UNITED STATES DISTRICT COURT
EASTERN DISTRICT OF MISSOURI

IN RE NUVARING PRODUCTS LIABILITY LITIGATION	: 4:08-md-1964-RWS : : ALL CASES : : Honorable Rodney W. Sippel

EXPERT REPORT OF SHELLEY ANN TISCHKAU, Ph.D.

I. Introduction

This is a report on the pharmacology of hormonal contraception, including specifically the pharmacology related to the synthetic steroid hormones, ethinyl estradiol (EE) and etonogestrel (ENG) delivered via vaginal administration by NuvaRing. This report also discusses the menstrual cycle, provides a history of hormonal contraceptives and the safety and efficacy of various types, i.e., generations of hormonal contraceptives. I have also addressed issues associated with steady state delivery of hormones and pharmacokinetic studies performed prior to the approval of the NuvaRing by the FDA, in particular problems associated with variability in serum EE levels in those studies that are not accurately reflected in the product label.

The opinions given in this report are to a reasonable degree of scientific certainty, based on the information I have reviewed to date. A list of the information provided to me is contained in Appendix A. I have also reviewed publicly available medical and scientific literature as well as relevant textbooks, the FDA website and other relevant internet resources. The opinions expressed in this report were derived using the same analytical processes that I routinely use in my profession as a University Professor and Research Scientist, including my education and experience in reproductive endocrinology and pharmacology. In addition, I reserve the right to supplement these opinions prior to trial.

II. Background and Qualifications

My formal training in reproductive endocrinology was completed in the world-renowned reproductive biology training program at the University of Illinois at Urbana-Champaign. I completed rigorous coursework in endocrinology and reproductive biology, to include the biochemistry and pharmacology of steroid hormones, including their function in the female menstrual cycle. Through this program, I was also exposed to cutting edge research in many aspects of female, as well as male reproduction. My Ph.D. dissertation research focused on the physiological control of follicle development and ovulation in the ovary, with particular emphasis on the role of progesterone in regulating neuroendocrine components of the ovulatory cycle. The cyclical nature of the ovulatory cycle revealed in this work led me to explore biological timing mechanisms, in an effort to ascertain how these might affect female reproduction. Thus, my postdoctoral work focused on neural regulation of biological timing, namely the control of circadian rhythmicity. As an independent investigator, I have merged these areas of expertise to gain understanding of the natural fluctuation and cyclicity of endocrine function, including how daily rhythms in hormone secretion are important for maintenance of homeostasis. A current line of research ongoing in my laboratory explores how the central biological clock in the brain controls the natural daily pattern of function in the female reproductive cycle. I have published extensively in well-respected, peer-reviewed journals, including *Science*, *Nature*, *Neuroendocrinology*, *Biology of Reproduction* and *PLoS One*, in the areas of neuroendocrinology and biological rhythms.

I also derive significant expertise from the teaching component of my profession. As a Visiting Assistant Professor at the University of Illinois at Urbana-Champaign, I taught a graduate

level course in advanced reproductive endocrinology. More than 50% of the lecture material for this course was focused on female reproductive endocrinology. Particularly relevant to this report, I developed and delivered lectures on the neuroendocrine control of the female menstrual cycle, on production of steroid hormones by the ovary, on the mechanism of action of steroid hormones, on the systemic metabolism of steroid hormones, and on the use of hormone-based contraceptives. I also taught neuroendocrinology of female reproduction in a freshman discovery course and a special topics course on the control of the female reproductive cycle by the circadian timing system. I was hired in 2007 by the Department of Pharmacology at Southern Illinois University School of Medicine specifically because of my expertise as a reproductive endocrinologist. I am the director of the Endocrine-Reproduction-Gastrointestinal (ERG) unit, which comprises one-quarter of the curriculum for the second year medical students. A major focus of the second year of the medical curriculum is Pharmacology. I have developed curricular components to include a 2 h lecture on methods of contraception. Most of that lecture is devoted to the use of hormone-based contraceptives, including oral contraceptives, injectables, patches, and NuvaRing. Second and third generation progestins, and the risks associated with the use of these compounds is specifically addressed in that lecture. Efficacy, benefits and side effects of contraceptive use are also discussed.

III. Overview of the Reproductive System – Menstrual cycles

Production of fertilizable ova is a meticulously choreographed affair that requires precise timing between the ovary, the hypothalamus of the brain and the pituitary gland, referred to as the menstrual cycle. The human menstrual cycle is, on average, 28 days in length and can be divided into two phases. A neuronal clock in the hypothalamus generates bursts of the hypothalamic hormone, gonadotropin releasing hormone (GnRH). During the first half of the menstrual cycle, the follicular phase, GnRH is secreted in slow pulses, which are decoded in the pituitary gland to cause the release of follicle stimulating hormone (FSH). FSH acts on the ovary to induce the growth of a cohort of small follicles within the ovarian stroma. As growth of the follicle pool progresses, the developing follicles produce estrogens, the major form of which is 17β-estradiol. Estrogen feeds back to the hypothalamus and pituitary to inhibit the production of FSH and luteinizing hormone (LH) in a negative feedback manner. At some point one follicle in the pool becomes the dominant follicle and grows rapidly, while the other follicles in the pool regress. As the dominant follicle in the pool reaches maturity it produces robust amounts of estrogen in a rapid manner. As estrogen levels climb quickly, the hypothalamus and pituitary respond by changing their responsiveness to estrogen. The GnRH pulse generator changes its speed. GnRH is now secreted in rapid pulses. Rapid pulses are decoded by the pituitary gland to cause the release of LH. Instead of estrogen acting to inhibit LH, the rapid elevation of estrogen causes a rapid secretion of LH. This positive feedback effect of estrogen occurs only on the day of ovulation and results in dramatic secretion of LH into the blood. This LH surge induces ovulation, the release of a mature ovum from the ovary and marks the beginning of the second phase of the menstrual cycle, the luteal phase. Upon release of the ovum, the follicle cells become luteinized, forming a structure known as a corpus luteum. The corpus luteum is an endocrine structure which exists to prepare the woman's uterus for the possibility of receiving a fertilized egg and thereby, to establish and maintain a pregnancy. The

corpus luteum achieves this objective through the secretion of progesterone. Progesterone also acts to suppress the secretion of LH; this suppression of LH by progesterone will occur as long as progesterone levels remain elevated.

Thus, if a pregnancy occurs and progesterone levels are maintained, LH will continually be suppressed. If fertilization and an ensuing pregnancy do not occur, the corpus luteum regresses due to a lack of LH support, and then progesterone levels drop. As progesterone is required for maintenance of mature uterine environment, the drop in progesterone levels causes a deterioration of the uterine lining. The sloughing of the uterine lining, known as menses, completes the cycle. Thus, it is apparent that the menstrual cycle requires a fluctuation in steroid hormone levels, with climbing estrogen levels occurring midcycle to induce ovulation. Progesterone levels are low during follicle development and are high during the luteal phase. Thus, hormonal contraceptives were developed with the idea that maintenance of constant, elevated levels of estrogen and progesterone would inhibit the naturally occurring cyclic fluctuations in the gonadotropins, thereby inhibiting follicle development and, more importantly, the LH surge and subsequent ovulation.

IV. Hormonal Contraceptives – the physiology of hormones – their development

Estrogens and progestins are naturally occurring hormones with a variety of physiological effects in women. These include primary reproductive effects that are both developmental, and physiological in the adult. They include the control of the neuroendocrine axis to regulate the ovulation, and cyclical preparation of the reproductive tract for acceptance and maintenance of pregnancy. Additionally, these endogenous steroids exert actions on carbohydrate, protein, lipid and mineral metabolism. Both estrogens and progestins exert their actions through binding with intracellular receptors that are members of the nuclear receptor superfamily. Upon binding hormone, these receptors dimerize and bind to specific DNA sequences on target genes to either activate or inhibit the subsequent production of their cognate proteins.

The year 2010 marked the 50th anniversary of the first oral contraceptive. As early as 1921 animal studies demonstrated that ovaries transplanted from pregnant animal could cause infertility in rabbits and guinea pigs. By 1937, it was established that the ovarian hormone responsible for this infertility, due to its inhibitory effects on the ovulatory process, was progesterone. The discovery that steroids could be synthesized in the lab paved the way for the development of synthetic estrogens and progestins. Although progesterone was first synthesized in the lab in the 1930's its efficacy as an oral contraceptive was limited to its poor absorption when given orally. A major breakthrough occurred with the development of the first synthetic progestin, norethindrone, which demonstrated high progestongenic activity with oral administration. A close isomer of norethindrone, norethynodrel, was developed a year later. These compounds successfully suppressed ovulation in all women without breakthrough bleeding and, thus norethynodrel was used in the first clinical contraceptive trials. A subsequent discovery determined that the original northynodrel compound was contaminated with mestranol, a synthetic estrogen. Removal of the mestranol from the preparation increased the incidence of breakthrough bleeding. The addition of mestranol to the purified norethynodrel proved a successful formulation and was finally developed

as combination oral contraceptive pill, Enovid, containing 5 mg norethynodrel and 75 mg mestranol. It was approved by the FDA in 1957 for treatment of menstrual disorders and finally in 1961 for use as an oral contraceptive. Subsequently, other combination oral contraceptives were marketed in the 1960's, including Anovlar, which was the first pill to contain ethinylestradiol (50 ug + 4 mg norethindrone).

The initial observation estrogen reduced breakthrough bleeding became the basis for using the combination of an estrogen and a progestin in later pills. However, estrogens can inhibit ovulation independent of progestins. Estrogens suppress FSH resulting in inhibition of follicular growth. Thus, in the constant presence of estrogens, it is not possible for ovarian follicles to reach a stage of maturity sufficient to elicit ovulation. Progestins, provide a contraceptive effect by directly suppressing the ovulatory LH surge. Furthermore, they thicken the cervical mucus to hinder sperm migration into the fallopian tube and block maturation of the uterine endometrium such that it will be less receptive to implantation. Progestins can cross react with other steroid receptors, including androgen receptor and glucocorticoid receptor. Estrogens promote the synthesis of several hepatic proteins and have a well-established prothrombotic effect.

Many changes have occurred such that current forms of hormonal contraceptives are vastly different from the original pill. Important modifications include reduction of hormonal dosages, introduction of new progestins, development of various schemes of estrogen-progestin administration, and development of alternative routes of administration.

The first evidence of increased risk for thromboembolism related to estrogen in oral contraceptives was surfaced less than ten years after their introduction when epidemiological studies in the United Kingdon revealed an increased susceptibility to thromboembolic events in contraceptive users. It is now firmly established that estrogens in general, and ethinylestradiol in particular, stimulates synthesis of several clotting factors. However, even at low doses, oral contraceptives remain prothrombotic.

Development of levonorgestrel (LNG), a second generation progestin, allowed the reduction of the progestin dosage. In general, the second generation progestins are androgen derivatives. They tend to be more androgenic, but less estrogenic than the first generation progestins. Oral contraceptives containing doses of LNG from 250 mg to 100 mg in later years, combined with 50, 30 or 20 mg ethinylestradiol were marketed at the end of the 1960s and are still the most frequently used.

In an effort to minimize the adverse androgenic effects associated with first and second generation progestins, two new progestins, gestodene and desogestrel, were derived from LNG and marketed staring in the early 1990's. They belong to the 'third generation progestins'. Both have been combined with ethinylestradiol in pills that were originally touted as the drug of choice due to their increased specificity for the progesterone receptor and decreased affinity for the androgen receptor. However, in 1995–1996 three papers published in The Lancet, revealed that the relative

risk of venous thromboembolism associated with the use of third generation pills was twice that of second generation pills.

Currently, there are eight types of synthetic progestins, produced primarily as chemical derivatives of testosterone (19-nortestosterone derivatives), which are commonly divided into two families: estrange and gonane. The estrane family which typically is considered first generation progestins consists of norethindrone and other progestins that metabolize to norethindrone. These include norethindrone acetate and ethynodiol diacetate. The gonane family can be further subdivided into two groups, based on their classification as a second or third generation progestin. The older gonanes are second generation progestins, including levonorgestrel and norgestrel. They have varying degrees of androgenicity and estrogenicity. The newer gonanes are the third generation progestins, which include desogestrel, norgestimate and etonogestrel. Drospirenone is a 17a spironolactone derivative that is considered a fourth generation progestin. Third (and fourth) generation progestins are highly selective for the progesterone receptor with minimal androgenic properties.

V. Risks of Hormonal contraceptives

Hormonal contraceptives are an effective means of birth control. The failure rate is less than 1% with ideal use and approximately 3% with typical use. Initiation of hormonal contraceptive therapy is associated with menstrual irregularity. Breakthrough bleeding and spotting are common during the first 3 months of administration of most hormonal contraceptives; in 3 large clinical trials, 2—11.7% experienced breakthrough bleeding during the first year of use. Dosing is continued under the prescribed conditions, menstrual irregularity generally subsides. Amenorrhea, oligomenorrhea, and temporary infertility are also reported in combination hormonal contraceptive users.

By far the most severe adverse effects associated with hormonally-based contraceptive use are thromboembolic disorders. The risk for development of deep venous thrombosis and/or pulmonary embolism is approximately 3—6 times greater in oral contraceptive users. These risks are increased in women who smoke. The dose of the hormone and the type of hormone are both important factors in determining risk for thromboembolic events. Estrogens, including EE, decrease levels of antithrombin-III and increase the production of blood clotting factors VII, VIII, IX and X. The type and dose of progestin, in combination with the estrogen contributes significantly to embolic risk. Typically, the first and second generation progestins, which have significant androgenic activity, will act to counterbalance the prothrombotic effects of estrogen. The same is not true for the third generation progestins, such as ENG. The vast majority of scientific literature and evidence suggests that inclusion of the third generation progestins in combination with EE doubles the risk of adverse thrombotic events.

Other cardiovascular system problems are also reported. Hypertension may also occur with hormonal contraceptives use. Its prevalence increases advanced age and with increased duration of us. Blood pressure typically returns to normal after discontinuation of EE:ENG therapy, but close monitoring is recommended in patients at risk for hypertension. The WHO Collaborative Study of

Cardiovascular Disease and Steroid Hormone Contraception found a 5-fold increased risk of myocardial infarction associated with oral contraceptive use. However, this increased risk may have reflected the increased number of smokers in this international trial. There was no increased risk of myocardial infarction in women who were non-smokers with normal blood pressure.

Smoking is well known to add risk to hormonal contraceptive therapy. As much as a There is a 10—12 fold increased risk of myocardial infarction is found in patients who use hormonal contraceptive therapy and smoke. An observational study presented at the European Society of Cardiology Congress in 2007 suggests that 10 years of oral contraceptive use may also increase risk of atherosclerosis. These data would be expected to apply similarly to women who use NuvaRing.

VI. Fundamentals of Pharmacokinetics

Successful drug therapy requires not only the known pharmacological action of a substance on a specific receptor found in a particular target tissue, but also must include an assessment of a variety of other factors. The mechanism of drug interaction with its receptor and the relationship between the drugs and its systemic effect are defined as pharmacodynamics. Upon entry into a biological system, however, the body begins immediately to interact with the drug through processes of absorption, distribution, metabolism or biotransformation and ultimately, elimination. These processes, defined as pharmacokinetics, are also important in the consideration of a drug's usefulness. An understanding of all these processes within the body is essential to the development of drugs that will both effective and safe to use.

Pharmacodynamic relationships, including the affinity, specificity and action of a drug with its targeted receptor are critical in determining the efficacy of a drug, and in determining what kinds of adverse effects the drug might have. Synthetic steroid hormones used as contraceptives, like their endogenous counterparts, typically act on intracellular receptors of the nuclear receptor superfamily. Synthetic steroids can act as agonists or antagonists on the intended receptor. In the case of synthetic steroids used in hormonally based contraceptives, the synthetic estrogen compound will act as an agonist on estrogen receptors (ER), whereas the progestin will act as an agonist on progesterone receptors (PR). Thus, binding of the synthetic estrogen to the ER will activate the ER; progestin binding to the PR will likewise activate the PR. Upon activation, ER and PR act as transcription factors by binding to DNA elements and either turning on or turning off known target genes. However, there can be cross reactivity between the synthetic steroids and other receptors. That is to say that the synthetic steroids can bind to other members of the nuclear receptor superfamily, or to other types of receptors. This event is more common with progestins than with estrogens. Synthetic progestins often cross react with androgen receptors and/or glucocorticoid receptors, which can lead to unintended effects. Typically, progestins have a much lower affinity for the androgen or glucocorticoid receptor than for the PR, thus, binding to these alternative receptors becomes more prominent as the dose of the progestin is increased.

In determining the appropriate dose of a drug that is safe to use, studies that examine the affinity of the drug for its target receptor are performed. Quantification of the amount of drug

required to achieve a desired response is typically done by establishing a dose-response relationship, where dose is the amount of the drug that is given and the response can be measured in a number of different ways. Many drugs, including steroid hormones, have a graded response that varies from a minimum to a maximum. The dose response curve typically shows increasing responsiveness with increasing dosage until it reaches a plateau. These types of dose response curves are used as a part of the determination of the therapeutic dose for any given drug.

Because it is not possible to know the exact concentration of a drug at its site of action and it can be difficult to measure the maximal response of various physiological parameters within a complex organism, an alternative approach for choosing an appropriate therapeutic dose of a drug is to determine the plasma or serum concentration. The plasma or serum concentration is defined as the concentration of the drug that is found in the plasma or serum at any given time. Plasma or serum concentration will vary with absorption, release and the rate of metabolism and elimination of the drug. The maximal concentration of the drug, or Cmax, is defined as the highest concentration achieved with a single dose of the drug. The time required to achieve Cmax, defined as Tmax, will vary according to the route of administration. Tmax is shortest when drugs are administered IV, and considerably longer when drugs are administered orally, epidermally or vaginally. However, for all drugs, a concentration profile (concentration of the drug in the blood over time) can be observed. Measuring the area under the curve (AUC) for the concentration vs. time plot gives an idea of how much drug is found in the body over time and is often used to determine dosing regimens.

Pharmacokinetics is also extremely important in determining the efficacy and safety of drugs, and importantly, in determining the appropriate dose to use. Regardless of the route of administration, all drugs must cross cell membrane in order to gain access to the body. Most drugs, including hormonally-based contraceptives, pass through membranes and gain access to the circulatory system where they are transported throughout the body. Drugs that are nonpolar, with low molecular weight and high lipid solubility, such as steroids, are readily transported across lipid-rich membranes. Thus, these drugs are readily absorbed by the body. Once absorbed, the body goes to work on these drugs immediately. Thus, several other pharmacokinetic parameters must be considered when developing a dosing regimen, including clearance, volume of distribution, bioavailability and half-life ($T_{1/2}$). Drug clearance is an independent pharmacokinetic parameter that does not depend on volume of distribution, $T_{1/2}$ or bioavailability. By definition, clearance is the volume of blood that is cleared of drug per unit time, which can be calculated from the following equation:

$$CL = \frac{rate\ of\ elimination\ of\ drug}{plasma\ concentration\ of\ drug}$$

Clearance is an important factor to consider when trying to establish a maintenance concentration, or target plasma concentration for a drug. The target drug concentration is often defined as the steady state concentration, although this term may be something of a misnomer,

since a constant level of drug in the blood is rarely achieved. The steady state concentration is inversely proportional to the clearance for any given maintenance dose of a drug.

Steady state is also affected by the $T_{1/2}$ of a drug. $T_{1/2}$ is defined as the amount of time required for the concentration of a drug to decrease by 50%. $T_{1/2}$ is a dependent pharmacokinetic factor. It depends on clearance and the volume of distribution. $T_{1/2}$ determines how long it takes to reach a steady state.

Bioavailability (F) is the fraction of a drug that reaches the systemic circulation after administration. By definition, bioavailability of an intravenously administered drug is 100%. However, when drugs are administered by other routes, including orally, transdermally and vaginally, only a fraction of the drug reaches the systemic circulation. Several chemical and physical factors can act to reduce bioavailability for drugs administered by these routes. These include poor solubility, incomplete absorption, pH, metabolism by bacteria in the GI tract, efflux of the drug, and rapid metabolism of the drug during its first pass through the liver. Bioavailability is determined by comparing the AUC for the drug administered through the chosen route in comparison to the same drug injected intravenously according to the following equation:

$$F = \frac{(AUC)oral}{(AUC)iv} \; x \frac{(dose)iv}{(dose)oral}$$

When absorption is not 100%, drugs must pass a stringent bioavailability test to assure that bioavailability remains constant between lots and formulations of the drug. Thus, the plasma concentration-time data are critical in determining the steady state concentration of the drug. From these data, the pharmacokinetic calculations of area under the curve and slope can be determined and then used to derive clearance, volume of distribution $T_{1/2}$ and bioavailability.

VII. Vaginal vs. Oral Delivery

Combination oral contraceptive regimes rely on the activities of estrogens and progestins to suppress ovulation. Generally, they provide very good cycle control and are highly effective at preventing unwanted pregnancies. Oral forms of these contraceptives have been available in the United States since the 1960's. As a class, this group of drugs is one of the most widely prescribed pharmaceutical agents in the industrialized world. However, there are several problems associated with the oral delivery of hormones, including patient compliance to the dosing regimen, a reliance on the GI tract for absorption, which can be incomplete or at least variable, and the effect of first pass metabolism by the liver. The first obstacle faced by an orally delivered drug is absorption through the GI tract. Although mechanisms that govern GI drug absorption are known, the process is complex and modified by several confounding factors; which include but are not limited to the following: 1) molecular anatomy of the drug and its concentration, 2) the pK_a of the drug, which governs its degree of ionization at the varying pH values throughout the GI tract), 3) lipid solubility, 4) chemical or physical interactions with other drug preparations and with food, 5) the disintegration and dissolution rates of pills, 6) the rate of passage of the drug through the GI tract, 7) fluid volume within the GI tract, 8) osmolality and pH of the GI tract, 9) intestinal blood and lymph

flow, 10) functional integrity of the gastric and intestinal epithelium, and 11) drug biotransformation within the intestinal lumen by microflora, or within the mucosa by host enzyme systems. Because oral dosing is the most common route of administration, decades of research has been performed to ensure the efficacy and safety of drugs that are administered through this route. Once absorbed via the GI tract, the drug passes first through the liver where it metabolism and biliary excretion can occur before it ever reaches the systemic circulation. If metabolism or biliary excretion is large, the bioavailability of a drug is substantially reduced, which is commonly referred to as the first pass effect. An example of a chemical substance relevant to this report where the first pass effect is substantial is the native hormone progesterone. In early studies, researchers observed that progesterone was capable of blocking ovulation. However, when the native hormone was introduced through oral administration, the highly lipophilic steroid passes into the liver, where it is biotransformed and excreted before reaching the systemic circulation. Modifications of the progesterone molecule to reduce biotransformation during the first pass through the liver provided a synthetic hormone capable of inhibiting ovulation when given orally.

In attempt to overcome issues with bioavailability as influenced by GI absorption and first pass metabolism, pharmaceutical companies have developed alternative forms of delivery for estrogens and progestins, including injectables, transdermal patches and vaginal rings. The NuvaRing is one example of a device that has been developed to overcome the problems associated with the oral delivery route. Insertion of the NuvaRing into the vagina can be easily performed by the end-user without the assistance of healthcare professionals, which allows greater reproductive freedom to women. Once in place, the device releases hormone for 21 days, thereby increasing compliance because there is no need to remember to take a pill every day. The vaginal route of delivery does overcome the problems associated with first pass metabolism by the liver. However, the claim that absorption through the vagina will avoid all the problems associated with absorption through the GI tract seems overstated.

In contrast to the oral mucosa or the GI tract, the vagina is highly dynamic with respect to its physiology, which creates significant uncertainty with respect to retention and absorption of drugs, as well as their metabolism and elimination from the vagina. The vagina does, however, offer a favorable environment for the absorption of drugs. It has a large surface area, is highly vascular and permeable. Several drawbacks to include cultural sensitivity, personal hygiene, gender specificity, local irritation, intercourse and menstrual cycle changes. Vaginal physiology must be considered. The vaginal epithelium undergoes cyclic changes, under the influence of hormones, particularly the female sex steroid estrogen and progesterone. These cyclic changes have dramatic effects on epithelial thickness and porosity, pH and amount and composition of vaginal secretions. The vaginal absorption of steroids is affected by the thickness of the vaginal epithelium. Vaginal absorption of estrogen is reported to be higher in postmenopausal women (whose vaginal epithelium is thinner) compared to premenopausal women.

Epithelial thickness is increased in the late follicular phase of the menstrual cycle due to estrogen-stimulated proliferation of the cells in the basal layers. In parallel, the number od desmosomes increase and intercellular junctions increases, which, in effect, binds the cells more tighylt to each other, creating more a barrier to absorption. In the luteal phase, where progesterone

dominates, the epithelium become loose and porous, and consequently, has increased permeability. There is increased absorption of even high molecular weight hydrophilic drugs during this phase.

The volume, viscosity and pH of vaginal fluid have a considerable influence on vaginal drug absorption. Elevated volume increases absorption of drugs that are not water soluble, although the increased volume may also cause leaking of the drug from the vagianl cavity. Excessively viscous cervical mucus may also hinder drug absorption. An in vitro study reported release of prostaglandin E2 from vaginal preparations varies depending on the pH of the media. Thus, any change in the vaginal pH may affect the release profiles of pH sensitive drugs from vaginal formulations. Furthermore, the vaginal epithelium has a lower enzyme activity compared to the GI tract; however, there is also menstrual cycle variation in the presence of these vaginal enzymes. The variations in the enzyme activity with cyclic and hormonal changes affect vaginal drug delivery.

Permeation of drugs through the vaginal epithelium will occur via either by passing between adjacent epithelial cells (paracellular) or across cell membranes (transcellular). Cyclic changes in the vaginal epithelium alter both of these prime routes of drug uptake. Generally, hydrophilic compounds use the paracellular route, whereas, hydrophobic compounds, like estrogen and progesterone, prefer the transcellular route. However, these generalizations regarding drug absorption may be altered with the cyclic changes in the vaginal epithelium, which has a substantial effect on vaginal absorption. Therefore, it stands to reason that in the design of pharmaceuticals that are delivered through the vaginal routs, significant attention should be paid to cyclic changes in the vaginal environment during the research and development process.

VIII. The Pharmacokinetic/Pharmacodynamic Studies on NuvaRing

FDA approval of NuvaRing in October 2001 was granted subsequent to a single, small clinical trial (Clinical Trial 34218) conducted to determine the pharmacokinetic profiles of EE and ENG delivered on a daily basis to users of this device. Clinical Trial 34218 was conducted using an open label, randomized cross-over design in 16 subjects to compare NuvaRing with the oral contraceptive, Marvelon, also manufactured by Organon. This study is the sole support for the serum concentration levels referenced in the NuvaRing product insert provided to physicians from the time of FDA approval through the present day.

The 16 subjects in Clinical trial 34218 were randomized into two groups, with 8 subjects in each group. Group I used the oral contraceptive, which contained 0.150 mg desogestrel (DSG) and 0.030 mg ethinyl estradiol (EE) per pill, followed by a 7 day pill free period, followed by 35 day use of the NuvaRing (daily release rate of 0.120 ENG and 0.015 EE), and finally an intravenous injection of 0.150 mg ENG and 0.015 mg EE. Group II used NuvaRing first for 35 days, followed by Marvelon and then the intravenous injection.

Clinical trial 34218 was designed to evaluate pharmacokinetics, including in vivo absorption rate and bioavailability (F) of ENG and EE released from the NuvaRing. Pharmacokinetics of ENG and EE were assessed on the final day of Marvelon intake, throughout the entire NuvaRing treatment period, and subsequent to the ENG/EE injection. Pharmacodynamics associated with NuvaRing use were determined before, during and after the treatment period by analysis of serum hormone levels and vaginal ultrasound. Blood samples were collected 1) on the last day of Marvelon intake (-5 min

and after 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 48 and 72 h), 2) on the day of NuvaRing insertion (-5 min and after 6, 8, 12, 16, 24, 48 and 72 h); 3) every other day through the NuvaRing use period; 4) upon removal of the NuvaRing (-5 min and after 3, 6, 12, 24, and 48 h); and 5) during the ENG/EE injection period (-5 min and after 5, 10, 15, 30 and 45 min and after 1, 1.5, 2, 4, 6, 8, 12, 16, 24, 36, 48, 72, 96 and 120 h). From these samples, serum hormone concentrations for ENG and EE were determined. Concentration vs. time plots were constructed for each subject. Concentration vs. time plots were used to determined various pharmacokinetic parameters, including Cmax, Tmax, steady state concentration, AUC, $T_{1/2}$, bioavailability and clearance. Pharmacokinetic data are included in Appendix B of the report for Clinical Trial 34218.

The conclusions derived by the researchers from this experimental trial can be summarized as follows: NuvaRing use for 35 days resulted in inhibition of ovulation and suppression of follicle growth in the ovary, as assessed both by vaginal ultrasonography of the ovary and serum gonadotropin levels. ENG concentrations for the NuvaRing continuously increased during the first week, followed by a subsequent weekly decrease of approximately 100 pg/ml. Mean ENG concentrations after one week of NuvaRing use was similar to the average steady state concentration attained with Marvelon use. The absorption rate of ENG steadily decreased throughout the period of use. $T_{1/2}$ for ENG was consistent regardless of the route of administration. Bioavailability of ENG was higher during NuvaRing use than for Marvelon. On average, EE concentrations rose much more rapidly compared to ENG to reach CMax 2-3 days after insertion of NuvaRing, followed by a steady decrease in levels throughout the remainder of the trial period. Bioavailability was similar for EE with NuvaRing and Marvelon.

However, in my opinion, the conclusions drawn from this study do not accurately reflect all of the actual pharmacokinetic data obtained during Clinical Trial 34218. Careful analysis of the pharmacokinetic data presented in Appendix B of that trial shows that some data points were omitted from the final analysis without reasonable cause or any scientific basis, or at least without reasonable explanation. Figure 3 of Appendix B is a graphical representation of serum concentrations of ENG and EE over the course of the study. The significant amount of variability in serum EE concentrations is particularly striking. When examining the profiles of EE serum concentrations, there seem to be two distinct patterns for EE. Several of the subjects (subject 0006, 0008, 0011 and 0016) experienced a "burst effect" characterized by a rapid increase in serum EE on the first day of NuvaRing use, followed by a drop to levels that were less variable throughout the remainder of the study (See Appendix 2 below for examples of subjects with a burst profile). For example, EE levels for Subjects 0006 and 0011 measured as high as 81 pg/ml and 51 pg/ml, respectively within 6 h after initial insertion of the NuvaRing. The second pattern includes a mid-use surge of EE levels, which was fairly extreme in two Subjects. Serum EE reached 42.5 pg/ml on day 23 of NuvaRing use in Subject 0007 and 62.0 pg/ml after 9 days in Subject 0010 (See Appendix 3 below for examples of Subjects who experienced unexplained midcycle surges in EE). Dr. Sam, Organon's in-house biochemist, testified that the data of these specific data points were not included in the calculation of serum EE found in Figure 4 of Appendix B, which is reflected as Figure I

in the NuvaRing product label. The narrative portion of Clinical trial 34218 addresses the omission of serum EE data points as follows:

> "The following serum concentrations were considered to be too low (Subject 0007: ENG concentration of 92.4 pg/Ml at 16 h after Marvelon® intake) or too high (Subject 0007: EE concentration of 42.5 pg/Ml after 23 days of CCVR use; Subject 0010: EE concentration of 62.0 pg/Ml after 9 days of CCVR use)."

The scientific basis for the exclusion of these data is not made explicitly clear in this report. Although some variability is expected in biological data, it is my opinion that it is not reasonable to simply discard data points because they appear "too high" or "too low". Statistical tests are available to examine individual points in a data set to determine whether they represent true anomalous readings that warrant exclusion from the analysis. These practices were clearly not followed in this report with regards to the handling of the data in Clinical trial 34218. What is clear is that the data reflected in Figure 4 of Appendix B, and therefore, in Figure 1 of the product label does not represent a true "n=16" because of the unexplained omission of certain data points.

The variability in serum EE is also reflected in the Tmax values reported in Tables 5-1 and 5-2 of Appendix B. Table 5-2 reports mean Tmax values for the 16 subjects to be 59.26 h, with a standard deviation of 67.53 h. A standard deviation that is higher than the mean clearly demonstrates that Tmax, the time to reach a maximal serum concentration of EE, was widely variable among study participants. Careful examination of Tmax for individual subjects in Table 5-1 again shows that there is a large amount of variation among subjects. For 6 of the subjects (0006, 0008, 0011, 0014, 0015 and 0016), Tmax was reached approximately 6-8 h after insertion of the NuvaRing. For 6 others (0001, 0002, 0004, 0007, 0009 and 0013), Tmax was reached 47-70 h after insertion, and finally, for 2 subjects Tmax was not reached until greater than 200 h after insertion of the NuvaRing. Thus, the variability in reaching Tmax was over 8 days, which is 1/3 of the time the ring is approved for use. This amount of variability is significant. These data clearly reflect a wide range in accumulation of EE in the serum, which may reflect differences in release, absorption and/or metabolism of EE among individual women. As these parameters will affect the efficacy and safety of drug use, it seems reasonable to expect that further pharmacokinetic studies would be performed to address potential population differences in serum EE levels.

In contrast to the variability in profiles of serum EE concentration over the course of the trial, patterns of serum ENG levels among participants were more consistent. Typically serum ENG levels rose over the course of several days, with Cmax most often reached on or about day 5. After reaching a peak on this day levels slowly declined until the end of the study. Interestingly, and in direct contrast to the EE data, there was not a single incidence where a "burst" effect was observed for serum ENG levels. It is my opinion that this discrepancy is unusual and should have been investigated. Potential reasons for differences in Cmax for the two synthetic hormones include: 1) vast differences in the rate of absorption or 2) differences in the rate of release of the hormone from the carrier. Analysis of the data provided in the Clinical trial suggests that there are significant

differences in the absorption rate. However, the rate of absorption of ENG is, in fact, higher than EE (Table 5-2), which suggests ENG is absorbed more readily than EE. This idea is further supported by differences in absolute bioavailability, which were 102.86±12.82% for ENG and 55.60±12.88% for EE. Collectively, these data suggest that EE is absorbed more slowly and less efficiently than ENG. Thus, the fact that "burst" effects were observed for EE but not for ENG remain unexplained in terms of the capacity of these compounds to be absorbed via the vaginal epithelium.

IX. The NuvaRing Label is Not Consistent with the findings in Clinical Study No. 34218

Careful analysis of Clinical Trial 34218, which is the basis for the data presented on the NuvaRing product label, suggests significant discrepancies between the product label and the complete data presented in the Clinical Trial. Of specific concern is the unexplained omission of data points for serum EE concentrations deemed anomalous for reasons that seem without solid scientific basis. The Clinical Trial clearly demonstrates significant variability in serum EE profiles over time in the 16 subjects studied. Specifically, there are "bursts" of EE in certain subjects that appear within 6-8 h of NuvaRing insertion, as well as mid-cycle spikes of serum EE in other subjects. Clinical Trial 34218, which is the sole study to represent the pharmacokinetic parameters of NuvaRing, revealed very high "bursts" of EE measuring 81 pg/ml and 51 pg/ml, respectively for Subjects 0006 and 0011, within 6 h after initial insertion of the Ring. This "burst" effect is not reflected on the product label.

A second source of variability in EE concentrations occurs throughout the course of NuvaRing use as reflected in the widely divergent profiles of EE serum concentrations among the 16 subjects as displayed in Figures 3-1 through 3-16 of the Clinical Trial. Of particular concern are mid-cycle spikes of EE in subjects 0002 (38.8 pg/ml) on Day 21 of NuvaRing use, Subject 0007 (42.5 pg/ml) after 23 days of NuvaRing use and Subject 0010 (62.0 pg/ml) after 9 days. Dr. Sam, Organon's in-house biochemist, testified that these specific data points from the latter two subjects, which showed the highest readings of EE, were not used in calculating the mean serum concentration of EE reflected in Figure 1 (i.e., the graph) of the NuvaRing label. In my assessment of the data presented in Clinical Trial 34218, I performed calculations on the mean serum EE concentrations and found that the numbers presented in Figure 1 do not reflect inclusion of all data points. Some of the data points presented in that Figure represent fewer than the 16 subjects as repeatedly indicated on the product label. Other variation in the amount of EE experienced by several other subjects, Subjects 0002 and 0009 with EE serum concentration levels at 35 pg/ml, while Subjects 0004 and 0005 had levels around 25 pg/ml, demonstrate clear inter-subject variability among participants in Clinical Trial 34218, yet as acknowledged by Organon's expert, Dr. Sam, much of this information is not present in the label. Thus, it is apparent that Organon was not completely transparent in their communications to the FDA regarding excluding the high EE levels of these two subjects in their calculation of the mean serum concentration levels presented in the NuvaRing label.

There are several aspects of the product label that are inconsistent with Clinical Trial 34218 and, therefore, are inaccurate representation of the only clinical data available regarding the pharmacokinetics of the hormones as delivered by NuvaRing. First, bioavailability data were derived

from 9 of 16 subjects for ENG and 5 of 16 subjects for EE. The reason for exclusion of certain subjects and inclusion of others for calculation of this critical pharmacokinetic parameter is not clear. It is particularly troubling that the bioavailability of EE is based solely on 5 Subjects. Although not explicitly stated, it is likely that the wide variability in serum EE levels seen when data from individual subjects are compared, contributed to the exclusion of the majority of the data when calculating bioavailability. The label does not disclose the extensive variability in the delivered dose of EE in these 16 subjects. The label represents that the pharmacokinetic profile of EE is at a steady state, as evidenced in Figure 1 of the NuvaRing label. Examination of Figure 1 in the product label, which presents the serum EE concentrations over time, compared to the same data as presented in Clinical Trial 34218 (Figure 4 in Appendix B) suggests that evidence of this burst effect is minimized on the product label. In the Clinical Trial (Figure 4, Appendix B), the y-axis for EE ranges from 0-35 pg/ml. Under these circumstances, the "burst" effect is clearly evident. However, when these same data are presented on the product label, the y-axis for EE is extended to 90 pg/ml, which in effect compresses the data so that any variability, including the "burst" effect, is minimized (See Appendix 1 below for a comparison between the actual data and the data shown on the product label). Furthermore, Figure 1 does not represent a true "n=16" since data points were excluded from the calculations as presented in the Figure. Thus, Figure 1 in the product label is misleading.

The "burst" effect was not acknowledged in the 1999 Clinical Expert Report, which provided the foundation for the original product label in 2001. However, the 2006 Clinical Expert Report does report the "burst" effect. Furthermore, Titia Mulders, a researcher and scientist who worked for Organon in the development of the NuvaRing®, acknowledged that the footnote regarding the "burst" effect" was omitted from the 1999 Clinical Expert Report. Additionally, Dr. Mulders agreed that by excluding the data, physicians could not have known that hormonal burst data was not disclosed on the package insert for NuvaRing® (573:10). Additionally, the published journal article (co-authored by Dr. Mulders) does not report any information about the burst effect or the EE spikes throughout the cycle. (570:22).

The pharmacokinetic profile of etonogestrel and ethinyl estradiol during use of NuvaRing® is shown in Figure 1.

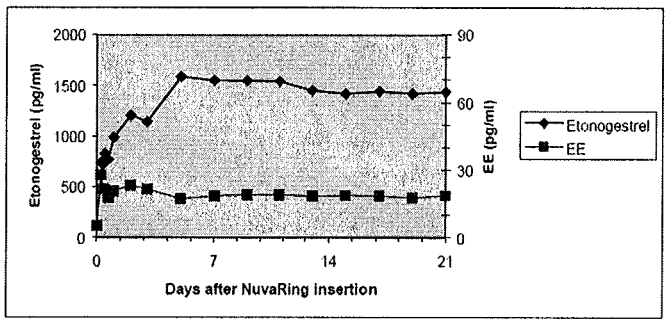


Figure 1. Mean serum concentration-time profile of etonogestrel and ethinyl estradiol during three weeks of NuvaRing® use.

TABLE I: MEAN (SD) SERUM ETONOGESTREL AND ETHINYL ESTRADIOL CONCENTRATIONS (n=16).

	1 week	2 weeks	3 weeks
Etonogestrel (pg/mL)	1578 (408)	1476 (362)	1374 (328)
Ethinyl estradiol (pg/mL)	19.1 (4.5)	18.3 (4.3)	17.6 (4.3)

TABLE II: MEAN (SD) PHARMACOKINETIC PARAMETERS OF NuvaRing® (n=16).

Hormone	C_{max} pg/mL	T_{max} hr	$t_{1/2}$ hr	CL L/hr
Etonogestrel	1716 (445)	200.3 (69.6)	29.3 (6.1)	3.4 (0.8)
Ethinyl Estradiol	34.7 (17.5)	59.3 (67.5)	44.7 (28.8)	34.8 (11.6)

C_{max} - maximum serum drug concentration
T_{max} - time at which maximum serum drug concentration occurs
$t_{1/2}$ - elimination half-life, calculated by $0.693/K_{elim}$
CL - apparent clearance

Similarly, the data presented in Table I of the product label does not represent a true n=16, as stated in the title of the table. Careful consideration of the data presented in Appendix B of Clinical Trial 34218 clearly demonstrates that certain data points were omitted, as outlined extensively above. Specifically, data points were excluded for ENG on days 2 and 11; and for EE on days 2, 9, 11 and 13. As these same data were used to calculate the serum concentrations reported in Table I, it is clear that data were selectively withdrawn. In my opinion, there is no clear scientific basis for the exclusion of these data.

Finally, Table II is also an inaccurate representation of Clinical Trial data, in that it also does not account for all data collected from all sixteen subjects. Whereas the ENG data are accurate and appear to reflect all subjects, EE data are derived from an n=13. Careful examination and comparison of the data in this table with all the data collected during the Clinical Trial suggest that Subjects 0004, 0011 and 0015 were not included in the pharmacokinetic data presented in Table II of the product label. Furthermore, neither Table I nor table II in the product label accounts for "burst" release of EE. The significant variability in Tmax, is however, listed on the label. Tmax values are reported as 59.26 h, with a standard deviation of 67.53 h. A standard deviation that is

higher than the mean clearly demonstrates that Tmax, the time to reach a maximal serum concentration of EE, was widely variable among study participants and reflect significant variability in the secretion, absorption or metabolism of EE. The variability in reaching Tmax was over 8 days, which is 1/3 of the time the ring is approved for use. This amount of variability seems unreasonable, or at the very least, warrants further investigation.

The summary of the pharmacokinetic parameters for NuvaRing as presented on the product label is incomplete, and significantly inconsistent with the actual data found in Clinical Trial 34218. The exclusion of data points without sound scientific justification does not represent acceptable method of handling of the available pharmacokinetic data. Thus, by inaccurately representing the real data, the label misinforms healthcare professionals who might seek to prescribe this product to their patients.

X. Variability in the EE absorption levels from the Ring

Clinical Trial 34218 is the only complete pharmacokinetic study performed with the actual NuvaRing. Two other studies (34225 and 34226) assessed pharmacokinetic parameters peripherally and these findings, although generally supportive of the main pharmacokinetic study, did not add substantial new information. However, previous clinical trials performed with a silastic prototype suggest a larger variation in hormone release can be expected in vaginal rings compared to oral tablets. Examination of the data obtained on serum hormone concentrations reveals striking inter-subject variability in EE profiles throughout the time of ring use. There are two apparent sources of this variation, suggested by marked differences in the overall profile of serum hormone concentration. The first source of variability is a "burst" effect observed in several subjects. Although acknowledged in the narrative for the Clinical Trial for two of the subjects (0006 and 0011, see Appendix 2 for the profiles for these two subjects), review of Tmax data for individual subjects suggests that this burst effect may be present, perhaps to a lesser extent, in at least 4 other subjects (0008, 0014, 0015 and 0016). For these 6 subjects Tmax was reached within 6-8 h of NuvaRing insertion. For six other subjects (0001, 0002, 0004, 0007, 0009 and 0013) Tmax was reached 47-70 h after insertion. Strikingly, Tmax was not reached in 2 patients (0005 and 0010) until more than 200 h after NuvaRing insertion.

This same "burst" effect was also observed in Clinical Trial 34237, which compared some aspects of pharmacokinetics among the NuvaRing, a transdermal contraceptive and an oral contraceptive. There is a significant difference in the serum levels of EE in the patch vs. ring groups, which is interesting and suggests that vaginal absorption of the hormone is actually reduced compared to transdermal absorption, even when consideration is given to the differences in hormone content between the patch and ring. However, I don't necessarily agree with the conclusion in the associated publication from Clinical Trial 34237, that vaginal application results in a more constant level. Within an individual subject there is less variation, although the "burst effect" and midcycle spikes are also present in this study. However, there was more inter-subject variability in the ring group. Coefficients of variation for AUC_{0-21} was 15.5% for patch, 23.8% for ring and 13.0 % for COC. Thus, while the amount of hormone that is delivered via the ring is clearly lower, the

inter-subject variability is nearly doubled compared to the oral contraceptive and increased by 50% compared to the patch.

These dramatic differences in the serum profile for EE found in two different pharmacokinetic studies suggest the possibility that secretion, absorption and/or metabolism of EE changes in different women across the course of NuvaRing therapy. Significant variability in this delivery method has been acknowledged by Dr. Sam, the expert biochemist from Organon. Dr. Sam has testified that there is variability in serum EE levels among the subjects in the Clinical Trial. However, as far as I am able to discern from the available studies, the source of this variability has never been investigated. Because NuvaRing uses a novel delivery method for the administration of hormonal contraceptive, it would seem plausible to expect that studies would have been conducted that might explore how the vaginal environment might influence release of the hormone from the carrier and/or absorption of hormone through the vaginal epithelium. It is acknowledged in Clinical Trial 34218 that the absorption rate of both EE and ENG decrease across the course of exposure to the NuvaRing. However, the cause of this change has never been explored.

It is my opinion, that the vaginal environment, particularly the content of vaginal secretion, vaginal Ph and the cytology of the vaginal epithelium would definitely influence the absorption of hormone. Vaginal Ph changes across the human lifespan. Prior to puberty vaginal Ph is elevated, but as adult levels of estrogen are produced vaginal Ph drops; vaginal Ph again rises in postmenopausal women. Normal adult vaginal Ph, in women with regular menstrual cycles ranges from 3.5 to 4.5, but is inversely related to estrogen levels. That is, when estrogen is elevated, Ph is lower. Therefore, it is readily apparent that vaginal Ph changes across the course of a normal menstrual cycle. Moreover, vaginal Ph is significantly affected by changing levels of estrogen. Adverse effects reported by users of vaginal rings include changes in discharge, increased lacerations and ulceration of the vagina. Any of these events can significantly alter the local environment within the vagina, including alteration of fluid volume and Ph. Thus, it stands to reason that vaginal pH might be affected by the presence of the hormones released from the NuvaRing. The pKa for EE is very high, and therefore will be more readily absorbed when the Ph of the vagina becomes higher. The fact that Ph is decreased in the presence of increased estrogen, therefore, suggests that an increased presence of EE within the vagina, which could lower Ph, could also then decrease the absorption of EE. This provides a scientific explanation of the decreased absorption of EE and ENG over the course of treatment as described in Clinical Trial 34218. Given the observed increased variability in absorption of hormones from rings compared to oral tablets, it is prudent to explore how vaginal Ph might affect absorption. Yet, the manufacturer did not perform any testing along these lines in the time before, or since approval of the drug by the FDA. It would seem extremely relevant to explore changes in vaginal Ph given what is known about the effects of Ph on absorption of drugs in the GI tract.

Finally, vaginal cytology may also affect absorption of hormone secreted from the NuvaRing. Similar to the uterus, vaginal cytology changes over the course of the menstrual cycle. Under the influence of estrogen, the vaginal wall thickens. It is further thickened progesterone so that it can, when under the influence of progestin for an extended period, become a barrier to absorption.

Long term use of the ring, could have profound effects on vaginal cytology. It is known, for example, that changes in uterine cytology occur with contraceptive use and that re-establishment of proper uterine cytology is the rate limiting factor in the return to fertility upon cessation of contraceptive use. The cytology of the reproductive tract, to include the vagina, is profoundly affected by synthetic estrogens and progestins such as those contained within the NuvaRing. However, changes in vaginal thickness have not been reported for NuvaRing. Given that the vagina itself is a known target for estrogens and progestins, and that these hormones have marked effects on the cytology of this structure in ways that could influence absorption of hormone, it seems prudent that an investigation of the influence of NuvaRing on vaginal cytology would be performed. However, the manufacturers did not perform such study either prior to or subsequent to FDA approval.

XI. Effects of progestins as a counterbalance to the prothrombotic effects of estrogens

It is well established that synthetic estrogens used in contraceptive preparations, such as the EE found in the Nuvaring, are prothrombotic, even when the doses are reduced, and especially when the estrogens are unopposed by the presence of a progestin. Increasing estrogenicity is associated with increased risk of venous thromboembolism (VTE). Although the major function of the progestin component of a hormonal contraceptive is inhibition of the neuroendocrine axis and ovulation, progestins also act to inhibit the prothrombotic effects of estrogen. It is clearly established that 2nd generation progestins are more effective than their 3rd generation counterparts in suppressing estrogenicity, thereby reducing the relative risk of VTE.

Recent studies have focused on the production of sex hormone binding globulin (SHBG) as a marker of relative estrogenicity, and thus VTE risk, of contraceptive preparations. Oral intake of EE alone results in a profound dose-dependent increase in SHBG. The concomitant presence of a progestin with the estrogen results in varying degrees of SHBG decrease, dependent on the dose and type of progestin, which can be expressed as differences in anti-estrogenic potency of various progestins (18–24). Therefore, the estrogenicity of a given combined estrogen/progestin contraceptive may be predicted by the effects on SHBG, which may be expressed as the sum of the *estrogenic* effect of EE and the *anti-estrogenic* effect of the progestin. Collectively, the available data also suggest that the timing of the presence of the progestin also contributes to the SHBG levels, and thus estrogenicity. Monophasic preparations, where EE and the progestin are present for the full 21 day period have decreased SHBG (estrogenicity) compared to bi-phasic and tri-phasic preparations, where the estrogen is sometimes present unopposed.

A cross-over study where all participants completed 2 cycles with NuvaRing, the Otho Evra Patch and Microgynon (an LNG containing oral contraceptive) indicated that NuvaRing use carried a higher resistance to APC, as well as higher SHBG levels, compared to the LNG-containing oral contraceptive. This study not only confirmed the clear association between individual changes in APCr and SHBG levels during treatment, but also confirmed that NuvaRing had a higher pro-thrombotic effect than a second generation oral contraceptive and the route of administration did not reduce this risk. The study further confirmed the use of SHBG as a marker for thrombotic risk of

all hormonal contraceptives. Finally, the study concluded that because of this increased risk of VTE, doctors were advised not to prescribe NuvaRing as a first choice in contraceptive.

In my opinion, there is yet another parameter present in the pharmacokinetic data from Clinical Trial 34218 that suggests the potential for increased estrogenicity in the NuvaRing. The pharmacokinetic profile of serum concentrations of EE and ENG in the NuvaRing demonstrate striking differences in Tmax, the time to reach the maximal concentration of hormone are vastly different for EE and ENG. As concluded in Clinical Trial 34218, for Nuvaring, the Tmax for EE is 2-3 days, whereas the Tmax for ENG is 8-9 days. Thus, progestin levels rise much more slowly compared to EE levels with NuvaRing use. Thus, the estrogenicity of the NuvaRing may actually not be constant over the course of a single round of therapy. The counerbalcing effect of the progestin is likely compromised until it reaches its peak 5 full days after the estrogen. These data suggest that estrogenicity and therefore VTE risk may be elevated even further on days 4-8 of use. Importantly, these differences in Tmax are not even considered by the manufacturer of the NuvaRing.

XII. Steady state delivery of combination hormones is not better.

The developers of transvaginal and transdermal pharmaceutical products often use the argument that these products will provide a better therapeutic approach because a more constant steady state, "homeostatic" level, of the drug will allow for a decreased overall drug exposure leading to fewer side-effects. Despite the wide-spread use of this theory, there is, in fact, little scientific evidence to support this claim. Within human physiology, there are very few incidences where systemic hormones exist in a constant steady state. Rather, the physiology of humans, as well as most other organism that exist on this planet, is expressed as a function of time. Biological events ranging from gene expression to human behavior, fluctuates with a near 24 h oscillation that is described as circadian rhythms. Although long recognized as a function of life, the role of circadian rhythms in health and disease, and in particular relevance to this report, in pharmacology, is only beginning to be appreciated. Biological circadian rhythms contribute to the occurrence of serious and life-threatening medical events, like myocardial infarction and stroke, and the manifestation and severity of symptoms of chronic diseases, like allergic rhinitis, asthma, and arthritis. Moreover, body rhythms can significantly affect responses of patients to diagnostic tests and drugs. Rhythmicity in disease pathophysiology and a desire to control adverse effects in medications where the therapeutic window is narrow of disease by administering drugs at the time of day when they can be most effective provides a basis for considering chronobiology in the development of drugs. For many drugs, the time of day of administration is very important. Recent studies indicate that administration of theophylline for asthma, for example, is more effective when the drug is given at night. Thus, steady state infusion of drugs, while commonly cited as better, may not, in fact be based on a firm scientific foundation. Time of day can influence the responsiveness of the human body to a drug. So why have the drug present when the body is not able. The idea of steady state delivery of drugs is long overdue for a re-evaluation and should not be, by any reasonable scientist, taken as a proven fact. Much more research is required before this blanket statement can be blindly applied.

XIII. Conclusion – NuvaRing carries a higher risk than second generation contraceptives; there are safer alternatives.

After a thorough review of the available data, it is my opinion that the vaginal contraceptive, NuvaRing, carries a higher risk for the development of serious adverse side effects, including VTE, than other, more well-established contraceptives. The reasons for this conclusion are delineated below.

1. NuvaRing is a 3rd generation progestin, which carries a higher risk for the occurrence of thromboembolic events. To my knowledge, the only available studies to indicate that 3rd generation progestins are as safe as 2nd generation products were all performed by the pharmaceutical company responsible for developing the product. Independent studies indicate that the use of 3rd generation progestins, in combination with an estrogen, carry a 2-fold elevated risk for the occurrence of thromboembolic events. 3rd generation progestins are characterized by their high affinity for progesterone receptor and, importantly, their low androgenicity. Although developed to decrease untoward side effects associated with the androgenicity of 2nd generation progestins, this lack of androgenicity decrease the ability of these compounds to counterbalance the effects of estrogens on production of clotting factors. A cross-over study where all participants completed 2 cycles with NuvaRing, the Otho Evra Patch and Microgynon (an LNG containing oral contraceptive) indicated that NuvaRing use carried a higher resistance to APC, as well as higher SHBG levels, compared to the LNG-containing oral contraceptive. This study not only confirmed the clear association between individual changes in APCr and SHBG levels during treatment, but also confirmed that NuvaRing had a higher pro-thrombotic effect than a second generation COC and the route of administration did not reduce this risk. The study further confirmed the use of SHBG as a marker for thrombotic risk of all hormonal contraceptives. Finally, the study concluded that because of this increased risk of VTE, doctors were advised not to prescribe NuvaRing as a first choice in contraceptive.

2. The variability in the delivered dose of EE demonstrated in Clinical Trial 34218 suggests that further pharmacokinetic testing regarding EE profiles and its effects should have been conducted. Given the fact that NuvaRing is a novel product and that delivery of hormones vaginally causes increased variability, there was insufficient testing performed. No studies were performed to determine the most effective dose of EE. Studies were not performed to account for variability that could have been introduced by inherent variability in the vaginal environment. Furthermore, Clinical study 85012, performed on silastic forms of the device (not the actual NuvaRing) show less variability and no apparent burst effect, although there were fewer data points collected, suggesting that the carrier selected for the final NuvaRing product may contribute to variability in delivery.

3. The "burst" effect observed in serum EE levels is a cause for concern. Elevated levels of estrogen are known to increase risk of VTE. The data clearly demonstrate that a

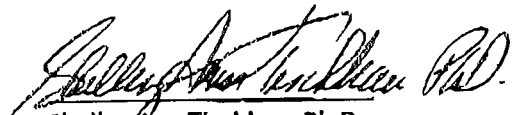
substantial subset of women using NuvaRing in the pharmacokinetic trials experienced as rapid increase in serum EE on the first day of use. Furthermore, the Tmax for EE was 2-3 days as reported in the Clinical Trial summary. In contrast, Tmax for ENG occurred 5 days later. In my opinion, this leaves a significant time frame where estrogen may be unopposed by progestin, which could contribute to the development of VTE in certain women. With long-term use, these women will experience 5 days each month where the increased risk for VTE is present. At the very least, this "burst" effect requires further investigation.

4. Due to the variability of the PK parameters among the Clinical Trial subjects, NuvaRing is more pro-thrombotic in certain women than an oral dose of the same progestin and estrogen, and such variability is not explained or adequately tested in the Clinical Trials.

It is my opinion that the product label fails to provide accurate and complete data concerning important data from clinical trials and the label is misleading to prescribing healthcare professionals.

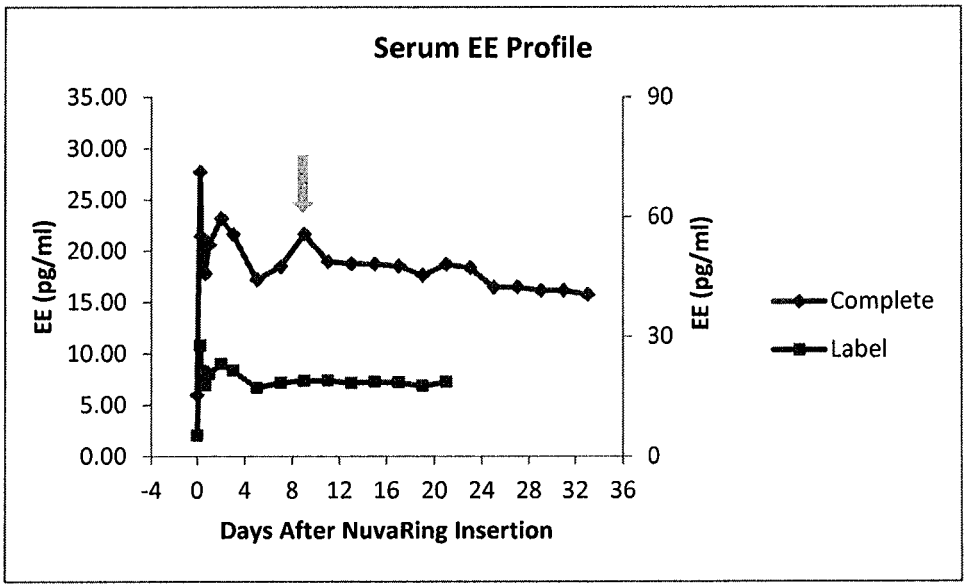
It is my opinion that NuvaRing was not tested and studied adequately – (1) There was no attempt made to determine cause of the "burst effect" seen in Study 34218. Moreover, such "burst effect" was not seen in Study 85012 with the Silastic Ring, implying that the type of delivery system is responsible.

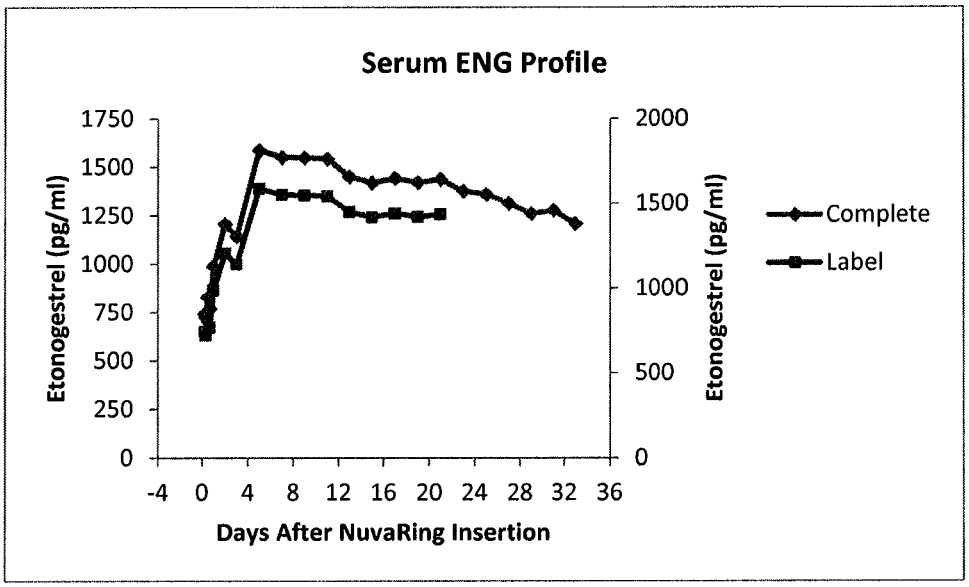
Additional opinions are provided throughout the report as well. All of my opinions are expressed within a reasonable degree of scientific certainty. I reserve the right to supplement my report with any additional materials that I may become aware of in the future.



Shelley Ann Tischkau, Ph.D.

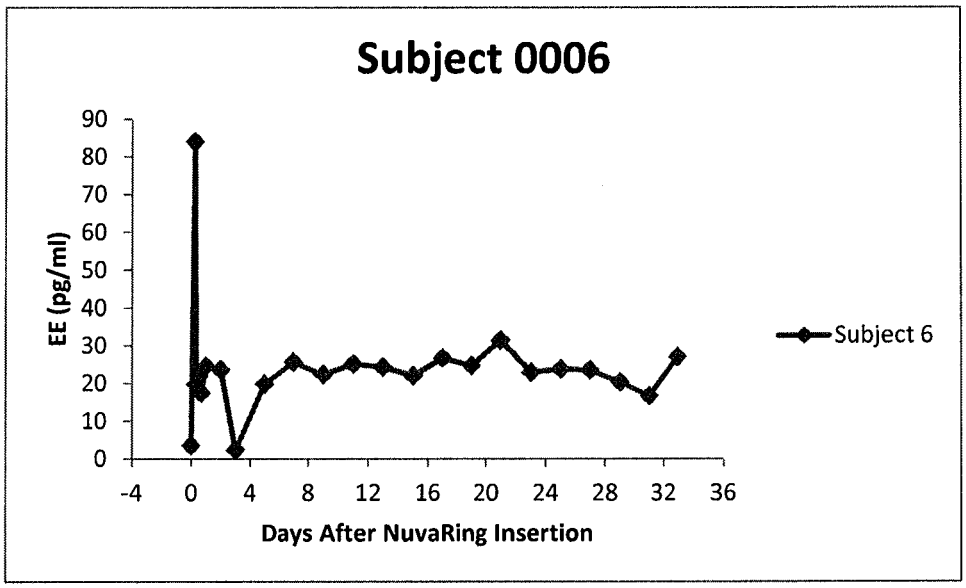
APPENDIX 1

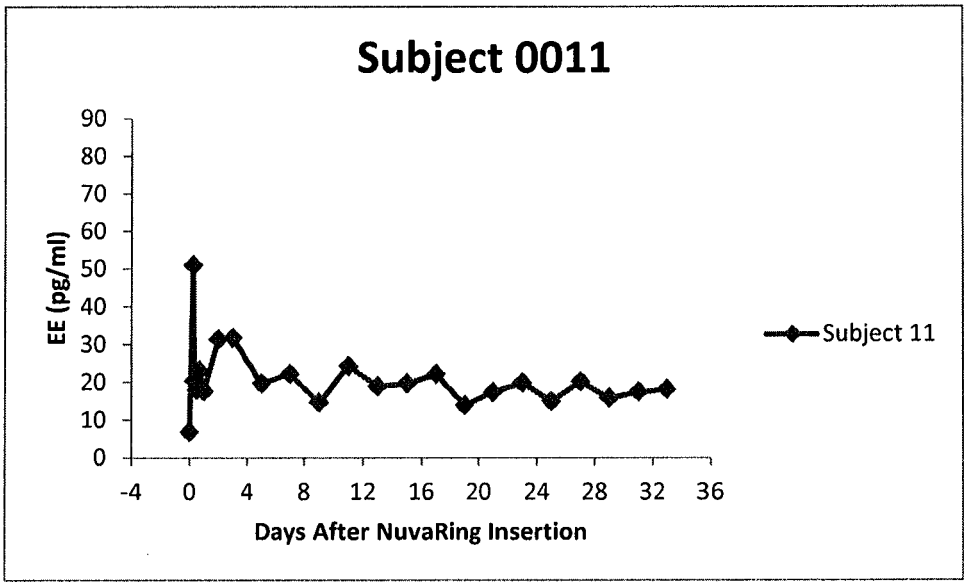




Data for serum EE (upper panel) and ENG (lower panel) as shown on the product label in comparison to the actual data from Clinical Trial 34218. Orange arrow in upper panel indicates where the exclusion of a data point alters the appearance of the profile for EE to mask an increase that occurs midcycle.

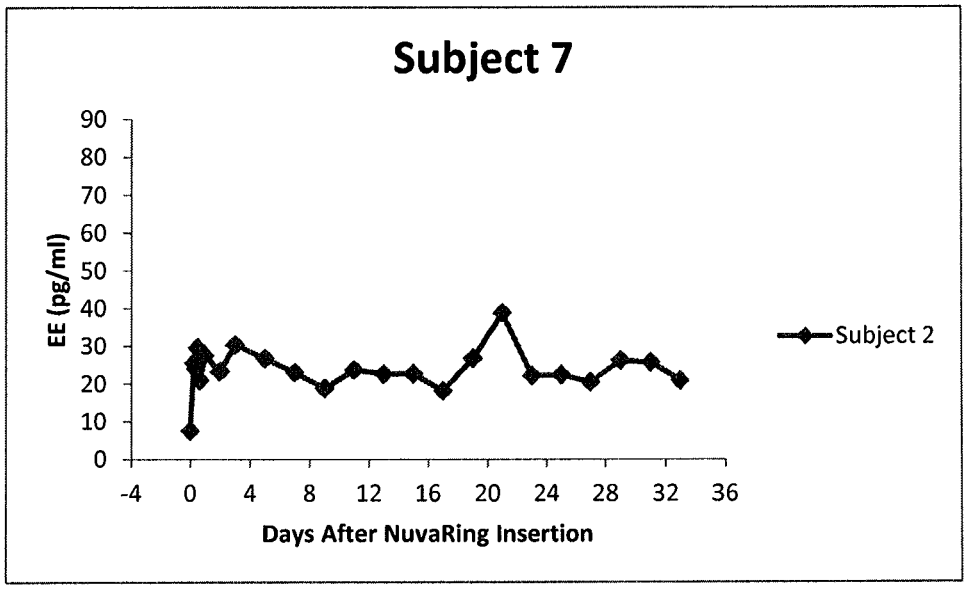
APPENDIX 2

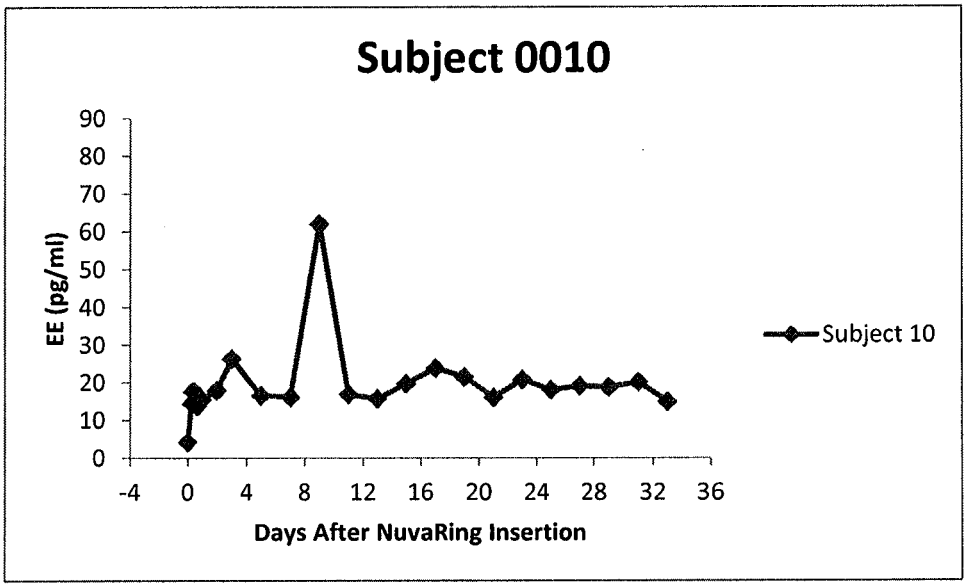




Serum EE profiles for 2 Subjects that experienced "burst' effects of EE.

APPENDIX 3





Serum EE profiles for 2 Subjects that experienced unexplained surges in EE during NuvaRing use.

Appendix A

Materials Considered by Dr. Shelley Tischkau

In addition to the materials specifically referenced in my report, the other materials I have considered are:

Literature

Editorial: Sex Hormone Binding Globulin: Inhibitor or Facilitator (or Both) of Sex Steroid Action? The Journal of Clinical Endocrinology & Metabolism; 2006 91(12) 1764-1766.

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Kemmeren, Third generation oral contraceptives and risk of venous thrombosis: meta-analysis, 2001 BJM, 323:131-34.

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Lidegaard, Hormonal contraception and risk of venous thromboembolism: national follow-up study, 2009 BMJ, 339, 2890:1-8.

Lidegaard, Oral contraceptives and the risk of venous thrombosis, New England Journal of Medicine, 2002 344:1527-37.

Lidegaard. Oral contraceptives and venous thromboembolism: a five-year national case-control study, Contraception 65 (2002) 187–196

Lidegaard. Oral Contraceptives and thrombosis, Acta Obstetricia et Gynecologica Schandinavica. 1999 78:142-49.

Lidegaard. Oral Contraceptives and Venous Thromboembolism. Contraception 1998; 57:291–301

Mulders. Pharmacokinetics of etonogestrel and ethinylestradiol released from a combined contraceptive vaginal ring. 2000 (Published article re: Study 34218).

NuvaRing (Etonogestrel/Ethinyl Estradiol Ring) Human Pharmacokinetic and Bioavailability Summary

Odlind; Can changes in sex hormone binding globulin predict the risk of venous thromboembolism with combined oral contraceptive pills? Acta Obstricia ct Gynecologica Scandinavica; Human Reproduction; 2002 81:482-490.

Rad, Comparative effects of a contraceptive vaginal ring delivering a nonandrogenic progestin and continuous ethinyl estradiol and a combined oral contraceptive containing levonorgestrel on hemostatis variables, American Journal of OBGYN, 2006 195.

Rosing, Oral contraceptives and venouts thrombosis: different sensitivities to activated protein C in women using second-and third-generation oral contraceptives; British Journal of Haemotology; 1997 97:233-238.

Roumen. Comparison of uterine concentrations of ethinyl estradiol and etonogestrel after use of a contraceptive vaginal ring and an oral contraceptive. American Society for Reproductive Medicine, Vol. 85, No. 1 2006.

Sitruk-Ware, New progestagens for contraceptive use, Human Reproductive Update, 2005 12 No. 2, 169-78.

Spitzer, W.O., Third generation oral contraceptives and risk of venous thromboembolic disorders: an international case-control study. 1996 BMJ, 312:8388

Suissa et al., First-time use of newer oral contraceptives and the risk of venous thromboembolism, Contraception, 1997 56:141-146.

Tans, A randomized cross-over study on the effects of levonorgestrel- and desogestrel containing oral contraceptives on the anticoagulant pathways, 2000 Thromb Haemost, 84:15-21.

Tans, Activated protein C resistance determined with thrombin generation-based test predicts for venous thrombosis in men and women; 2002 122:465-470.

Tchaikovski, Mechanisms of Estrogen-induces venous thromboembolism; Thrombosis Research. 2010

Van Vliet, Association between sex hormone-binding globulin levels and activated protein C resistance in explaining the risk of thrombosis in users of oral contraceptives containing different progestogens. 2005

Van Vliet, Letters to the Editor, Effects of the contraceptive vaginal ring, the contraceptive transdermal patch and combine oral contraceptives on markers of hemostasis; Contraception, 2010 81:88-90.

Van Vliet, Letters to the Editor, Sex hormone-binding globulin: an adequate surrogate marker for venous thromboembolism in women using new hormonal contraceptives; Contraception, 2009 79:328-330.

Vanderbroucke, Oral contraceptives and the risk of venous thrombosis, New England Journal of Medicine, 2001 344:1527-35.

Vlieg, The venous thrombotic risk of oral contraceptives, effects of oestrogen dose and progestogen type: results of the MEGA case-control study, 2009 BMJ, 339, 2921:1-8.

Wihelmus. Study Article No. 34237: Comparison of ethinylestradiol pharmacokinetics in three hormonal contraceptive formulations: the vaginal ring, the transdermal patch and an oral contraceptive. 2005

Other Documents

Clinical Pharmacology Summary

Clinical Trial No. 34218, Appendices

Clinical Trial No. 34225, Appendices

Clinical Trial No. 34226, Appendices

Clinical Trial No. 34227, Appendices

Clinical Trial No. 34228, Appendices

Clinical Trial No. 34232, Appendices

Clinical Trial No. 34233, Appendices

Clinical Trial No. 34234, Appendices

Clinical Trial No. 34235, Appendices

Clinical Trial No. 34237, Appendices

Clinical Trial No. 85012, Appendices

Clinical Trial No. 86016, Appendices

1999 Expert Report on the Clinical Documentation of NuvaRing (Combined Contraceptive Vaginal Ring, Org 37681; 12-08-99 ORG1650733-ORG1650787)

1999 Draft Expert Report on the Clinical Documentation of NuvaRing (ORG1650733-ORG1650787)

2006 Expert Report on the Clinical Documentation of NuvaRing

2006 Appendix A- To the Expert on the Clinical Documentation of NuvaRing (ORG0445254-ORG0445360)

In-vivo/in-vitro correlation for NuvaRing, ORG1049753

Medical Officer's Review 10-6-00

Medical Officer's Review, Addendum to 12-18-00

NuvaRing Label (2001)

NuvaRing Package Insert (2008)

NuvaRing Patient Information Package Insert 10-2007

NuvaRing Physician Information Package Insert 10-2007

SDG Release Report No. 2096. Pharmacokinetics of ORG 3236 and ethinylestradiol released from different types of contraceptive vaginal rings, in comparison with an oral preparation containing 150mg Org 2969 and 30mg ethinylestradiol. 10-14-87 ORG0958857-ORG0958914

Vertrouwelijk: SDG Release Report No.2059. An open comparative study to evaluate the contraceptive properties of two vaginal rings containing different amounts of Org 3236 (3-keto desogestrel) plus ethinylostradiol versus an oral preparation containing 0,150 mg Org 2969 (desogestrel) plus 0,030 mg ethinyloestradiol, in healthy female volunteers (Dr. D. Apter, Dr. U-H Stenman-Finland); 8-21-87 NDA-0018041-NDA-0018201

Vertrouwelijk: SDG Release Report No. 2060. An open comparative study to evaluate the contraceptive properties of a vaginal ring with a daily in vitro release rate of 0, 100 mg Org 3236 (3-keto desogestrel) plus 0, 015 mg ethinyloestradiol versus an oral preparation containing

0,150mg Org 2969 (desogestrel) plus 0, 030 mg ethinyloestradiol in healthy female volunteers. (Dr. D. Apter, Dr. U-H Stenman-Finland); 8-21-87 NDA-0018202-NDA-0018337

Deposition Testimony

1. Deposition transcript of Dr. A.P. (Tom) Sam, Exhibits 1-10, Defense Exhibits 1-2
2. All deposition transcripts for Dr. Mulders

SHELLEY ANN TISCHKAU, PhD

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Mechanicsburg, IL 62545
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Southern Illinois University School of Medicine
801 N. Rutledge Rm 3333
P.O. Box 19629
Springfield, IL 62794-9629
Phone: (217) 545-6524
e-mail: stischkau@siumed.edu

EDUCATION:

May 1995 Ph.D. Physiology. University of Illinois at Urbana-Champaign, Urbana, IL
Thesis Title: Physiology of the Germinal Disc Region of Preovulatory Follicles in the Chicken Ovary
Advisor: Janice M. Bahr, Ph.D.

May 1991 M.S. Physiology. University of Illinois at Urbana-Champaign (UIUC), Urbana, IL
Project: Mechanisms of Sexual Dimorphism in the Non-genomic Actions of Progesterone in the Rat Brain
Advisor: Victor D. Ramirez, M.D.

May 1989 M.S. Biology. Truman State University, Kirksville, MO
Thesis Title: Characterization of Transepithelial Molecular Transport of L-arginine and L-leucine by Gut Segments of the Pacific Hagfish, *Eptatretus stoutii*.
Advisor: George J. Schulte, Ph.D.

May 1986 B.S. Biology. Truman State University, Kirksville, MO
Honors: Magna cum laude

POSTGRADUATE EDUCATION AND TRAINING:

Sept. 1995-1999 Postdoctoral Fellow. University of Illinois at Urbana-Champaign
Advisor: Martha U. Gillette, Ph.D.

PROFESSIONAL APPOINTMENTS:

Southern Illinois University School of Medicine

Assistant Professor, Department of Pharmacology, 2007-present. Position is 50% research, 40% teaching, 10% service.

- Unit Director, 2nd Year medical curriculum, 2009-present. Oversee and direct the Endocrine-Reproduction-Gastrointestinal block curriculum for the 2nd year medical students.

Adjunct Assistant Professor, Department of Medical Microbiology, Immunology and Cell Biology, 2010-present.

University of Illinois at Urbana-Champaign

Assistant Professor, Department of Veterinary Biosciences, 2001-2006. Position was 70% research, 30% teaching and service

- Co-Director, Program in Translational Biology and Medicine, College of Veterinary Medicine, 2005-2006. Directed the seminar series in translational biology. Developed curriculum for the graduate program.
- Faculty Affiliate, Neuroscience Program, 2001-2006.
- Core Faculty Member, Interdisciplinary Environmental Toxicology Program, 2003-2006
- Core Faculty Member, Reproductive Biology Training Program, 2004-2006

Visiting Assistant Professor, Department of Animal Sciences, 1999-2001.

Research Assistant Professor, Department of Cell and Structural Biology, 2000-2001.

Postdoctoral Fellow, Department of Cell and Structural Biology, 1995-1999.

Predoctoral Fellow, Department of Molecular and Integrative Physiology, 1991-1995.

Graduate Teaching Assistant, School of Life Sciences, 1989-1991

Parkland College

Instructor, Department of Health and Physical Education, Parkland College, Champaign, Il, 1992-1996.

Assistant Volleyball Coach, Parkland College, 1992-1995.

Truman State University

Graduate Teaching Assistant, Division of Science, 1986-1989.

TEACHING EXPERIENCE:

Southern Illinois University School of Medicine

Medical Curriculum Teaching:

Toxicology (Lecture), 2007-present
Nutrition and Obesity (Integrated Resource Session), 2008-present
Fertility and Contraception (Lecture), 2008-present
Pregnancy (Integrated Resource Session), 2007-present
Abnormal Uterine Bleeding (Integrated Resource Session) 2007-present
Tutor for ERG Unit (2008-present)

Pharmacology Graduate Curriculum Teaching:

Pharm 545 Toxicology

Course co-coordinator, 2008-present
Lecture on Reproductive Toxicology, 2008-present
Pharm 550a Principles of Pharmacology
Lecture of Principles of Toxicology, 2007-present
Pharm 550b Principles of Pharmacology
Lecture on Female Reproductive Pharmacology, 2007-present
Lecture on Oral Contraceptives, 2007-present
Lecture on Pregnancy and Parturition, 2007-present
Lecture on Obesity and Metabolic Syndrome, 2010-present
Pharm 574 Advanced Neuropharmacology
Lecture on Descending Neural Pathways, 2007
Lecture on Circadian Rhythms, 2007
Lecture on Cell Biology Principles, 2010
Course co-coordinator
MBMB 530 Advanced Cell Biology
Lecture on Regulation of Transcription, 2007-present

Department of Medical Education
Problem Based Learning Workshop, Faculty, 2011

Other Teaching:
University of Illinois at Springfield Course
Lecture on Reproductive Pharmacology (2007-2010)

University of Illinois at Urbana-Champaign
Veterinary Medical Curriculum Teaching:
VB610 Veterinary Neurobiology, course coordinator (2001-2006)
VB610 Veterinary Neurobiology (2001-2006)
Course coordinator
32 Lectures, Neuroanatomy, Developmental Neurobiology, Neurophysiology, Muscle Physiology
Lab coordinator, Neuroanatomy lab
VB605 Veterinary Histology (2003-2006)
6 lectures/labs on Embryology

Graduate and Undergraduate Teaching:
PHYSL510 Advanced Topics in Reproductive Biology (2003)
Course coordinator
PHYSL455 Advanced Reproductive Endocrinology (1999-2001)
Course coordinator
Taught all lectures
PHYSL301 Cell and Membrane Physiology (1999-2000)
6 lectures on Nervous System Physiology
PHYSL402 Comparative Animal Physiology (1998-2001)
6 Lectures on Comparative Endocrinology
PHSYL199 Reproductive Biology Discovery Course (1999-2007)
2 lectures on Neuroendocrinology
BIOL120 The Cell (Honor's Biology) (1989-1990)
Teaching Assistant – prepped and taught all labs
BIOL122 The Organism (Honor's Biology) (1990-1991)

Teaching Assistant – prepped and taught all labs
BIOL110 Principles of Biology II (1991)
Teaching Assistant – taught all labs
PHYSL101 Introduction to Human Physiology (1992)
Teaching Assistant – taught all labs, tutor group

Truman State University
Undergraduate and Graduate Teaching:
BIOL101 Introductory Biology (1987-1988)
Taught all lectures and labs
CHEM101 Introductory Chemistry (1989)
Taught all lectures and labs
BIOL315 Human Physiology (1985-1988)
Teaching Assistant – prepped and taught all labs
BIOL330 Embryology and Developmental Biology (1986-1989)
Taught 10 lectures, prepped and taught all labs

MEMBERSHIPS IN SCIENTIFIC SOCIETIES:

American Association for the Advancement of Science (1990-present)
Sigma Xi (1992-present)
Society for the Study of Reproduction (1993-present)
Abstract Reviewer (2001-2002)
Platform Session Chair - Follicular Development (1994)
Mini-Symposium Co-Chair (2005)
By-laws committee (2003-2006)
Society for Research in Biological Rhythms (1996-present)
Executive Board (ex officio, 2010-present)
Newsletter Editor (2008-present)
Program Committee (2008, 2010)
Bi-Annual Meeting Facilities Chair (2008, 2010)
Communications Committee (2007-present)
Annual Meeting Session Chair – SCN (2006)
Women in Neuroscience (2001-present)
Society for Neuroscience (1996-present)
Endocrine Society (1993-present)
Society of Toxicology (2005-present)
American Society for Pharmacology and Experimental Therapeutics (2008-present)
International Association of Medical Science Educators (2009-present)

HONORS AND AWARDS:

College of Veterinary Medicine Helen and Norman Levine Research Award Finalist (2005)
College of Veterinary Medicine Norton Teaching Award Finalist (2005)
Membership in UIUC College of Veterinary Medicine Teaching Academy (2004-2006)

U of I List of Teachers Ranked Excellent by their Students (1989-1991, 1999-2006)
ISCAVMA Teaching Excellence Award (2004, 2006)
NIH Postdoctoral Fellowship, Individual National Research Service Award (1998-1999)
NIH Postdoctoral Fellowship, Individual National Research Service Award (1996-1998)
U of I Graduate College Travel Award (1994, 1995)
Constance Campbell Research Award (1993, 1994)
NIH Predoctoral Fellowship, Systems and Integrative Biology Training Grant (1993-1994)
NIH Predoctoral Fellowship, Reproductive Biology Training Grant (1991-1993)
Outstanding College Students of America (1987)
Truman State University (TSU) President's Honorary Scholarship (1982-1986)
Dean's List 8 semesters (1982-1986)
TSU Wray Reiger Award, Outstanding Student in Biology (1984)
Beta Beta Beta Biological Honor Society (1984-1986)
Cardinal Key National Honor Sorority (1984-1986)
TSU Varsity Athletic Scholarship (1982-1986)
TSU Varsity Volleyball Letter Winner (1982-1986)

ADMINISTRATIVE SERVICE-INSTITUTIONAL SERVICE:

Southern Illinois University

School of Medicine

Year 2 Curriculum Committee (2007-present)
- USMLE Step 1 Subcommittee (2010-present)

Laboratory Animal Care and Use Committee (2007-present), Vice Chair (2010-2011), Chair (2011-present)
- LACUC Policy Review Subcommittee (2009 and 2011)

Department of Pharmacology

Graduate Program Committee (2007-present)
Seminar Program Coordinator (2008-present)
Annual Pharmacology Awards Selection Committee (2010)
LAN Manager Search Committee (2011)

University of Illinois at Urbana-Champaign

University

Post-genomics Initiative Faculty Search Committee (2002, 2003)
Faculty Senate (2004-2006)
HHMI Translational Research Initiative Committee (2005)

College of Veterinary Medicine

Admissions Committee (2005-2007)
Executive Committee (2005-2007)
Comparative and Translational Research Program Steering Committee (2005-2007)
Research Advisory Committee (2005-2006)
Department of Veterinary Biosciences Head Search Committee (2005)
Faculty Search Committee, Anesthesiology, Dept. of Clinical Medicine (2006)
Blue Coat Ceremony, Dept. of Veterinary Biosciences Representative (2004-2006)

White Coat Ceremony, Class of 2007
Committee on Committees (2003)
College of Veterinary Medicine Teaching Academy (2004-2007)

Department of Veterinary Biosciences
Courses and Curriculum Committee (2003-2006), Chair (2005-2006)
Anatomy Preparator Search Committee (2005)
Seminar Committee for Fields Program in Reproductive Biology (2003-2007)
Faculty Search Committee (2006)

Neuroscience Program
Executive Committee (2005-2006)
Graduate Admissions Committee (2003-2004)

Reproductive Biology Training Program
Discovery Course Committee (2005-2007)
Graduate Student Association President (1993-1995)

Truman State University
Beta Beta Beta Biological Society, President (1985-1986)
Cardinal Key National Honor Sorority, Secretary (1985-1986)

ADMINISTRATIVE SERVICE-LOCAL AND NATIONAL:

Grant Review Panels
Member, NSF Review Panel (Neural Systems Cluster) (2009-present)
Reviewer, NIH Challenge Grants (2009)
Reviewer, *ad hoc,* NIH Review Panel (ZRG F03A) (2005-2006)
AAAS, Reviewer for competitive grants program (2001-present)
Lalor Foundation, Reviewer for postdoctoral fellowship program (2002-2008)
UIUC Environmental Toxicology Scholars Review Panel (2005-2006)
SIU-CRC Excellence in Academic Medicine ad-hoc grant reviewer (2007-present)
SIU-CRC Near-miss Award ad-hoc reviewer (2007)

Journals and Books
Journal of Neuroscience, Reviewer
Experimental Neurology, Reviewer
European Journal of Neuroscience, Reviewer
Biology of Reproduction, Reviewer
Sleep Research Online, Reviewer
Journal of Comparative Endocrinology, Reviewer
Physiology & Behavior, Reviewer
Physiological Genomics, Reviewer
American Journal of Physiology, Reviewer
Endocrinology, Reviewer
Journal of Biological Rhythms, Reviewer

Chronobiology International, Reviewer
Neurobiology of Disease, Reviewer
General and Comparative Endocrinology, Reviewer
Brain Research, Reviewer
Journal of the American Association of Laboratory Animal Science, Reviewer
Journal of Circadian Rhythms, Reviewer
Molecular and Cellular Endocrinology, Reviewer
Pharmacology, Biochemistry and Behavior, Reviewer

Other Review Panels

Society for the Study of Reproduction, Abstract Reviewer, Ovary (2003)
Northwestern Reproductive Biology Mini-symposium, Student Presentation Judge (2004-2005)
Southern Illinois University School of Medicine Trainee Research Symposium, Judge (2007)
Society for Research on Biological Rhythms, Trainee Research Day, Abstract Reviewer (2008, 2010)

GRADUATE AND UNDERGRADUATE RESEARCH INSTRUCTION:

PhD Students:

2002-2007	Jason Hickok, Dept of Cell & Developmental Biology, UIUC Project: Gene Expression Profiling in Native GnRH Neurons
2003-2006	Motoko Mukai, Dept of Veterinary Biosciences, UIUC Project: Role of Aryl Hydrocarbon Receptor in Circadian Rhythms
2007-present	Sumedha Karmarkar, Dept of Pharmacology, SIU-SOM Project: Role of Mitogen Activated Proteins Kinases in Endogenous Neuroprotection of Suprachiasmatic Nucleus Neurons
2010-present	Mingwei Sun, Dept of Pharmacology, SIU-SOM Project: Rev-erbα as a Mediator of the Circadian Regulation of Metabolism
2010-present	Cassie Jaeger, Dept of Pharmacology, SIU-SOM Project: Interactions of Sleep Fragmentation and Obesity in the Induction of Endoplasmic Reticulum Stress

Master's Degree Students:

1999-2001	Julie Pendergast, Program in Biology, UIUC Project: Protein Kinase G II Expression in the Suprachiasmatic Nucleus
2007-2009	Kanchan Paradkar, Department of Pharmacology, SIU School of Medicine Project: Regulation of EMT-6 Cell Growth by Melatonin and Sleep Fragmentation

Undergraduate Students:

2001-2002	Kirsten Holthusen (UIUC, Animal Sciences, BS 2002)
2001-2002	Alexander Landers (UIUC, Bioengineering, BS 2002)
2001-2003	Claire Zimmerman (UIUC, Animal Sciences, BS 2003)
2002-2004	Jared Cohen (UIUC, Molecular and Cellular Biology, BS 2004)
2002-2003	Kelly Barry (UIUC, Animal Sciences, BS 2003)
2003-2005	Benjamin Haley (UIUC, Honor's Biology, BS 2005)

2003-2005 Jackie Kanoon (UIUC, Animal Sciences, BS 2005)
2003-2005 Mia Layne (UIUC, Molecular and Cellular Biology, BS 2005)
2004-2005 Jennifer Howell (UIUC, Animal Sciences, BS 2005)
2003-2005 Emily Poon (UIUC, Molecular and Cellular Biology, BS 2005)
2005-2007 Kara Escutia (UIUC, Animal Sciences, BS 2007)
2005-2007 Divya Chandiramani (UIUC, Molecular and Cellular Biology, BS 2008)
2006-2007 Jessica Anderson (UIUC, Molecular and Cellular Biology, BS 2009)
2007 Dana Armour (Illinois Wesleyen University, BS 2007)
2008 Caitlin Allen (Illinois College, BS 2010)
2008-2009 Cassie Jaeger (Millikin University, BS 2010)
2009 Leigh Groszek (University of Illinois at Springfield, BS 2011, expected)
2010 Susan Coryell (University of Illinois at Springfield, BS, 2011, expected)
2011 Natalie Allen (Illinois College, BS 2012, expected)

UIUC Summer Research Opportunities Program:

Jared Cohen (UIUC, 2002)
Eliza Salvo (Purdue University, 2003)
Lucius Jerome Hales (Jackson State University, 2004)
Aurora Cruz-Torres (University of Puerto Rico, 2005)
Kara Escutia (University of Illinois, 2005)
Pilar Silva (University of Puerto Rico, 2006)

THESIS COMMITTEES, MS and PHD:

Sabra Abbott (Molecular & Integrative Physiology, UIUC, PhD 2005)
Project: Cholinergic Regulation of Sleep Centers and the Circadian Clock
Jamie Stark (Veterinary Biosciences, UIUC, PhD 2006)
Project: Physiological Implications and Mechanisms of Estrogen Protection Associated with Myocardial Ischemia/Reperfusion Injury
Archana Koosuru (Veterinary Biosciences, UIUC, MS 2005)
Project: Effects of Resvertrol on Uterine Growth and Development
Christopher Zugates (Cell & Structural Biology, UIUC, PhD 2007)
Project: Studies of Post-Embryonic *Drosophila* Brain Development
Laura Pace (Neuroscience Program, UIUC, PhD 2007)
Project: Role of Protein Kinase G Signaling in the Suprachiasmatic Circadian Clock
Sufang Wang (Cell & Structural Biology, UIUC, PhD 2008)
Project: Role of Atypical Protein Kinase C in Light Signaling and Cytoskeletal Rearrangement in the Suprachiasmatic Nucleus
Lilia Zurkovsky (Neuroscience Program, UIUC, PhD 2009)
Project: Estrogen Modulation of Place Learning through Estrogen Receptors in the Hippocampus
Molly Kent (Neuroscience Program, UIUC, PhD 2009)
Project: Estrogenic Effects on Spatial Learning Tasks Involves Multiple Memory Systems
Patty Kandelapas (Neuroscience Program, UIUC, PhD 2009)
Project: Molecular Mechanisms of Melatonin Action on the Rat Suprachiasmatic Nucleus: an *In Vitro* Analysis
Stephen Johnson (Pharmacology, SIU-SOM, PhD 2009)

Project: A Biochemical and Pharmacological Characterization of a Novel Neuroactive Peptide from the Neotropical Hunting Ant *Dinoponera australis*

Joe Jeffry (Pharmacology, SIU-SOM, PhD 2009)
Project: Modulation of Synaptic Transmission at the Trigeminal Nucleus Caudalis by TRPV1

Jacob Neumann (Pharmacology, SIU-SOM, PhD 2011)
Project: Modulation of Ryanodine Receptors in Cardiac Muscle

Bethany Karman (Comparative Biosciences, UIUC, PhD, expected 2012)
Project: The Role of the Aryl Hydrocarbon Receptor in Ovarian Follicle Development

Xuejing Zhang (Medical Microbiology, Immunology, Cell Biology, SIU-SOM PhD expected 2012)
Project: Role of Thromboxane A2 Receptor in Breast Cancer

Bopanna Kalappa (Pharmacology, SIU-SOM, PhD expected 2012)
Project: Modulation of α7 Nicotinic Acetylcholine Receptors in the Hippocampus

Tejbeer Kaur (Pharmacology, SIU-SOM, PhD expected 2012)
Project: Role of STAT1 Signaling in Cisplatin-induced Hearing Loss

Jamila Hangadoumbo (Med Micro, Immunology, Cell Biology, SIU-SOM, PhD expected 2013)
Project: Role of Pregnane X Receptor in Breast Cancer

Sandeep Sheth (Pharmacology, SIU-SOM, PhD expected 2013)
Project: Signal Transduction Mechanisms downstream of TRPV1 in cancer cells

Mruvil Abooj (Pharmacology, SIU-SOM, PhD expected 2014)
Project: TRP Receptors in Neuropathic Pain

Rebecca Brockman (Comparative Biosciences, UIUC, PhD expected 2015)
Project: Circadian rhythmicity in Aromatase Knockout Mice

GRANT SUPPORT (Principal Investigator unless otherwise noted):

Completed Support

NS10170-01 NIH, National Research Service Award. "Role of cGMP in the Circadian Clock", 1996-1998. Postdoctoral fellowship awarded to examine the role of cGMP and protein kinase G signal transduction mechanisms in light-induced phase resetting of the circadian clock. Proposal score was in the first 1%.

NS10170-03 NIH, National Research Service Award, Postdoctoral Fellowship. "Role of cGMP in the Circadian Clock", 1998-1999. Competitive renewal of postdoctoral fellowship awarded to examine the role of the circadian clock gene, *timeless*, in light-induced phase resetting of the circadian clock.

NS22155, Co-Investigator, NIH RO1 "Physiological Substrates of a Circadian Oscillator" (Martha Gillette, PI), 2000-2004. Awarded to examine interactions of PACAP and glutamate as mediators of light-induced phase resetting of the circadian clock.

UIUC College of Veterinary Medicine Governor's Venture Technology Fund. "Circadian Control of Gonadotropin Releasing Hormone". 2001. $25,000 awarded to examine interactions of SCN neurons with the hypothalamic gonadotropin-releasing hormone neurons.

UIUC College of Veterinary Medicine Governor's Venture Technology Fund. "Circadian Control of Gonadotropin Releasing Hormone". 2002. $10,000 extension of previous award.

UIUC College of Veterinary Medicine Governor's Venture Technology Fund – Molecular Pharmacology Program. 2002. "Development of Single-Cell Microarray Technology". $7,000 awarded to develop technology to perform DNA microarray analysis from single mammalian neurons.

UIUC Research Board. "Endogenous Protection from Ischemic Brain Insult". 2002-2003. $25,000 awarded to build a system to analyze circadian rhythmicity *in vivo*.

UIUC College of Veterinary Medicine, Veterinary Medical Research Funds. "Transcriptional Analysis of GnRH Neurons". 2004-2005. $30,000 awarded to perform microarray analysis on GnRH neurons.

Field Endowment, College of Veterinary Medicine. Transcriptional Analysis in the Ovary of the *Clock* Mutant Mouse. 2003-2004. $6,000 awarded to perform microarray analysis on ovaries from *Clock* mutant mice.

USDA HATCH FUNDS "Luteinizing Hormone Regulation of Circadian Clock Genes in the Avian Ovary". 2005-2006. $20,000 awarded to examine circadian clock gene function in the avian ovary.

NIH ES07326-01 Training Program in Endocrine, Developmental and Reproductive Toxicology. 2005-2010. $1,399,352. Role: Preceptor (15 total faculty members)

ES012948, NIH R21, "Environmental Toxins Disrupt Ovarian Circadian Rhythms". 2005-2007. Submitted under PA-03-107, the NIH Exploratory/Developmental Research Grant Award. $275,000 awarded to examine the effects of dioxins on ovarian circadian clock genes.

ACS/IRG, "Aryl Hydrocarbon receptor regulation of the tumor suppressor, Per1", 2007-2008. SA Tischkau, PI

NIH K99/R00, Pathways to Independence Award, "Assessment of Psychostimulant Addiction Risk Following Developmental PCB Exposure". H Sable, PI, SA Tischkau, Co-Mentor

SIU-Near Miss, "Mechanisms of circadian clock-regulated excitotoxic resistance", 2010, K. Bottum PI, SA Tischkau, Co-Investigator.

Current Support

NIH R01, "PCBs, PBDEs, Hearing Loss and Attention/Impulsivity: Mechanistic Studies in Animals". 2007-2012. SL Schantz, PI. SA Tischkau, Co-Investigator.

NIH 1 R01 ES017774-01, "Circadian Clock Disruption: A Mechanism for Dioxin-induced Metabolic Syndrome" 2010-2014, SA Tischkau, PI; 25% Commitment.

SIU-CRC Excellence in Academic Medicine Award, "Mechanisms of Endogenous Neuroprotection in the Suprachiasmatic Nucleus", 2011, SA Tischkau, PI.

Pending Support

NSF, "Regulation of GnRH neurons by estradiol and the circadian clock", Kuehl-Kovarik, C, PI, SA Tischkau, Co-I, 10% commitment

NSF, "Characterization of a native alpha7-containing nicotinic acetylcholine receptor in the hypothalamus", Uteshev, V, PI, SA Tischkau, Co-Investigator, 10% commitment

NIH R01 ES021371 "Diet and Environmental Toxicants Interact in Development of Metabolic Syndrome", SA Tischkau, PI, 20% commitment.

EXTRACURRICULAR ACTIVITIES:

Volleyball Coach – Head Coach, Blue Lightning Volleyball Club, 2007-present
Member - United States Tennis Association, 1989-present
USTA League Team Tennis, 1990-present
Urbana Open Tennis Champion, 1995, 1997, 2000
Assistant Volleyball Coach, Parkland College, 1992-1995
Volleyball Instructor, Parkland College, 1992-1996
PrimeTime Volleyball Club Coach, 1996-1999
TSU Women's Softball, 1984-1986
TSU Intercollegiate Volleyball Team 1982-1986
Champaign-Urbana Park District Softball, 1990-2002

COMMUNITY SERVICE:

Volunteer Coach, High School Winter Volleyball Program (2005-present)
Tournament Director, Lincolnwood High School Winter Volleyball (2007-present)
Volunteer, USTA Challenger Tennis Tournament (1999-present)
Illinois State Science Fair Judge (1995, 1997, 1999)
Brain Awareness Week volunteer (1997-2007; 2011)
Volleyball Coach, Holy Cross School, Champaign, IL (1995-1996)
Neuroscience Workshop for High School Health Students, Urbana High School (2002-2007)
Illinois State Science Fair Coordinator (2010-present)

PUBLICATIONS:

Book Chapters:

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Tischkau SA and VD Ramirez. 1993. A specific membrane binding protein for progesterone in the rat brain: sex differences and induction by estrogen. **Proc Natl Acad Sci** 90:1285-1289. PMID: 8433988.

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Krager SL, SW Karmarkar, KM Bottum and **SA Tischkau**. 2011. Caspase activation after excitotoxic insult in suprachiasmatic nucleus neurons. (In preparation for submission to **Brain Research**).

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Tischkau SA. 1996. Physiology of the Germinal Disc Region of Preovulatory Follicles in the Chicken Ovary. Department of Molecular and Integrative Physiology Seminar Series, University of Illinois at Urbana-Champaign.

Tischkau SA, AM Fox, LE Faiman and MU Gillette. 1997. A circadian clock control point in the late night: Regulation by protein kinase G. 1st Annual Sleep and Circadian Rhythms DataBlitz. **Annual Meeting of the Society for Neuroscience**. New Orleans, LA.

Tischkau SA and MU Gillette. 1998. Winding the Circadian Clock: a *Timeless* Tale of Kinase Regulation? Department of Molecular and Integrative Physiology Annual Retreat, University of Illinois at Urbana-Champaign.

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Tischkau SA. 2000. Glutamatergic Regulation of Molecular Circadian Timekeeping. Neuroscience Program. University of Illinois.

Tischkau SA. 2001. Signaling Components in Glutamatergic Regulation of the Molecular Circadian Clockworks. Department of Biology, Grand Valley State University.

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Tischkau SA. 2001. Protein Kinase G: Critical Signaling Component in the Biological Clock. Department of Biology, Florida State University.

Tischkau SA. 2001. Critical Signaling Components in Regulation of the Circadian Clock. Department of Physiology, Southern Illinois University School of Medicine.

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Tischkau SA. 2003. Protein Kinase G Signals State Change: Critical Regulation of Glutamatergic Signaling in the Circadian Clock. Department of Pharmacology, University of Virginia.

Tischkau SA. 2003. Glutamate Regulates Circadian Timing through Protein Kinase G. Department of Biology, University of Illinois at Springfield.

Tischkau SA. 2003. Circadian Clock Genes in the Female Reproductive System. Bi-annual State of Illinois Reproductive Biology Retreat. University of Illinois.

Tischkau, SA. Reproductive Rhythms. 2003. ACCA Biology Seminar: Biorhythms. Benedictine University, Lisle, IL.

Tischkau SA and BN Klementiev. 2004. Keeping time in the rat ovary: Expression of circadian clock genes. **Society for the Study of Reproduction.** Baltimore, MD.

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CR Zimmerman and **SA Tischkau.** 2005. Expression of circadian clock genes in gonadotropin-releasing hormone neurons. **Society for the Study of Reproduction.** Cincinnati, OH.

Tischkau SA. 2005. Emergence of Avian Vacuolar Myelinopathy in Wild Birds. Wildlife Rounds. University of Illinois College of Veterinary Medicine.

Tischkau SA. 2005. Shedding Light on Biological Timing: Establishing a Circadian Link to Reproduction. Reproductive Biology Training Program. University of Illinois at Urbana-Champaign.

Tischkau SA. 2005. Nocturnal Checkpoint Regulation of the Mammalian Circadian Clock by Protein Kinase G. 2005 Gordon Conference on Chronobiology. Salve Regina University. Newport, Rhode Island.

Tischkau SA. 2006. Protein Kinase G: The Next Circadian Clock Gene? Systems and Integrative Biology Training Grant, School of Molecular and Cellular Biology. University of Illinois at Urbana-Champaign.

Tischkau SA. 2006. The Chronobiology of Fertility: the Brain, the Ovary and the Environment. Department of Biology, University of Illinois at Springfield.

Tischkau, SA. 2006. The Chronobiology of Fertility: the Brain, the Ovary and the Environment. Department of Pharmacology, Southern Illinois University School of Medicine.

Tischkau, SA. 2007. Circadian Clock Control of the Hypothalamic-Pituitary-Gonadal Axis. Interdisciplinary Neuroscience Program Seminar Series, University of Missouri at Columbia.

Tischkau SA. 2007. Mechanisms of Circadian Rhythmicity: Light, Clock Genes and Physiological Targets. Biology Program Seminar Series. Truman State University.

Tischkau, SA. 2007. Issues in Reproductive Pharmacology. Department of Chemistry, University of Illinois at Springfield.

Tischkau, SA. 2008. Circadian Regulation of Neuroendocrine Targets: Role of Aryl Hydrocarbon Receptor, Neuroscience Research Institute Seminar Series, Indiana University School of Medicine.

Tischkau SA. 2008. Protein Kinase G Regulation of Glutamatergic Signaling in the Circadian Clockworks. Department of Biology, Rutgers University.

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SA Tischkau. 2009. Aryl Hydrocarbon Receptor Activation by Tryptophan Photoproducts Alters Circadian Light Responses. **International Congress on Photobiology**. Dusseldorf, Germany

SA Tischkau. 2009. Aryl Hydrocarbon Receptor Activation Alters Light Responsiveness of the Circadian Clock. **Midwest Symposium on Advances in Chronobiology**. Northwestern University.

SA Tischkau. 2010. Interactions of Aryl Hydrocarbon Receptor with the Molecular Circadian Clockworks. Department of Medical Microbiology, Immunology and Cell Biology. Southern Illinois University School of Medicine. Springfield, IL.

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ABSTRACTS

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Stark JT, **SA Tischkau** and DR Gross. 2003. Estrogen decreases apoptotic and total cell death following cardiac arrest and resuscitation. **Experimental Biology Annual Meeting.** San Diego, CA.

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Hickok JR and **SA Tischkau**. 2004. Circadian clock regulation of gonadotropin releasing hormone neurons: the neural network that drives reproduction. **Understanding Complex Systems Symposium**. Department of Physics, University of Illinois at Urbana-Champaign.

Hickok JR and **SA Tischkau**. 2004. Transcriptional Profiles of GnRH Neurons: Implications of Circadian Input into the hypothtalamic-pituitary-gonadal axis. **25th Annual Reproductive Biology Minisymposium**, Northwestern University. Note: J. Hickok received 1st place research award for presentation.

Klementiev BK and **SA Tischkau**. 2004. Entraining the Ovarian Clock: LH regulates clock gene expression in the rat ovary. **25th Annual Reproductive Biology Minisymposium**, Northwestern University.

Bottum KM, E Poon, M Comiskey and **SA Tischkau**. 2004. Endogenous neuroprotection in the suprachiasmatic nucleus. **Critical Care Conference**. Cleveland, OH.

Cohen JA and **SA Tischkau**. 2004. Circadian variation in hippocampal damage incurred by global ischemia insult. **Colgate-Palmolive Student Symposium**. University of Illinois at Urbana-Champaign.

Hales LJ and **SA Tischkau**. 2004. Circadian expression of *Period1* in the immortalized GT1-7 cell line. **Summer Research Opportunities Program Symposium**. University of Illinois at Urbana-Champaign.

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Poon, E, K Bottum and **S. Tischkau**. 2005. Endogenous Neuroprotection in the suprachiasmatic Nucleus. **CMB Symposium**, University of Illinois at Urbana-Champaign.

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Hickok, J and **S. Tischkau**. 2005. What is a GnRH neuron, anyway? **Reproductive Biology Mini-symposium**, Northwestern University.

Hickok, J and **S. Tischkau**. 2005. Gene Expression Profiles in GnRH Neurons. **Biennial Illinois Reproductive Biology Conference**, Monticello, IL.

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Mukai, M., Lin, TM., Peterson, RE., Cooke, PS., **Tischkau, SA**. 2005. Potential Role of Aryl Hydrocarbon Receptor in Light Regulation of Circadian Rhythm, **Cell and Molecular Biology & Molecular Biophysics 18th Research Symposium**, Urbana, IL.

Escutia, K, M Mukai and **S. Tischkau**. 2005. AhR-Induced *cyp1a1* Expression in Liver after Light Exposure. **Biotechnology Center Poster Competition**. University of Illinois at Urbana-Champaign. Note: K. Escutia recognized as finalist for excellence award for presentation.

Cruz-Torres, A, J Hickok and **S Tischkau**. 2005. Regulation of GnRH neurons by estrogen status. **Summer Research Opportunities Program Symposium**. University of Illinois at Urbana-Champaign.

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Mukai M and **SA Tischkau**. 2006. Role of AhR in circadian rhythms: Effects of 2,3,8,8-tetrachlorodibenzo-p-dioxin (TCDD). **Gordon Conference on Environmental Endocrine Disruptors**, Il Ciocco, Lucca (Barga), Italy.

Mukai, M and **SA Tischkau**. 2006. Adverse effect of dioxins on the biological clock. **Phi Zeta Research Symposium, University of Illinois at Urbana-Champaign.** April 15, 2006. Note: M. Mukai received 1st place award for presentation.

Howell RE, JM Bahr and **SA Tischkau**. 2006. Characterization of the Avian Circadian System using cBmal1, cCry1, cPer2 and the Effect of Luteinizing Hormone. **Annual Meeting of the Society for the Study of Reproduction.** Omaha, Nebraska.

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Cohen JA, JT Stark, KM Bottum, DR Gross, **SA Tischkau**. 2007. The Hippocampus Shows Circadian Variation in Susceptibility to Global Ischemia. **International Stroke Conference.** San Francisco, CA.

SA Tischkau, BN Karman, SL Krager and C Xu. Aryl Hydrocarbon Regulation of Circadian Rhythms. 2008. **Semi-annual Neuroscience Retreat, Southern Illinois Chapter of the Society for Neuroscience**.

Bottum KM, R Trammell, J Lipcammon, A Annamalai, G Costin, **S Tischkau** and D Resch. 2008. Serum brain derived neurotrophic factor is not a good biomarker of depression. **Society for Neuroscience Annual Meeting**, Washington, DC.

Tischkau S, J Cohen, J Stark, K Bottum and D Gross. 2008. Estrogen status is associated with damage from global ischemia in hippocampal CA1 neurons. **Society for Neuroscience Annual Meeting**, Washington, DC.

Karmarkar SW, KM Bottum, **SA Tischkau**. 2009. Diurnal variation in NMDA receptor subunits and pro-survival factors in the hippocampus. **Society for Neuroscience Annual Meeting,** Chicago, IL.

Xu CX, SL Krager, **SA Tischkau**. 2009. Disruption of CLOCK/BMAL1 transcriptional activity is responsible for aryl hydrocarbon receptor-mediated regulation of the Period1 gene. **Society for Neuroscience Annual Meeting,** Chicago, IL.

Karmarkar SW, KM Bottum and **SA Tischkau**. 2009. Diurnal variation in NMDA receptor subunits and pro-survival factors in the hippocampus. **University of Illinois at Springfield. 9th Annual Research Symposium**. Note: SW Karmarkar won 2nd place in the oral presentation category for this work.

Karmarkar SW, KM Bottum and **SA Tischkau**. 2009. Diurnal variation in NMDA receptor subunits and pro-survival factors in the hippocampus. **Southern Illinois University School of Medicine. 20th Annual Research Symposium**.

Karmarkar SW, SL Krager, KM Bottum, **SA Tischkau**. 2010. Mechanisms of Neuroprotection in the Suprachiasmatic Nucleus. **Society for Neuroscience Annual Meeting**. San Diego, CA.

Karmarkar SW, SL Krager, JA Cohen, JT Stark, **SA Tischkau** and KM Bottum. 2011. Caspase activation and MAPK pathways are important in endogenous neuroprotection in suprachiasmatic nucleus neurons. **Annual Meeting of the American Stroke Association.** Los Angeles, CA.

Karmarkar SW, SL Krager, KM Bottum and **SA Tischkau.** 2011. Mechanisms of neuroprotection in the SCN2.2 neurons. **University of Illinois at Springfield. 11th Annual Research Symposium**. Note: SW Karmarkar won 1st place in the oral presentation category for this work.

Karmarkar SW, SL Krager, KM Bottum and **SA Tischkau.** 2011. Mechanisms of neuroprotection in the SCN2.2 neurons. **Southern Illinois University School of Medicine. 22nd Annual Research Symposium**. Note: SW Karmarkar won 1st place in the oral presentation category for this work.

Fee Schedule and Prior Testimony
Dr. Shelley Tischkau

Fee Schedule

Hourly: $300.00

Prior Testimony

Dr. Tischkau has not been previously deposed or given prior testimony.