was found in the largest quantity (82.0-86.1%), whereas α-zearalenol and zearalenone were found on smaller quantities (13.4-16.7% and 0.5-1.3%, respectively). The highest concentrations of zearalenol found in the milk ranged from 0.575 to 0.790 ppm. However, Vanyi *et al.* (1983) reported that the administration of 40 ppm ZEN during late pregnancy and early lactation did not produce the estrogenic syndrome in piglets suckled by sows eliminating the toxin in their milk. They concluded that the amount of toxin excreted in the milk (0.12 ppm) was not sufficient to induce or maintain the estrogenic syndrome in suckling pigs. In contrast to the above reports, Young *et al.* (1982) were unable to detect ZEN or its metabolites, α- and ß-zearalenol, in milk samples obtained at day 1 and 14 postfarrow. Similarly, Shreeve *et al.* (1978) were unable to detect residues of ZEN in sow milk within 12 hours of farrowing. Therefore, it is difficult to conclude in the present experiment that milk-borne ZEN would play an important role in the alteration of the reproductive tracts of the piglets. However, the possibility exists that these piglets may have obtained ZEN and/or tamoxifen from the sow's diet.

Alteration of behavioral sex differentiation was not observed in pigs exposed to estrogenic chemicals during gestation and lactation contrary to observations on female

rats (Gray *et al.*, 1985; Kumagai and Shimizu, 1982; Mess *et al.*, 1979). Williams *et al.* (1989) demonstrated that mice exposed to ZEN neonatally had ovary-dependent reproductive tract alterations which were characterized by an absence of corpora lutea, lack of stromal glands, accumulation of dense collagen in the stroma, and squamous metaplasia of the uterine luminal epithelium. In the study of neonatal exposure to estradiol and ZEN in hamsters, it was found that females were masculinized but not defeminized. However, males receiving estradiol treatment had smaller testes, seminal vesicles, and cauda epididymides and 57% had epididymal cysts (Gray *et al.*, 1985). These results demonstrated that a single exposure to a weakly estrogenic chemical like ZEN during a critical developmental period can cause the brain to differentiate in a manner inconsistent with the female's genetic sex. However, we did not observe the phenomena of defeminization and masculinization in this trial. This may be due to the fact that pigs differ from other mammals that have been investigated because sexual differentiation of reproductive behavior occurs during pubertal development and not during gestation (Ford, 1990).

In the current experiment, there was a little delay in the age of first observed estrus in the F1 gilts which were exposed to ZEN during gestation and lactation (Table 9). This result is similar to that reported by Edwards *et al.* (1987a) in that puberty was delayed by ingestion of ZEN. In the F1

gilts, the changes in ovarian and uterine weights persisted from weaning to early puberty in the ZEN treatment group. Furthermore, the results of histological examination of ovaries in the ZEN and tamoxifen groups (Table 10) indicated that these gilts had not gone though an estrus cycle as opposed to animals in the control and combination groups that showed evidence of recent or impending ovulations indicating ovarian cyclicity. Although these effects were not statistically significant, it may suggest that *in utero* and lactational exposure to ZEN could cause permanent alterations of the reproductive capability of swine. This hypothesis is supported by Ruzsas *et al.* (1979) who reported that rats consuming ZEN-contaminated diet during pregnancy and lactation had permanent changes in reproductive organs of their offspring. However, in the combination F1 gilts in the present trial, the ovarian and uterine weights as compared to control weights were in contrast to the comparisons reported at weaning. In addition, a decrease in uterine weight was also observed in the tamoxifen group (Table 9). The reason for this inconsistency is not clear, but ZEN is almost certainly not responsible for this change. It is possible that there were some significant changes occurring in the pituitary-gonadal axis of these pigs after withdrawal of tamoxifen. Therefore, the reversal of ovarian atrophy progressed after the withdrawal of ZEN plus tamoxifen from the diet, and the ovarian weight increased markedly. The evidence

of ovulation and a normal estrus cycle observed in the combination group further supported this possibility. On the basis of these considerations, the mechanisms by which tamoxifen acts alone as well as the interactions between tamoxifen and ZEN to impair reproduction remain equivocal.

Although prepucial and mammary development have been described in males exposed to ZEN (Koen and Smith, 1945; Stob *et al.*, 1962), we did not observed this clinical feature. However, there was a significant delay in the attainment of puberty by F1 boars farrowed by sows exposed to ZEN based on age of first mount (Table 11). Previous reports indicated that feeding 60 ppm ZEN for 8 weeks to mature boars did not affect libido (Ruhr, 1979) but that feeding moldy corn rations did (Bristol and Djurickovic, 1971). When compared with litter-mate controls, boars fed 40 ppm ZEN from 14 to 18 weeks subsequently had reduced libido scores and fewer treated animals showed mating behavior (Berger *et al.*, 1981). Changes in the weights of testes and epididymides were observed in the present study (Table 11). An interesting point is that the relative epididymides weight in the combination boars was 24% less than control value, whereas when the comparison was made in the 21-day-old male piglets, there was a 32% increase. This inconsistency in the combination group was similar to the inconsistency observed in the ovarian and uterine weights of the combination gilts.

There was a large variation in sperm concentrations among

the groups (Table 11). It seems that estrogenic compounds could accelerate the maturation of spermatogenesis when compared with the sperm production in normal 6-month-old boars. This is in agreement with Vanyi and Szeky (1980a) who observed that spermatogenesis began in ZEN-affected boars 1.5 to 2 months earlier than in the control animals. In contrast, Young and King (1986b) reported that feeding diets containing 9 ppm dietary ZEN to boars from 32 days up to 145 or 312 days did not affect libido and sexual behavior, but there was a tendency for boars to produce lower total and gel-free volumes of semen, with lower total motile sperm. Although *in utero* and lactational exposure to ZEN seems to cause testicular atrophy in the young boar and decreased libido in the mature boar, the effect of this mycotoxin on spermatogenesis and fertility in the boar is still unknown. Administration of the antiestrogen tamoxifen improved sperm count in humans (Willis *et al.*, 1977) and stimulated spermatogenesis in cockerels (Rozenboim *et al.*, 1986) has been reported. Evidence of higher sperm motility was observed in the tamoxifen group in the present experiment. Although some boars would have been classified as having greater reproductive potential than others, all boars were successfully bred with females (Table 12). Therefore, *in utero* and lactational exposure to ZEN and/or tamoxifen in this study did not appear to adversely affect the reproductive potential of mature boars.

In the study of relative binding affinity, our data

demonstrated that both ZEN and tamoxifen can compete with 17ß-estradiol for binding with uterine estrogen receptors (Figure 5). In a previous report, it was indicated that the biological effect (uterotropic activity) of ZEN is about 1000-fold less than that of 17ß-estradiol (Kumagai and Shimizu, 1982). However, from the competition curve, the binding affinity of ZEN to receptors is only 20-fold less than that of 17ß-estradiol. Our results are parallel to those reported by Kiang *et al.* (1978) in which the binding affinity of ZEN and tamoxifen was about 10-fold and 30-fold less, respectively, than that of estradiol in the calf uterine estrogen receptor. In their study, Kiang and coworkers further indicated that ZEN differs from either 17ß-estradiol or tamoxifen in three aspects: (1) a second wave of translocation occurred 6 to 12 hours following ZEN injection; (2) there was a much longer nuclear retention (over 24 hours) than in the case of 17ß-estradiol; and (3) following a depletion of estrogen receptors, ZEN induced an over-replenishment by 24 hours, whereas tamoxifen is reported to suppress the replenishment (Kiang *et al.*, 1978). As a result of these differences, it may provide some information in the different biological responses caused by ZEN and tamoxifen.

Another important consideration in biological activity is that the quantity of ZEN or tamoxifen available for receptor binding *in vivo* depends on the rate of metabolism and excretion. The binding affinity and biological activity of

variable metabolites of ZEN and tamoxifen are different. In addition, metabolism may be important in the elimination of chemicals from target tissues. These factors must be considered in predicting or analyzing the biological effectiveness of ZEN and tamoxifen.

## SUMMARY AND CONCLUSIONS

Experiments were conducted to determine the effects of *in utero* and lactational exposure to ZEN on the sexual development of pigs, the potential of tamoxifen as a treatment for ZEN toxicosis, and the relative binding abilities of ZEN and tamoxifen to porcine cytoplasmic estrogen receptors. Data from our experiment suggest that treatment of sows during gestation and lactation with low concentrations of ZEN and/or tamoxifen resulted in estrogenic effects which may affect subsequent reproductive efficiency. Ingestion of ZEN appeared to lead to an increase, although not significant, in fetal mortality. Sows receiving the ration containing ZEN and/or tamoxifen had lower ovarian weights and higher uterine weights when compared to controls. Gross and histopathologic examination of the reproductive tract suggested that the ovary was the primary site of injury in ZEN intoxication which results in maturation arrest and degeneration of follicles. Similar changes in ovarian and uterine weights were observed in the nursing F1 piglets which raises the possibility of transfer of ZEN and/or tamoxifen or their metabolites via the placenta or milk. Furthermore, ZEN-contaminated diets consumed by mothers during pregnancy and lactation may have induced permanent changes in the reproductive organs, and disorders in cyclicity and fertility of their female offspring, but the reproductive potential of male offspring did not appear to be at risk. The alterations in the genital

tract caused by ZEN and/or tamoxifen could have been due to a change in concentrations of estrogen-like compounds and would be expected to be more pronounced in the combination group pigs because they were exposed to both ZEN and tamoxifen and showed more estrogenic effects. The lack of an interaction between dietary tamoxifen and ZEN on various measurements of reproductive performance suggests that tamoxifen at the concentration used did not exert a protective effect against ZEN toxicoses but rather mimicked or augmented the estrogen activity of ZEN on the reproductive tract. Thus, in the pig, tamoxifen can be classified as an estrogen agonist. From the estrogen binding assay, it was demonstrated that there was a similar binding pattern for 17ß-estradiol, ZEN, and tamoxifen to porcine uterine estrogen receptor and the relative binding affinity to receptors was approximately 100:5:1, respectively.

# LIST OF REFERENCES

## LIST OF REFERENCES


Abbas, H. K., C. J. Mirocha and W. T. Shier. 1984. Mycotoxins produced from fungi isolated from foodstuffs and soil: Comparison of toxicity in fibroblasts and rat feeding tests. Appl. Envir. Microbiol. 48:654.

Aizawa, T. and G. C. Mueller. 1961. The effect *in vivo* and *in vitro* of estrogens on lipid synthesis in rat uterus. J. Biol. Chem. 236:381.

Amoroso, E. C. 1969. Physiological mechanisms in reproduction. J. Reprod. Fertil. 6(Suppl.):5.

Anderson, L. L. 1978. Growth, protein content and distribution of early pig embryos. Anat. Rec. 190:143.

Anderson, L. L. 1987. Pigs. In: E. S. E. Hafez (Ed.) *Reproduction in Farm Animals*. pp 324-344. Lea & Febiger, Philadelphia.

Anon. 1975. *MAFF. Boards of the Joint Consultative Organization for Research and Development in Agriculture and Food*. 2nd report, pp 19. London:HMSO.

Aulerich, R. J., S. J. Bursian, D. Tanaka, Jr. and R. J. Bonna. 1991. Effects of *in utero* and lactational exposure to zearalenone on subsequent sexual development and reproductive performance of mink. 1991 Progress Reports of Projects Supported by the Mink Farmers Research Foundation. Thiensville, WI.

Baird, D. T., A. Uno and J. D. Melby. 1969. Adrenal secretion of androgens and estrogens. J. Endocr. 45:135.

Ball, P., H. P. Gelbke and R. Knuppen. 1975. The excretion of 2-hydroxyestrone during the menstrual cycle. J. Clin. Endocr. Metab. 40:406.

Bennet, G. A. and O. L. Shotwell. 1979. Zearalenone in cereal grains. J. Amer. Chem. Soc. 56:812.

Berger, T., K. L. Esbenshade, M. A. Diekman, T. Hoagland and J.Tuite. 1981. Influence of prepubertal consumption of zearalenone on sexual development of boars. J. Anim. Sci. 53:1559.

Berisford, Y. C. and J. C. Ayres. 1976. Use of the insecticide naled to control zearalenone production. J. Agric. Food Chem. 24:973.

Binart, N., M. H. Catelli, C. Geynet, V. Puri, R. Hahnel, J. Mester and E. E. Baulieu. 1979. Monohydroxytamoxifen: An antiestrogen with high affinity for the chick oviduct oestrogen receptor. Biochem. Biophys. Res. Commun. 91:812.

Breuer, H. 1962. Metabolism and neutral estrogens. Vitams Horm. 20:285.

Breuer, H., J. Breuer, K. Dahm, R. Knuppen and W. D. Lehmann. 1969. Some newer aspects of estrogen metabolism. Adv. Biosci. 2:113.

Bristol, F. M. and S. Djurickovic. 1971. Hyperestrogenism in female swine as the result of feeding mouldy corn. Can. Vet. J. 12:132.

Bock, K. W., W. Lilienblum, G. Fisher, G. Schirmer and B. S. Bock-Henning. 1987. The role of conjugation reactions in detoxification. Arch. Toxicol. 60:22.

Boda, J. M. 1959. In: H. H. Cole and P. T. Cupps (Eds.) *Reproduction In Domestic Animals*. Academic Press, New York.

Borgna, J. L. and H. Rochefort. 1979. Occupation *in vivo* des recepteurs estrogens par des metabolites hydroxyles du tamoxifen. C. R. Hebd. Seanc. Acad. Sci. 287:1141.

Boyd, P. and J. Wittliff. 1978. Mechanism of *Fusarium* mycotoxin action in mammary gland. J. Toxicol. Environ. Health 4:1.

Buxton, E. A. 1927. Mycotic vaginitis in gilts. Vet. Med. 22:451.

Caldwell, R. W., J. Tuite., M. Stob and R. Baldwin. 1970. Zearalenone production by *Fusarium* species. Appl. Microbiol. 20:31.

Cameron, R. D. A. 1985. Factors influencing semen characteristics in boars. Aust. Vet. J. 62: 293.

Campen, C. A., J. Jordan and J. Gorski. 1985. Opposing biological actions of antiestrogens *in vitro* and *in vivo*: induction of progesterone receptor in rat and mouse uterus. Endocrinology 116:2327.

Cantley, T. C., D. A. Redmer and B. N. Day. 1982. The effects of zearalenone on sexual function in gilts. J. Anim. Sci. 55(suppl. 1):104.

Chafouleas, J. G., L. Lagace, W. E. Boulton, A. E. Boyd and A. R. Means. 1984. Changes in calmodulin and its MRNA accompany re-entry of quiescent ($G_0$) cells in the cell cycle. Cell 36:73.

Chang, K., H. J. Kurtz and C. J. Mirocha. 1979. Effect of the mycotoxin zearalenone on swine reproduction. Am. J. Vet. Res. 40:1260.

Christensen, C. M. 1979. Zearalenone. In: W. Shimoda (Ed.) *Conference on Mycotoxins in Animal Feeds and Grains Related to Animal Health*. Food and Drug Administration, Rockville, MD.

Christensen, C. M., C. J. Mirocha, G. A. Nelson and J. F. Quast. 1972. Effect on young swine of consumption of rations containing corn invaded by *Fusarium roseum*. Appl. Microbiol. 23:202.

Clark, J. H., J. Peck and J. N. Anderson. 1974. Oestrogen receptors and antagonizing steroid hormone action. Nature 251:446.

Cole, R. J. and R. H. Cox. 1981. Handbook of Toxic Fungal Metabolites. Academic Press, New York.

Colenbrander, B. and B. Kemp. 1990. Factors influencing semen quality in pigs. J. Reprod. Fert. 40(Suppl.):105.

Dailey, R. E., R. E. Reese and A. Brouwer. 1980. Metabolism of [$^{14}$C]zearalenone in laying hens. J. Agric. Food Chem. 28:286.

Diczfalusy, E. and P. Troen. 1961. Endocrine functions of the human placenta. Vitams Horm. 19:230.

Doman, I. 1970. Vulva and teat swelling syndrome of pigs of various ages and both sexes apparently of feed origin. Magy Allatorv Lapja 25:637.

Donaldson, M. D. C., M. G. Forest and J. M. Saez. 1981a. Effects of estradiol benzoate ($E_2B$) tamoxifen (tamox) and 1,4,6-androstatriene-3,17-dione (ATD) on rat testicular function. Pediatric Res. 15:81.

Donaldson, M. D. C., J. M. Saez and M. G. Forest. 1981b. Effects of the aromatase inhibitor 1,4,6-androstatriene-3,17-dione and the antiestrogen tamoxifen on rat testicular function. J. Steroid Biochem. 14:823.

Draughon, F. A. and D. C. Churchville. 1985. Effect of pesticides on zearalenone production in culture and in corn plants. Phytopath. 75:553.

Dziuk, P. 1991. Reproduction in the pig. In: P. T. Cupps (Ed.) *Reproduction In Domestic Animals*. pp 471-489. Academic Press, New York.

Dziuk, P. J., C. Polge and L. E. Rowson. 1964. Intra-uterine migration and mixing of embryos in swine following egg transfer. J. Anim. Sci. 23:37.

Eckert, R. L. and B. S. Katzenellenbogen. 1982. Physical properties of estrogen receptor complexes in MCF-7 human breast cancer cells. J. Biol. Chem. 257:8840.

Edwards, S., T. C. Cantley and B. N. Day. 1984. The effects of zearalenone on sexual function in gilts. J. Anim. Sci. 59(suppl. 1):320.

Edwards, S., T. C. Cantley, G. E. Rottinhaus, G. D. Osweiler and B. N. Day. 1987a. The effects of zearalenone on reproduction in swine. I. The relationship between ingested zearalenone dose and anestrus in non-pregnant, sexually mature gilts. Theriogenology 28:43.

Edwards, S., T. C. Cantley and B. N. Day. 1987b. The effects of zearalenone on reproduction of swine. II. The effect onpuberty attainment and postweaning rebreeding performance. Theriogenology 28:51.

Eriksen, E. 1968. Estrogen factors in moldy grain. Vulvovaginitis in hogs. Nord. Vet. Med. 20:396.

Etienne, M. and M. Jemmali. 1979. Effects of feeding maize contaminated with *Fusarium* on reproduction in sows. C. R. Acad. Sci. Paris Serie D 288:779.

Etienne, M. and M. Jemmali. 1982. Effects of zearalenone (F2) on estrous activity and reproduction in gilts. J. Anim. Sci. 55:1.

Eugenio, C. P., C. M. Christensen and C. J. Mirocha. 1970. Factors affecting production of the mycotoxin F-2 by *Fusarium roseum*. Phytopathology 60:1055.

Evans, E., P. P. Baskevitch and H. Rochefort. 1982. Estrogen receptor-DNA interaction: Difference between activation by estrogen and antiestrogen. Eur. J. Biochem. 128:185.

Fishman, J. and B. Norton. 1975. Catechol estrogen formation in the central nervous system of the rat.Endocrinology 96:1054.

Fitzpatrick, D. W., C. A. Picken, L. C. Murphy and M. M. Buhr. 1989. Measurement of the relative binding affinity of zearalenone, α-zearalenol and ß-zearalenol for uterine and oviduct estrogen receptors in swine, rats and chickens: an indicator of estrogenic potencies. Comp. Biochem. Physiol. 94C:691.

Ford, J. J. 1990. Differentiation of sexual behaviour in pigs. J. Reprod. Fert., Suppl. 40:311.

Friend, D. W., H. L. Trenholm, B. K. Thompson, P. S. Fiser and K. E. Hartin. 1986. Effects of feeding deoxynivalenol (vomitoxin)-contaminated wheat or corn on the feed consumption, weight gain, organ weight and sexual development of male and female pigs. Can. J. Anim. Sci. 66:765.

Friend, D. W., H. L. Trenholm, B. K. Thompson, K. E. Hartin, P. S. Fiser, E. K. Asem and B. K. Tsang. 1990. The reproductive efficiency of gilts fed very low levels of zearalenone. Can. J, Anim. Sci. 70:635.

Fromson, J. M., S. Pearson and S. Bramah. 1973a. The metabolism of tamoxifen (ICI46,474) Part I in laboratory animals. Xenobiotica 3:693.

Funke, P. J., U. W. Tunn, T. Senge and F. Neumann. 1981. Effect of tamoxifen and cyproterone acetate on experimentally induced epithelial and stromal proliferation of the canine prostate. Acta Endocr. 243(Suppl.):159.

Funke, P. J., U. W. Tunn, T. Senge, F. Neumann and B. Schenck. 1980. Effects of tamoxifen on experimentally induced canine prostatic hyperplasia. Urol. Res. 8:248.

Furr, B. J. A. and V. C. Jordan. 1984. The pharmacology and clinical uses of tamoxifen. Pharmac. Ther. 25:127.

Furr, B. J. A., J. S. Patterson, D. N. Richardson, S. R. Slater and A. E. Wakeling. 1979. Tamoxifen. In: M. E. Goldberg (Ed.) *Pharmacological and Biochemical Properties of Drug Substances*. Vol. 2, pp 355-399. American Pharmaceutical Association, Washington, D. C.

Geisert, R. D., R. H. Reneger, W. W. Thatcher, R. M. Roberts and F. W. Bazer. 1982. Estabolishment of pregnancy in the pig. 2. Cellular remodeling of the porcine blastocyst during elongation on day 12 of pregnancy. Biol. Reprod. 27:941.

Gerhard, I. and B. Runnerbaum. 1979. Comparison between tamoxifen and clomiphene therapy in woman with anovulation. Arch. Gynaekol. 227:274.

Gorski, J. 1964. Early estrogen effects on the activity of uterine ribonucleic acid polymerase. 239:889.

Gorski, J., W. V. Welshons, D. Sakai, J. Hansen, J. Walent, J. Kassis, J. Shull, G. Stack and C. Campfn. 1986. Evolution of a model of estrogen action. Recent Prog. Hormo. Res. 42:297.

Gray, L. E., J. M. Ferrell and J. S. Ostby. 1985. Alteration of behavioral sex differentiation by exposure to estrogenic compounds during a critical neonatal period: effects of zearalenone, methoxychlor, and estradiol in hamsters. Toxicol. Appl. Pharmacol. 80:127.

Greenman, D. L., R. G. Mehta and J. L. Wittliff. 1979. Nuclear interactions of *Fusarium* mycotoxins with estradiol binding sites in the mouse uterus. J. Toxicol. Environ. Health 5:593.

Hadley, M. E. 1988. Hormones and female reproductive physiology. In: *Endocrinology*. pp.428-451. Simon & Schuster, New Jersey.

Hansel, W., P. W. Concannon and J. H. Lukaszewska. 1973. Corpora lutea of the large domestic animals. Biol. Reprod. 8:222.

Harper, M. J. K. and A. L. Walpole. 1966. Contrasting endocrine activity of cis and trans isomers in a series of substituted triphenylethylene. Nature 212:87.

Harper, M. J. K. and A. L. Walpole. 1967. A new derivative of triphenylethylene: effect on implantation and mode of action in rats. J. Reprod. Fert. 13:101.

Heel, R. C., R. N. Brogden, T. M. Speight and G. S. Avery. 1978. Tamoxifen: A review of its pharmacological properties and therapeutics on the treatment of breast cancer. Drugs 16:1.

Herrick, J. B. and H. L. Self. 1962. Semen evaluation and recording data. In: *Evaluation of Fertility in the Bull and Boar*. pp.52-58. Iowa State Univ. Press, Ames.

Hidy, P. H., R. S. Baldwin, R. L. Greasham, C. L. Keith and J. R. McMullen. 1977. Zearalenone and some derivatives: Production and biological activities. Adv. Appl. Microbiol. 22:59.

Houpt, K. A. and I. R. Wolski. 1982. Domestic animal behavior for veterinarians and animal scientists. pp.1-356. Iowa State Univ. Press, Ames.

Hurd, R. N. 1977. Structure activity relationships in zearalenone. In: *Mycotoxins in Human and Animal Health*. J. V. Rodricks, C. W. Hesseltine and M. A. Mehlman, eds. Pathotox. Publishers, Forest Park II. pp.379-381.

Ito, H. and H. Hidaka. 1983. Antitumor effect of a calmodulin antagonism on the growth of solid sarcoma-180. Cancer Lett. 19:215.

Jordan, V. C. 1984. Biochemical pharmacology of antiestrogen action. Pharmac. Rev. 36:245.

Jordan, V. C. and K. E. Allen. 1980. Evaluation of the antitumour activity of the non-steroidal antioestrogen monohydroxytamoxifen in the DMBA-induced rat carcinoma model. Eur. J. Cancer Clin. Oncol. 16:239.

Jordan, V. C., M. M. Collins, L. Rowsby and G. Prestwich. 1977. A monohydroxylated metabolite of tamoxifen with potent antioestrogenic activity. J. Endocrinol. 75:305.

Jordan, V. C., C. J. Dix, K. E. Naylor, G. Prestwich and L. Rowsby. 1978. Nonsteroidal antiestrogens: their biological effects and potential mechanisms of action. J. Tox. Environ. Health 4:363.

Jordan, V. C. and B. Gosden. 1982. Importance of the alkylaminoethoxy side chain for the estrogenic and antiestrogenic actions of tamoxifen and trioxifen in the immature rat uterus. Mol. Cell. Endocrinol. 27:291.

Jordan, V. C. and M. E. Lieberman. 1983. Structural classification of estrogen agonists, partial agonists and antagonists. Pharmacologist 25:30 (Abstract).

Jordan, V. C. and G. Prestwich. 1977. Binding of [$^3$H]tamoxifen in rat uterine cytosols: A comparison of swinging bucket and vertical tube rotor sucrose density gradient analysis. Mol. Cell. Endocrinol. 8:179.

Kassiss, J. A. and J. Gorski. 1981. Estrogen receptor replenishment- Evidence for receptor recycling. J. Biol. Chem. 256:7378.

Katzenellenbogen, B. S., J. A. Katzenellenbogen and D. Mordecai. 1979. Zearalenones: Characterization of the estrogenic potencies and receptor interactions of a series of fungal ß-resorcylic acid lactones. Endocrinology 105:33.

Kawabata, Y., F. Tashiro and Y. Ueno. 1982. Synthesis of a specific protein induced by zearalenone and its derivatives in rat uterus. J. Biochem. 91:801.

Kaye, A. M., D. Sheratzky and H. R. Lindner. 1972. Kinetics of DNA synthesis in immature rat uterus: age dependence and estradiol stimulation. Biochem. Biophys. Acta. 261:475.

Kemp, J. V., H. K. Adam, A. E. Wakeling and R. Slater. 1983. Identification and biological activity of tamoxifen metabolites in human serum. Biochem. Pharmacol. 32:2045.

Kiang, D. T. and B. J. Kennedy. 1977. Tamoxifen (antiestrogen) therapy in advenced breast cancer. Ann. Internal Med. 87:687.

Kiang, D. T., B. J. Kennedy and S. V. Pathre and C. J. Mirocha. 1978. Binding characteristics of zearalenone analogs to estrogen receptors. Cancer Res. 38:3611.

King, W. J. and G. L. Greene. 1984. Monoclonal antibodies localize oestrogen receptor in the nuclei of target cells. Nature 307:745.

Kiessling, K. H. and H. Pettersson. 1978. Metabolism of zearalenone in rat liver. Acta Pharmacol. Toxicol. 43:285.

Kitagawa, M., F. Tashiro and Y. Ueno. 1982. Interaction between zearalenone, an estrogenic mycotoxin, and the estrogen receptor of the rat brain. Proc. Japan Assoc. Mycotoxicol. 15:28.

Klopper, A. and M. Hall. 1971. New synthetic agent for the induction of ovulation: Preliminary trials in women. Br. Med. J. 1:152.

Koen, J. S. and H. C. Smith. 1945. An unusual case of genital involvement in swine associated with eating moldy corn. Vet. Med. 40:131.

Koike, S., A. Nii, M. Sakai and M. Muromatsu. 1987. The steroid binding domain of porcine estrogen receptor. Biochemistry 26:2563.

Koseki, Y., D. T. Zava, G. C. Chamness and W. L. McGuire. 1977. Estrogen receptor translocation and replenishment by the antiestrogen tamoxifen. Endocrinology 101:1104.

Kuiper-Goodman, T., P. M. Scott and H. Watanabe. 1987. Risk assessment of the mycotoxin zearalenone. Reg. Toxicol Pharmacol. 7:253.

Kumagai, S. and T. Shimizu. 1982. Neonatal exposure to zearalenone causes persistent anovulatory estrus in the rat. Arch. Toxicol. 50:279.

Kurtz, H. J., M. E. Nairn, G. H. , C. M. Christensen and C. J. Mirocha. 1969. Histologic changes in the genital tracts of swine fed estrogenic mycotoxin. Am. J. Vet. Res. 30:551.

Lam, P. H.-Y. 1984. Tamoxifen is a calmodulin antagonist in the activation of cAMP phosphodiesterase. Biochem. Biophys. Res. Comm. 118:27.

Legenhausen, A. H. 1928. Poisoning due to mold on corn. Vet. Med. 23:29.

Legha, S. S. and S. K. Carter. 1976. Antiestrogens in the treatment of breast cancer. Cancer Treat. Rev. 3:205.

Lieberman, M. E. J. Gorski and V. C. Jordan. 1983. An estrogen receptor model to describe the regulation of prolactin synthesis by antiestrogens *in vitro*. J. Biol. Chem. 258:4741.

Lin, C. L. and H. L. Buttle. 1991a. Progesterone receptor in the mammary tissue of pregnant and lactating gilts and the effect of tamoxifen treatment during late gestation. J. Endocrinol. 130:251.

Lin, C. L. and H. L. Buttle. 1991b. Effect of oestradiol benzoate and tamoxifen on the growth of and induction of progesterone receptors in the uterus and mammary gland of immature pigs. J. Endocrinol. 130:259.

Long, G. G. and M. A. Diekman. 1984. Effect of purified zearalenone on early gestation in gilts. J. Anim. Sci. 59:1662.

Long, G. G. and M. A. Diekman. 1986. Characterization of effects of zearalenone in swine during early pregnancy. Am. J. Vet. Res. 47:184.

Long, G. G., M. A. Diekman and A. B. Scheidt. 1988. Effect of zearalenone on days 7 to 10 postmating on intrauterine environment and migration of embryos in sows. J. Anim. Sci. 66:452.

Long, G. G., M. A. Diekman, F. Tuite, M. Shannon and F. Vesonder. 1982. Effect of *fusarium roseum* corn culture containing zearalenone on early pregnancy in swine. Am. J. Vet. Res. 43:1599.

Long, G. G. and J. J. Turek. 1989. Effect of zearalenone on the growth of mouse embyros from blastocysts to the egg cylinder stage *in vitro*. Am. J. Vet. Res. 50:296.

Longcope, C., W. Widrich and C. T. Sawin. 1972. The secretion of estrone and estradiol-17ß by human testis. Steroids 20:439.

L'vova, L. S., Z. K. Bystryakova and T. I. Shatilova. 1981. Formation of mycotoxins during storage of freshly harvested corn. Prikl. Biokhim. Mikrobiol.17:766.

Macnab, M. W., R. J. Tallarida and R. Joseph. 1984. An evaluation of tamoxifen as a partial agonist by classical receptor theory- An explanation of the dual action of tamoxifen. Eur. J. Pharmac. 103:321.

Major, J. S., B. Green and P. J. Heald. 1976. Interactions of oestradiol-17ß and tamoxifen in the uterus of the pregnant rat. J. Endocr. 71:315.

Marois, M. and G. Marois. 1977. Action of an antioestrogen tamoxifen, on the uterus and the vagina of the ovariectomized rat. C. R. Seanc. Soc. Biol. 171:280.

Martin, P. M., K. B. Horowitz, D. S. Ryan and W. L. McGuire. 1978. Phytoestrogen interaction with estrogen receptors in human brest cancer cells. Endocrinology 103:1860.

McClellan, M. C., N. B. West, D. E. Tacha, G. L. Greene and R. M. Brenner. 1984. Immunocytochemical localization of estrogen receptors in the macaque reproductive tract with monoclonal antiestrophilins. Endocrinology 114:2002.

McNutt, S. H., P. Purwin and C. Murray. 1928. Vulvovaginitis preliminary report. J. Amer. Vet. Med. Assoc. 73:483.

Mercier-Bodard, C., A. Alfsen and E.-E. Baulieu. 1970. Sex steroid binding plasma protein (SBP). Acta Endocr., Copenh. 147(Suppl.):204.

Mess, B., C. Ruzsas, L. Woller and M. Biro-Gosztonyi. 1979. Alterations in reproductive functions of albino rats treated with a fungous toxin, zearalenone (F-2), in the adult age or during the neonatal period. Neuroendocrinol. Lett. 1:1.

Miller, J. K., A. Hacking, J. Harrison and V. J. Gross. 1973. Stillbirths, neonatal mortality, and small litters in pigs associated with ingestion of *Fusarium* toxin by pregnant sows. Vet. Rec. 93:555.

Mirocha, C. J. and C. M. Christensen. 1974. Oestrogenic mycotoxins synthesized by *Fusarium*. In: I. F. H. Purchase (Ed.) *Mycotoxins*. pp 129-148. Elsevier, Amsterdam.

Mirocha, C. J., C. M. Christensen and G. H. Nelson. 1968. Toxic metabolites produced by fungi implicated in mycotoxicoses. Biotechnol. Bioeng. 10:469.

Mirocha, C. J., C. M. Christensen and G. H. Nelson. 1971. F-2 (Zearalenone) estrogenic mycotoxin from *Fusarium*. In: S. Kadis, A. Ciegler and S. J. Ajl (Eds.) *Microbial Toxins*. Vol. 7:107-138. Academic Press, New York.

Mirocha, C. J., S. V. Pathre and C. M. Christensen. 1977. Zearalenone. In: J. V. Rodricks, C. W. Hesseltine and M. A. Mehlman (Eds.) *Mycotoxins in Human and Animal Health*. pp 345-364. Pathotix. Pub., Inc. Forest Park, IL.

Mirocha, C. J., S. V. Pathre and T, S, Robison. 1981. Comparative metabolism of zearalenone and transmission into bovine milk. Food Cosmet. Toxicol. 19:25.

Montani, M. L., G. L. Vaamonde, S. L. Resnik and P. Buera. 1988. Influence of water activity and temperature on the accumulation of zearalenone in corn. Intl. J. Food Microbiol. 6:1.

Nelson, G. H., D. M. Barnes, C. M. Christensen and C. J. Mirocha. 1970. Effect of mycotoxins on reproduction. Symp. Proc. 70-0 (Effect of disease and stress on reproductive efficiency in swine), pp. 90-97. Univ. of Nebraska.

Nelson, G. H., C. M. Christensen and C. J. Mirocha. 1973. *Fusarium* and estrogenism in swine. J. Am. Vet. Med. Assoc. 163:1276.

Nelson, J. A., R. F. Struck and R. James. 1978. Estrogenic activities of chlorinated hydrocarbons. J. Toxicol. Environ. Health 4:325.

Olsen, M. 1985. Zearalenone metabolism in animals. Subcellular distribution and properties of reducing hepatic enzyme and *in vivo* measurements of biotransformation. Acta University of Uppsala, *Comprehensive Summaries of Upplasa Dissertations from the Faculty of Science*. Vol. 15. Uppsala, Sweden.

Olsen, M. and K.-H. Kiessling. 1983. Species differences in zearalenone-reducing activity in subcellular fractions of liver from female domestic animals. Acta Pharmacol. Toxicol. 52:287.

Olsen, M., K. Malmlöf, H. Petterssen, K. Sandholm and K.-H. Kiessling. 1985a. Plasma and urinary levels of zearalenone. Acta Pharmacol. Toxicol. 56:239.

Olsen, M., H. Pettersson and K.-H. Kiessling. 1981. Reduction of zearalenone to zearalenol in female rat liver by 3α-hydroxysteroid dehydrogenase. Acta Pharmacol. Toxicol. 48:157.

Olsen, M., H. Pettersson, K. A. Sandholm and K.-H. Kiessling. 1985b. Quantitive liquid chromatographic method using fluorescence detection for determining zearalenone and its metabolites in blood plasma and urine. J. Assoc. Official Anal. Chem. 68:632.

Palyusik, M. 1973. Experimental vulvooedema of swine; fusariotoxicosis caused by *Fusarium graminearum*). Magyar Allat. Lapja 28:297.

Palyusik, M., B. Harrach, C. J. Mirocha and S. V. Pathre. 1980. Transmission of zearalenone and zearalenol into porcine milk. Acta Vet. Acad. Sci. Hungar. 28:217.

Pasqualini, J. R., N. Giambiagi, C. Sumida, B. L. Nguyen, C. Gelly, C. Mayrand and F. Lecerf. 1986. Biological responses of tamoxifen in the fetal and newborn vagina and uterus of the guinea-pig and in the R-27 mammary cancer cell line. J. Steroid Biochem. 24:99.

Pasqualini, J. R., C. Sumida and N. Giambiagi. 1988. Pharmacodynamic and biological effects of antiestrogens in different models. J. Steroid Biochem. 31:613.

Pathre, S. V. and C. J. Mirocha. 1976. Zearalenone and related compounds. In: J. V. Rodricks (Ed.) *Mycotoxins and Other Fungal Related Food Problems*. pp 179-227. American Chemical Society, Washington, D. C.

Patterson, J. S. 1981. Clinical aspects and development of antioestrogen therapy: A review of the endocrine effects of tamoxifen in animals and man. J. Endocrinol. 89:67P.

Perry, J. S., R. B. Heap and E. C. Amoroso. 1973. Steroid hormone production by pig blastocysts. Nature 245:45.

Pompa, G., C. Montesissa, F. M. DiLauro and L. Fadini. 1986. The metabolism of zearalenone in subcellular fractions from rabbit and hen hepatocytes and its estrogenic activity in rabbits. Toxicol. 42:69.

Pond, W. G. and J. H. Maner. 1984. Reproduction. In: *Swine Production And Nutrition*. pp.123-146. Animal Science Textbook Series.

Powell-Jones, W., S. Raeford and G. W. Lucier. 1981. Binding properties of zearalenone mycotoxins to hepatic estrogen receptors. Mol. Pharmacol. 20:35.

Price, K. R. and G. R. Fenwick. 1985. Naturally Occurring oestrogens in foods-A review. Food Additives and Contaminants 2:73.

Reddy, V. V. R., F. Naftolin and K. J. Ryan. 1974. Conversion of androstenedione to estrone by neural tissues from fetal and neonatal rats. Endocrinology 94:117.

Rochefort, H. and J. L. Borgna. 1981. Differences between oestrogen receptor activation by oestrogen and antioestrogen. Nature 292:257.

Rochefort, H., M. Garcia and J. L. Borgna. 1979. Absence of correlation between antiestrogenic activity and binding affinity for the estrogen receptor. Biochem. Biophys. Res. Commun. 88:351.

Rohr, H. P., G. Bartsch and B. Rotach. 1979. The effect of tamoxifen on the ventral prostatic lobe of the rat. Exp. Molec. Path. 31:344.

Rosenbaum, W., N. P. Christy and W. G. Kelly. 1966. Electrophoretic evidence for the presence of an estrogen-binding ß-globulin in human plasma. J. Clin. Endocr. Metab.26:1399.

Rozenboim, I., G. Gvaryahu, B. Robinzon, N. Sayag and N. Snapir. 1986. Induction of precocious development of reproductive function in cockerels by tamoxifen administration. Poultry Sci. 65:1980.

Ruhr, L. P. 1979. The effect of the mycotoxin zearalenone on fertility in the boar. Ph.D. Dissertation, Univ. of Missouri, Columbia.

Ruhr, L. P., G. D. Oswelier, C. W. Foley. 1978. The effect of zearalenone on fertility in the boar. Am. Assoc. Vet. Lab. Diagnost. 21st Ann. Proc. pp127-134.

Ruzsas, C., M. Biro-Gosztonyi, L. Woller and B. Mess. 1979. Effect of the fungal toxin (zearalenone) on the reproductive system and fertility of male and female rats. Acta Biol. Acad. Sci. Hung. 30:335.

Ruzsas, C., B. Mess, M. Biro-Gosztonyi and L. Woller. 1978. Effect of pre-and perinatal administration of the fungus F2-toxin on the reproduction of the albino rat. Dev. Endocrinol. 3:57.

Ryan, K. J. and O. W. Smith. 1965. Biogenesis of steroid hormones in the human ovary. Recent Prog. Horm. Res. 21:367.

Saez, J. M., A. M. Morera, A. Dazord and J. Bertrand. 1972. Adrenal and testicular contribution to plasma oestrogens. J. Endocr. 55:41.

Sakai, F., F. Cheix, M. Clavel, J. Colon, M. Mayer, E. Pommatau and S. Saez. 1978. Increases in steroid binding globulins induced by tamoxifen in patients with carcinoma of the breast. J. Endocrinol. 76:219.

Sarff, M. and Gorski, J. 1971. Control of estrogen binding protein concentration under basal conditions after estrogen administration. Biochemistry 10:2557.

Sharma, V. D., R. F. Wilson and L. E. Williams. 1974. Reproductive performance of female swine fed corn naturally molded or inoculated with *Fusarium roseum*, Ohio isolates B and C. J. Anim. Sci. 38:198.

Shipchandler, M. T. 1975. Chemistry of zearalenone and some of its derivatives. Heterocycles 3:471.

Shreeve, B. J., D. S. P. Patterson and B. A. Roberts. 1975. Investigation of suspected cases of mycotoxicosis in farm animals in Britain. Vet. Rec. 97:275.

Shreeve, B. J., D. S. P. Patterson, B. A. Roberts and A. E. Wrathall. 1978. Effect of mouldy feed containing zearalenone on pregnant sows. Br. Vet. J. 134:421.

Signoret, J.-P., B. A. Baldwin, D. Fraser and E. S. E. Hafez. 1975. The behaviour of swine. In: E. S. E. Hafez (Ed.) *The Behaviour of Domestic Animals*. pp 295-329. Williams & Wilkins, Baltimore.

Skidmore, J. R., A. L. Walpole and J. Woodburn. 1972. Effect of some triphenylethylenes on oestradiol binding *in vitro* to macromolecules from uterus and anterior pituitary. J. Endocrinol. 52:289.

Smith, T. K. 1980. Effect of dietary protein, alfalfa and zeolite on excretory patterns of 5', 5', 7', 7'[$^{3}$H] zearalenone in rats. Can. J. Physiol. Pharmacol. 58:1251.

Stabenfeldt, G. H., E. L. Akins, L. L. Ewing and M. C. Morrisette. 1969. Peripheral plasma progesterone levels in pigs during the oestrous cycle. J. Reprod. Fert. 20:443.

Steinbach, J. and D. Smidt. 1970. Cyclical phenomena in the female genital tract of swine- histological observations. J. Anim. Sci. 30:573.

Stob, M., R. S. Baldwin, J. Tuite, F. N. Andrews and K. G. Gillette. 1962. Isolation of an anabolic uterotropic compound from corn infected with *Gibberella zeae*. Nature 196:1318.

Stob, M. 1983. Naturally occurring food toxicants: Estrogens. In: M. Rechcigl, Jr. (Ed.) *CRC Handbook of Naturally Occurring Food Toxicants*. pp 81-100. CRC Press, Boca Raton, FL.

Sudo, K., F. J. Monsma and B. S. Katzenellenbogen. 1983. Antiestrogen binding sites distinct from the estrogen receptor: Subcellular localization, ligand specificity and distribution in tissues of the rat. Endocrinology 112:425.

Sumida, C. and J. R. Pasqualini. 1985. Receptors and biological responses of oestrogens, antiestrogens and progesterone in the fetal and newborn uterus. In: V. K. Moudgil (Ed.) *Molecular Mechanism of Steroid Hormone Action*. pp 471-504. Walter de Gruyter & Co. Berlin.

Sundlof, S. F. and C. Strickland. 1986. Zearalenone and zearanol-Potential residue problems in livestock. Vet. Hum. Toxicol. 28:242.

Sutherland, R. L. 1981. Estrogen antagonists in chick oviduct: Antagonist activity of eight synthetic triphenylenthylene derivatives and their interactions with cytoplasmic and nuclear estrogen receptors. Endocrinology 109:2061.

Sutherland, R. L., J. Mester and E. E. Baulieu. 1977. Tamoxifen is a potent "pure" antioestrogen in the chick oviduct. Nature 267:434.

Sutherland, R. L., L. C. Murphy, M. S. Foo, M. D. Green, A. M. Whybourne and Z. S. Krozowski. 1980. High affinity antioestrogen binding site distinct from the oestrogen receptor. Nature 288:173.

Sutton, J. C., W. Baliko and H. S. Funnel. 1980. Relation of weather variables to incidence of zearalenone in corn in southern Ontario. Can. J. plant Sci. 60:149.

Tashiro, F., Y. Kawabata, M. Naoi and Y. Ueno. 1980. Zearalenone-estrogen receptor interaction and RNA synthesis in rat uterus. In: H. J. Preusser (Ed.) *Medical Mycology*. 8(Suppl.):311-320. Fisher, New York.

Terenius, L. 1970. Two modes of interaction between oestrogen and antioestrogen. Acta Endocrinol. 64(Suppl.):47.

Terenius, L. 1971. Structure-activity relationships of antioestrogens with regard to interaction with 17β-oestradiol in the mouse uterus and vagina. Acta Endocrinol. 66(Suppl.):431.

Ueno, Y. and F. Tashiro. 1981. α-zearalenol, a major hepatic metabolite in rats of zearalenone, an oestrogenic mycotoxin of *Fusarium* species. J. Biochem. 89:563.

Vanyi, A., A. Azeky and S. E. Rumvaryne. 1976. Fusariotoxicoses. V. The effect of F-2 toxin on the sexual activity of female swine. Magy Allatorv Lapja 29:723.

Vanyi, A., A. Bata and G. S. Sandor. 1983. Metabolism of zearalenone in pregnant sows. Proc. Internat. Sympos. Mycotoxins. pp.311-315.

Vanyi, A., G. Danko and P. Aldsay. 1973. Fusariotoxicosis. I. Studies on the oestrogenic effect of *Fusarium* strains. Magy Allatorv Lapja 28:303.

Vanyi, A. and A. Szeky. 1980. Fusariotoxicoses. VI. The effect of F-2-toxin (zearalenone) on the spermatogenesis of male swine. Magy. Allat. Lapja 35:242.

Vermeulen, A., L. Verdonck, M. Van der Straeten and N. Orie. 1969. Capacity of the testosterone-binding globulin in human plasma and influence of specific binding of testosterone on its metabolic clearance rate. J. Clin. Endocr. Metab.29:1470.

Villee, C. A. 1969. Placental and fetal tissues:a biphasic system for the synthesis of steroids. Am. J. Obstet. Gynec. 104:406.

Voluntir, V., I. Popescu, I. Jivanescu, R. Moga Minzat, M. Purcel Vlah, S. Constantinescu and M. Filip. 1971. Aspects of stachybotryotoxicosis and fusariotoxicosis in swine. Rev. Zooteh. Med. Vet. 21:68.

Webel, S. K. 1978. Control of ovulation in pig. In: D. G. Crighton, N. B. Haynes, G. R. Foxcroft and G. E. Lamming (Eds.) *Control of Ovulation*. pp 421-434. Butterworths, London.

Welshons, W. V., M. E. Lieberman and J. Gorski. 1984. Nuclear localization of unoccupied oestrogen receptors. Nature 307:747.

Wilking, N. 1981. Antioestrogens and breast cancer. Thesis. University of Stockholm.

Wilking, N., L. E. Applegren, K. Carlstrom, A. Pousette and N. O. Theve. 1981. The distribution and metabolism of $^{14}C$-labelled tamoxifen in spayed female mice. Revs. Endocrine-related Cancer 9(Suppl.):223.

Wilking, N., L. E. Applegren, K. Carlstrom, A. Pousette and N. O. Theve. 1982. The distribution and metabolism of $^{14}C$ labelled tamoxifen in spayed female mice. Acta Pharmac. Tox. 50:161.

Williams, B. A., K. T. Mills, C. D. Burroughs and H. A. Bern. 1989. Reproductive alterations in female C57BL/Crgl mice exposed neonatally to zearalenone, on estrogenic mycotoxin. Cancer Letters 46:225.

Willis, K. L., D. R. London, M. A. Bevis, W. R. Butt, S. S. Lynch and G. Holder. 1977. Hormonal effects of tamoxifen in aligospermic men. J. Endocrinol. 73:171.

Williamson, J. G. and J. P. Ellis. 1973. The induction of ovulation by tamoxifen. J. Obstet. Gynaecol. Br. Commonw. 80:844.

Wolf, J. C. and C. J. Mirocha. 1973. Regulation of sexual reproduction in *Gibberella zeae (Fusarium roseum "Graminearum")* by F-2 (zearalenone). Can. J. Microbiol. 19:725.

Young, L. G. and G. J. King. 1986a. Low concentrations of zearalenone in diets of mature gilts. J. Anim. Sci. 63:1191.

Young, L. G. and G. J. King. 1986b. Low concentrations of zearalenone in diets of boars for a prolonged period of time. J. Anim. Sci. 63:1197.

Young, L. G., G. J. King, L. McGirr and J. C. Sutton. 1982. Moldy corn in diets of gestating and lactating swine. J. Anim. Sci. 54:976.

Young, L. G., R. F. Vesonder, H. S. Funnel, I. Simons and B. Wilcock. 1981. Moldy corn in diets of swine. J. Anim. Sci. 52:1312.