

Conducting Preclinical Abuse Liability Screening in Only One Sex: Making a Case for “Reasonable Exclusion”

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Abstract

The Animal Welfare Act (AWA; 1990) requires the reduction in use of purpose bred animal subjects in bona fide research conducted in drug development. The National Institutes of Health (NIH) Revitalization Act of 1993 also requires its Director to reduce the number of animals used in government funded research as well as promoting those specific study protocols that provide valid and reliable data using only one gender. The consensus between the pharmaceutical industry and FDA was that a valid and reliable set of abuse liability studies did NOT require the inclusion of both male and female subjects. In recent pre-study protocol reviews, FDA has required the inclusion of both males and female animals in all three core abuse liability assays, basically doubling the total number of animals used on a single study design. NIH/FDA policy does allow for exceptions to the new rule. We provide evidence to establish a defence of a more balanced approach to these study designs that complies with the AWA and the NIH Revitalization Act by reducing the use of laboratory animals in preclinical research and to align the study designs with current goals of the International Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).

Keywords: Abuse liability testing; Sex differences; Animal Welfare act; NIH revitalization act; Animal welfare; 3 R's reduction

Definition

We have adopted the current NIH language regarding the use of the terms sex- and/or gender-specific differences in an attribute. According to the NIH, the term “sex” refers to being male or female according to reproductive organs and biologic functions assigned by chromosomal complement. The term “gender” refers to socially defined and derived expectations and roles rooted in biology and shaped by the environment and experience.

Introduction

This review is about drug control. The issue is focused on sex differences in the results of three core preclinical assays required for FDA approval: drug discrimination, self-administration, and dependence liability. We do not contend that there are no sex differences. Rather, that those differences do not mitigate for or against schedule control actions regarding New Molecular Entities (NMEs).

All new compounds must be approved through submission of a New Drug Application (NDA) to the U.S. Food and Drug Administration or a Marketing Authorisation Application to the Committee for Medicinal Products for Human Use at the European Medicines Agency. All preclinical (animal and *in vitro*) and clinical (human) data are submitted to the agency for review.

Guidance in study conduct and design has generally been harmonized using a “Tiered Testing” approach by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). As a member state the U.S.,

through the FDA, has adopted the ICH Guidelines as the current “standard” for drug approval. In the tiered testing approach a minimal data set is first acquired and evaluated. A set of criteria are then defined to trigger the next tier or stage of testing, and several factors may influence the need for information beyond a minimal data set. The tiered approach requires that the expertise for data evaluation and final decision making is available, both to the regulatory authorities and the industry responsible for performing the studies.

Inclusion of both male and female animal subjects is not required for all study designs. FDA, through its acceptance of ICH Guides, requires that some preclinical studies for approval include both male and female subjects, while other studies do not. For example, safety pharmacology core battery studies (S7A: Cardiovascular, pulmonary and CNS) are generally conducted in only one sex. Clinical and experimental studies show that females clearly demonstrate sex-dependent differences in the electrocardiographic pattern of ventricular repolarization in animal species and in humans. These electrocardiographic (ECG) changes are associated with a longer rate-corrected QT (QTc) interval at baseline than males [1]. While not much is documented regarding drug differences between women and men, data from drug-induced adverse events have shown that women experience torsades de pointes, a potentially fatal arrhythmia, more frequently when compared to male cohorts [2]. Despite these discrepancies, the majority of all of the cardiovascular safety studies conducted for FDA submissions are done in male canines, NHPs, and swine [3]. Administrative precedent has been established that simply identifying a sex-difference in a critical parameter such as cardiovascular safety does not establish a regulatory basis for FDA to require preclinical cardiovascular studies in both males and females. In such cases, the study design is dictated by the experimental question at hand.

Other second tier assays described in ICH S7A [4] and M3-R2 [5], such as renal, gastrointestinal (GI: Motility and stomach emptying), and neurological (EEG, seizure sensitivity) are generally conducted in only one sex, as well. For example, since male albino rats generally show proteinuria, Tier II renal studies are generally conducted in only female rats, while GI and EEGs are generally only conducted in males.

All required preclinical (animal) data are generally gathered prior to Phase II clinical trials for non-CNS active compounds; that is, no further animal testing is generally required when initiating Phase II Clinical Trials. However, all CNS active compounds require three additional animal studies for submission to the drug approval process: drug discrimination, self-administration, and dependence liability studies.

As part of every New Drug Application (NDA) process the US FDA must review all relevant and supplied preclinical study data for identification of any relevant indicator that is predictive of schedule control actions under the US Controlled Substances Act (Title 21, Chapter 13, USCA), also known as the Controlled Substances Act [6]. Under U.N. Treaty Obligations and U.S. federal statutes, the FDA and Drug Enforcement Administration (DEA) independently review all available pharmacology, safety, toxicity, developmental, pharmacokinetic, and carcinogenicity data for any relevant scientific finding of fact that supports any one or all of the eight factors that contribute to the decision of labelling and schedule control actions.

Drug control and the NDA

Under the CSA (§811.c.) there are 8 factors that the DEA considers with respect to each drug or substance proposed by the Secretary of Health and Human Services (HHS) as part of the NDA approval and marketing process [6]. These 8 factors are generally irrelevant for peripherally acting or non-CNS active compounds, devices, or treatments. However, active substances with peripheral targets may enter the brain as well either as parent active substance or as a metabolite at relevant concentrations and interact at central targets. This could be a concentration dependent phenomenon, the result of applying different routes of administration or a consequence of metabolism. When available data give rise to a concern in this respect studies as explained in this document should be considered to further clarify the pharmacological profile of the product.

The 8-factors relevant to schedule control actions are:

1. The drugs actual or relative potential for abuse;
2. The scientific evidence of the pharmacological effect of the new drug, if known;
3. The state of current scientific knowledge regarding the drug or other like substances;
4. The history and current patterns of abuse (of any drug with similar structure or function);
5. The scope, duration, and significance of abuse related to the new drug or similar drugs already on the market;
6. Any risk of the new entity to public health;
7. The psychic or physical dependence liability of the new drug; and
8. If the new drug is an immediate precursor or prodrug of a drug already controlled in the CSA.

Peripheral-acting drug approvals do not need to address these 8-factors determinative of control. Two independent health agency data reviews are conducted by staff at FDA (in consultation, in part, with

National Institute on Drug Abuse [NIDA]) and DEA on all relevant preclinical data submitted by the Sponsor.

Drug control policy is “Sex-Blind”

Drug control policy reviewers (FDA-CSS staff at CDER; and DEA-Drug and Chemical Evaluation Section, Office of Diversion Control) closely scrutinize the data submitted to the FDA by the Sponsor regarding the three core battery tests (self-administration, drug discrimination, and dependence liability). If a new drug does or does not induce a dependence syndrome or express a drug discontinuation syndrome upon abrupt cessation of treatment in animals, or in humans participating in clinical trials, it is a necessary but not sufficient condition for drug control. “Legend drug” means drugs that are approved by the US FDA and require, by federal or state law, to be dispensed to the public only on prescription of a licensed physician or other licensed provider but are not controlled substances under the CSA. Many legend drugs produce drug dependence but are NOT controlled under the CSA, for example, propranolol and corticosteroids. There are also over-the-counter drugs such as caffeine; nicotine and alcohol that produce dependence but none are schedule controlled. Critical in the analysis of Factors 1 and 2 are the result of self-administration and drug discrimination assays. If any experimental animal (rat is the preferred species) self-administers the new drug to a degree that is statistically greater than vehicle in a standard self-administration assay, there is sufficient evidence suggestive of a potential for abuse following approval and marketed release. This determination for schedule control actions is based on data generated using male OR female subjects. Drug schedule control is placed on “drug substances” in the vast majority of cases. There is no current bifurcated schedule control based on the use or abuse patterns based on males or females. Moreover, there are no therapeutic drugs whose abuse has historically been limited to only male or only female subjects (See Substance Abuse Mental Health Services Administration data: Drug Abuse Warning Network (DAWN), Treatment Episode Data Set (TEDS), and the National Survey on Drug Use and Health (NSDUH) data). As FDA has summarized in their conversations with pharmaceutical industry representatives, self-administration for a single animal for a single day is suggestive of potential for abuse [7].

One goal of preclinical drug discrimination studies is to evaluate the potential similarity of the subjective effects of a novel compound against that of a known drug of abuse. If the compound is found to have similar subjective effects to that of a controlled substance, it may have implications for future drug development and schedule control action. For example, if animals trained to discriminate cocaine from saline are administered a test compound, whether or not this compound engenders “cocaine-like” responding is of interest to both the Sponsor as well as regulatory agencies. In this case, whether or not the compound engenders “cocaine-like” responding in males but not in females would be irrelevant, and more importantly not required or advocated, under the current regulatory guidelines. It would provide a positive indicator that the compound may have “cocaine-like” properties. While the effective sensory/perceptual thresholds (limen or ED₅₀) between male and female rat subjects have been reported across most pharmacological classes of training drugs, if males or females engender complete generalization to the drug of abuse selected for the training drug, schedule control review will be initiated.

International Drug Control Treaties on the Single Convention on Narcotic Drugs of 1961 (as amended by the 1972 Protocol; United Nations, 1972) [8], the Convention on Psychotropic Substances of 1971

(United Nations, 1971) [9] and the United Nations Convention against Illicit Traffic in Narcotic Drugs and Psychotropic Substances of 1988 (United Nations, 1988) [10], have established a structured set of rules and processes by which the participating states must collaboratively support, defend, and maintain drug control schedules that establish a “closed system” to addictive or dependence-producing pharmaceuticals and natural products (alkaloids) that allows for sufficient supply for legitimate patient use, minimal access to drugs that lack medical use, as well as sufficient supplies for the conduct of bonafide research. The policies are set into motion without any reference to sex- or race-related differential drug intake or health threat, but they do carve out exceptions for access to dangerous drugs that lack general acceptance for medical use based on religious beliefs (i.e., ayahuasca, Khat, mescaline, and marijuana).

Current NIH initiatives/Perspectives on drug control

The current thinking within the US Federal Public Health Policy [11] and the European Monitoring Centre for Drugs and Drug Addiction [12] in regard to risk assessment, in general, is that the agencies must consider actual, not just ideal (medically indicated) use; the analysis must go beyond the clinical study, the risk assessment must consider how people actually use drug substances outside the scope of medical practice which includes consideration of cognitive and behavioural factors affecting human judgment and decision-making [13]. Drug control policy decisions are set into motion during the NDA review and approval process when there are predictive data from any subset of preclinical and clinical studies conducted prior to NDA approval. The NDA approval is based on a preponderance of pharmacology, general toxicity, developmental, carcinogenicity, pharmacokinetic, and safety assessments studies.

Drug abuse liability assessment is only one aspect required for NDA approval as part of Tier II testing for CNS active compounds, only. Moreover, current drug control policy is sex-neutral. If there is diversion of drug product in clinical trials by males or females, or positive preclinical behavioural data that have been shown to have moderate to strong predictive validity for human abuse potential, such as increases in locomotor activity or self-administration in either male or female rats, then drug scheduling reviews are initiated. Two key CSS members, Silvia Calderon and Michael Klein [14] are quoted as saying:

The FDA/CDER guidance provides a considerable degree of scientific flexibility regarding models and methodology; because the assessment of abuse potential of a drug is an evolving area of research.....There is no mention in the CSA of the type of medical and scientific data required to assess abuse potential for scheduling purposes.

The purpose of this review is to establish a rationale for standardized preclinical protocols related to the three core behavioural assays used to assess a new drug's abuse liability that must be submitted to support NDA approval: 1) Drug discrimination, 2) Self-administration, and 3) Dependence potential assessments.

Discord between statutory, regulatory, and administrative policies regarding animal abuse liability studies

In the United States the Animal Welfare Act [15] and Regulations (Public Law 89-544; USDA 1985) [16] has established a system of self-regulation and regulatory oversight that binds researchers and federal institutions using animal research subjects. Both researchers and institutions have affirmative duties to comply with both statutory and

regulatory requirements for a rigorous program of animal care and use while allowing flexibility in fulfilling the responsibilities of bonafide research on non-human subjects. The Three R's represent a practical method to ensure compliance for the proper care, use, and humane treatment of laboratory animals produced for or used in research, testing, or teaching. Replacement, Reduction, and Refinement (3 R's) have become an international accepted approach to ensure compliance with both statutory and regulatory processes [17]. Reduction in the number of animals used in research is a federally mandated strategy to obtain comparable levels of information from the use of fewer animals or for maximizing the information from a given number of animals so that in the long run fewer animals are needed to acquire the same scientific information. Historically, male animal subjects have been utilized in the vast majority of all drug discrimination and self-administration studies published in peer-reviewed scientific journals. As discussed above, regulatory precedent has been established that simply identifying sex-differences in one aspect of drug safety assessments (i.e., QT prolongation) does not mitigate for the inclusion of both sexes in cardiovascular safety under ICH S7A [4] and S7B [18].

The National Institutes of Health (NIH) has published a decision to require NIH grant applicants to “report their plans for the balance of male and female cells in all future grant [sic] applications, unless sex-specific inclusion is unwarranted” [19]. Under the NIH policy, male and female animals and human patients should be used in the preclinical and clinical trial phases of drug development. Clayton and Collins [20] announced the NIH plans to address the issue of sex inclusion across biomedical research multi-dimensionally-through program oversight, review and policy, as well as through collaboration with stakeholders. Under the statutory limits of the CSA as well as the International Drug Control Treaty obligations, the determination of a relative abuse liability must be based on valid and reliable data that are scientifically sound, legally defensible and timely, relevant to schedule control reviews conducted by both FDA and DEA as part of the NDA approval and drug labelling regulatory requirements [21]. The FDA's Draft Guidance Document on Abuse Liability [22] clearly states that:

The sample size in animal studies should be adequate to accurately characterize the ability of the drug to induce the particular behaviour of interest. The number of animals included in a study depends on the anticipated effect size and the desired power of the statistical test used.

By inference, since the CSA [6] requires decisions based on legally defensible, valid and reliable data, Clayton and Collins [20] have clearly established the NIH/FDA policy on sex in preclinical abuse liability studies should include the statistical evaluation of differences in these data sets based on power analysis and the anticipated effect size for the specific 3 assays required by the agency for NDA review. This requirement, by definition, increases the total number of animals that must be used to comply with these new regulatory demands.

It is the intent of the NIH to include females and males on studies to ensure that valid and reliable data are generated that can support the legally defensible nature of these sex-differences under the applicable statutes, policies, and regulations regarding drug approval and drug control processes. Clayton and Collins [20] noted that such biases could mislead future clinical studies and, ultimately, clinical practice and argued that NIH needed to promote balanced representation of both sexes in preclinical research. Plans for future policies drew feedback from the scientific community supporting the view that consideration of Sex as a Biological Variable (SABV) could potentially influence the reproducibility, rigor, and generalizability of research findings in biomedical research.

The Secretary of Health and Human Services administers over both NIH and FDA. The need for enhanced collaboration between NIH and FDA has never been more pressing, given new scientific opportunities in translational research, new public health challenges, far-reaching economic changes at the national and global level, and fundamental changes to the U.S. health care system. Under the Secretary of HHS a “Joint Leadership Council” has been formed that works together to help ensure that regulatory considerations form an integral component of biomedical research planning, and that the latest science is integrated into the regulatory review process. Such collaboration and integration advances the development of new products for the treatment, diagnosis, and prevention of common and rare diseases and enhances the safety, quality, and efficiency of the clinical research and medical product approval enterprise. The formation of the Leadership Council represents a commitment on the part of both agencies to forge a new partnership and to leverage the strengths of each agency toward this common goal. The Leadership Council is chaired by the NIH Director and FDA Commissioner. Currently, seven members are chosen by NIH from among the NIH Institute and Centre Directors and senior staff, and seven members are chosen from among FDA’s Centre Directors and senior staff.

Under the universal oversight of all science-based agencies under HHS purview the NIH sex-inclusion policy has been adopted by the review process through Controlled Substances Staff (CSS) at the Centre for Drug Evaluation and Research (CDER). Through the Leadership Council the FDA has extended and generalized this over-inclusive use of both male and female subject requirements to privately funded research endeavours in the preclinical development of NMEs intended for the NDA process. It should be noted here that the Animal Welfare Act [15,16] requirements to reduce the number of animals on preclinical studies only applies to the FDA and NIH initiatives to include both male and female animal research subject in preclinical study designs. It obviously does not apply to human clinical trials.

Gender Representation	Representation is scientifically...	
	Acceptable	Unacceptable (Bar to award)
Both genders included	G1A	G1U
Females only	G2A	G2U
Males only	G3A	G3U
Unknown (Cannot be known)	G4A	G4U

Table 1: NIH decision tree for inclusion of women plan-acceptable and unacceptable plans for representation.

As shown in Table 1, under the current NIH policy regarding human clinical trials there is a decision tree for the inclusion of both male and females that allows for exceptions. The exclusionary criteria are:

1. If strong evidence exists for significant differences in intervention effect AND study design or analysis can answer primary question(s) separately for each sexes AND can detect significant difference in intervention effect, then both sexes should be included.
2. If strong evidence exists for NO significant sex differences in intervention effect, then both sexes (Code G1), females only (Code G2) or males only (Code G3) can be used.

3. If there is no clear evidence for NO significant sex differences in intervention effect and study design and analysis plans will permit valid analysis of a differential intervention effect (statistical sex differences), then both genders are included (Code G1), or
4. If one sex is excluded because inclusion is inappropriate with respect to their health or because the research question is relevant to only one sex, then females only (Code G2) or males only (Code G3) is allowed.

Under the “NIH Revitalization Act of 1993” [23] the Director of the NIH is, in part, directed to establish:

1. Methods of research and experimentation that reduce the number of animals;
2. Methods of such research and experimentation that produces less pain and distress in such animals, and
3. For establishing the validity and reliability of the methods used in animal studies
4. For encouraging the acceptance by the scientific community of such methods that have been found to be valid and reliable.

Under the federal statutes the Director of the NIH is legally required to take such actions as may be appropriate to convey to scientists and others who use animals in biomedical or behavioural research or experimentation information respecting the methods found to be valid and reliable under subsection (a) (2) of the statute. This requirement extends to the Animal Welfare Act of 1990.

In the NIH initiative statement, McCullough et al., [19] clearly stated that their review targeted NIH funded grant applicants and it allowed for “reasonable exclusion” or “defined exceptions” to the grant application process. However, they pose the reasonable question, “...at what point would we determine that sex imbalance in disease incidence would no longer constitute reasonable grounds for exclusion of both sexes in preclinical studies?”

The policies, implemented in January 2016, are discussed further in the following NIH Guide Notices (<http://grants.nih.gov/grants/guide/notice-files/NOT-OD-15-102.html>; <http://grants.nih.gov/grants/guide/notice-files/NOT-OD-15-103.html>). The NIH leaders raised concerns regarding an over-reliance on male animals in preclinical research-particularly for diseases occurring more frequently in women and for diseases that manifest differently in men and women [20]. They noted that such biases could mislead future clinical studies and, ultimately, clinical practice and argued that NIH needed to promote balanced representation of both sexes in all preclinical research. While this appears to be a noble cause, these recommendations are now carrying over to areas that were not outlined in the original NIH policy changes, and can ultimately pose ethical and procedural challenges for pharmaceutical companies and Contract Research Organizations (CROs) that conduct bonafide preclinical research under the administrative or regulatory powers of the FDA. The scientific rationale for these policy changes is context-dependent, and does not necessarily hold true for the types of assessments typically conducted throughout the drug development process (e.g., abuse liability assessments).

As previously noted, through the Joint Leadership Council the FDA has extended this NIH initiative to preclinical toxicology protocols involving drug development projects funded by private pharmaceutical companies. In doing so, FDA has set administrative procedures established by a non-regulatory agency (NIH) for grantees and placed

those requirements onto the privately held pharmaceutical registrants under the US Food and Drug Administration without due process, public comment, and administrative reviews. The Office of Information and Regulatory Affairs (OIRA) is a statutory part of the Office of Management and Budget within the Executive Office of the President. OIRA is the United States Government’s central authority for the review of Executive Branch regulations, approval of Government information collections, establishment of Government statistical practices, and coordination of federal privacy policy. The development of Federal regulations and other related documents issued by the US Government Agencies, such as the FDA must allow for the public to read and comment on proposed regulations that govern their performance, such as FDA registrants.

A recent paper by Guizzetti et al., [24] announced that NIH expects that SABV will be factored into research designs, analyses, and reporting in vertebrate animal and human studies to the fullest extent possible. Under the Leadership Council that initiative has been put into practice by its protocol reviews through the CSS staff at CDER. However, the original NIH initiative did not intend to require that every NIH-supported preclinical study include equivalent numbers of males and females in every experiment (see exclusion criteria, above). Perhaps more importantly, the authors note that some sex differences that emerge may not be meaningful or interesting. Guizzetti et al., [24] also highlighted that resource scarcity and expense may also legitimately limit the ability to study both sexes, as in the case of nonhuman primate research.

Drug Control Policy

Under current federal regulatory and administrative requirements established by both the FDA and DEA, the data from preclinical abuse liability testing of NMEs are reviewed in conjunction with the totality of the voluminous preclinical pharmacology, toxicity, safety, carcinogenicity, efficacy, and pharmacokinetic data. The three core battery of tests required under the FDA draft guidance document [22] do not stand alone in the review of the 8 factors determinative of schedule control reviews by FDA, DEA, and the National Institute on Drug Abuse (NIDA). All available data are considered in the independent schedule control 8-factor analyses conducted by FDA and DEA. Schedule control actions are sex-neutral; positive markers for abuse potential in either male or female animals will initiate the 8-factor analysis for schedule control actions. It is even questionable whether current binding International treaties or United States Code (21 USCA, Chapter 13) allow for bifurcated schedule control based on sex. Moreover, it is unclear as to the legal ramifications of this strategy in practice—would males be allowed to legally consume and buy certain substances while females are not? This point brings home the potential ambiguity of CSS staff at FDA establishing regulatory requirements for the automatic inclusion of both sexes in preclinical abuse liability assessments, thus preventing customization of research protocols.

Current abuse liability policy perspectives at FDA

Recommendations to conduct abuse liability screening in tandem with Phase I or Phase II clinical trials occur for the following reasons:

A) At this juncture of the development program the equivalent animal therapeutic plasma concentrations are known.

B) In most cases, the mechanism of action of the test article has been well characterized as well as the identification of the test articles’ action on one of the 8 neurotransmitter systems “associated with abuse

potential” (dopamine, norepinephrine, serotonin, gamma-aminobutyric acid (GABA), acetylcholine, opioid, N-methyl-D-aspartate (NMDA), or cannabinoid) [22].

1) Under the draft guidance document, receptor binding data should be submitted as a part of the pharmacology-toxicology section of the NDA and should also be included in, or hyperlinked to, the abuse potential assessment section of the NDA.

C) A full pharmacokinetic profile of both male and female subjects in pharmacology, toxicology, carcinogenicity, efficacy, and in some cases safety assessments, has been completed and data available to support the selection of the most sensitive sex for testing.

1) Toxicokinetic studies are performed for new drugs, CNS-acting and peripheral acting drugs primarily to assist in the interpretation of toxicity studies, and as such, the test system selected (rat, NHP) will necessarily be the same as that used for the toxicity study.

D) The Good Manufacturing Practice (GMP)-final product to be used in clinical trials is available for use in the abuse liability screening.

E) The Phase I targeted doses have been selected and, many times, initial human pharmacokinetic profiles are available.

It is highly recommended that all abuse liability protocols be submitted to the agency for pre-study review, with the knowledge that these reviews are not binding on the agency or the Sponsors. In a series of recent CSS reviews of abuse liability protocols requested by small and large pharmaceutical clients, CSS called for the automatic inclusion of both male and female rats in every preclinical abuse liability study citing “there may be pharmacokinetic differences between male and females with this test article.” This seems to signify a paradigm shift at CSS, and would appear to be directly related to the aforementioned NIH recommendations for those researchers applying for federal grant funding.

Pharmacokinetics and abuse liability

In a review on this particular subject matter, Meibohm et al., [25] concluded that sex-related differences in pharmacokinetics have frequently been considered as potentially important determinants for clinical effectiveness of drug therapy. The mechanistic processes underlying sex-specific pharmacokinetics can be divided into molecular and physiological factors. Major molecular factors involved in drug disposition include drug transporters and drug-metabolizing enzymes. Meibohm et al., [25] highlighted that while sex-related differences in pharmacokinetics have been identified for numerous drugs, the differences are generally only subtle. For a few drugs (e.g. verapamil, beta-blockers and selective serotonin reuptake inhibitors), sex-related differences in pharmacokinetics have been shown to result in different pharmacological responses, but their clinical relevance remains unproven.

Schwartz [26] agreed with Meibohm et al., [25] and pointed to the fact that on average, males are larger than females. Body size differences result in larger distribution volumes and faster total clearance of most medications in males compared to females, and body fat may increase distribution volumes for lipophilic drugs in females. Total drug absorption does not appear to be significantly affected by sex although absorption rates may be slightly slower in females. Bioavailability after oral drug dosing, for CYP3A substrates in particular, may be somewhat higher in females when compared to male cohorts. Bioavailability after transdermal drug administration does not appear to be significantly affected by gender; nor does protein

binding. Renal processes of glomerular filtration, tubular secretion, and tubular reabsorption appear to be faster in males compared to females whether considered on mg/kg or total body weight basis. Schwartz concluded that the relative role of sex on pharmacokinetics as compared to genetics, age, disease, social habits and their potential interactions in the clinical setting is not yet fully known but should be routinely considered and further studied. In differentiating between preclinical pharmacokinetics and toxicokinetics, Welling [27] has concluded that while the pharmacokinetic preclinical-clinical data link is useful and sometimes very important, it is seldom essential during a drug development program.

In the major markets of the developed world PK information is now routinely included in approved drug labelling [28]. Most often the PK sex difference data are derived from small clinical pharmacology studies with typically 12-24 healthy subjects. Studies with small patient numbers may be adequate to detect large sex-based differences in clearance; however, if the sex-based PK difference is small, the relatively small size of most clinical pharmacology studies makes it difficult to interpret small differences observed, or to confirm if there is no difference in PK [28]. Whether or not preclinical data is sufficient to accurately predict drug effects in humans may have little influence on the final decision to proceed with clinical studies and on which direction such studies might take; that is, preclinical PK data has minimal regulatory weight with respect to drug control scheduling.

Fadiran and Zhang [29] from the FDA have recently highlighted a survey of clinical pharmacology data contained in 300 NDAs submitted to the US FDA between 1994 and 2000 found that 163 (54%) NDAs had sex-based PK information [28]. Of the 163 drugs, 51 (31%) showed a possible sex effect, i.e., a PK sex difference of greater than 20%. The survey results showed that [28]:

- The majority (90%) of PK sex differences were less than 40%.
- Except for one drug, where PK sex difference was greater than 40%, women consistently showed higher plasma exposure.
- Regardless of the disposition pathways involved, more than 50% of the drugs studied showed PK differences of less than 20%.

As detailed by Faridan and Zhang [29], a more recent survey of the U.S. FDA labels of 69 NMEs, drugs, and biologics approved by the FDA between September 2007 and August 2010 showed that out of 52 NMEs with sex-based PK information (in either the approved labelling or the clinical pharmacology review) the majority (38/52, 73%) had no sex difference in PK. Four NMEs reported PK difference less than 20%, 10 reported PK difference greater than 20% but only 1 NME reported a >40% PK difference. No sex-based difference in dosage recommendation was made based on the observed PK sex difference because the differences were not clinically relevant [29].

Similar to cardiovascular, pulmonary, and CNS safety assessments, demonstrating a sex-dependent difference in PK does not, by itself, require inclusion of males and females in preclinical safety studies under the adopted ICH safety assessment guidelines.

Drug control and pharmacokinetics: In general, the Controlled Substances Act schedules drug “substances”. Some dose formulations have been scheduled based on the compositional elements of the substance, but the majority of drug control placement in a given schedule are independent of any pharmacokinetic, pharmacodynamics, or drug bioavailability established in preclinical or clinical pre-NDA studies. Any differential kinetics or bio distribution of the drug substance based on sex has minimal regulatory weight in drug control decisions.

For example, Oxy-IR™ or Roxicodone™, are immediate release formulations of oxycodone that are recommended for the treatment of moderate to severe pain that requires opiate analgesic control (i.e., breakthrough pain). In contrast, OxyContin™ and MS-Contin™ are indicated for the treatment of moderate to severe pain of long duration. Their onset of action is slower in controlled or extended release formulations and their half-life extends to 10-12 h. Despite these significant differential ADME profiles, all oxycodone and morphine substances are in the same schedule, CII. While the effects of extensive, rapid, and slow metabolizers of cytochrome P450 2D6 isoenzymes on the elimination rates of opiates have been based on both race and sex, all of these drug substances are in the same schedule in the CSA.

Similarly, the differential kinetics and biodistribution of transdermal (patch; Duragesic™), IV dose administrations (Sublimaze™), transmucosal (Oralet™), and oral fentanyl administration (Actiq™) in humans and animals had no determinative factor in placing all formulations of fentanyl into the same schedule of the CSA-CII.

Another example of differential pharmacokinetic profiles to schedule control is demonstrated by the C-II status of lisdexamphetamine (Vyvanse™), dextroamphetamine (Dexadrine®, Adderall™, Obetrol™), and cocaine. All three substances are CNS active stimulants. Lisdexamphetamine is a prodrug of amphetamine and requires absorption in the gut and the conversion to dextro-amphetamine and l-lysine by the hydrolytic activity of red blood cells and is not metabolized by cytochrome P450 enzymes. Lisdexamphetamine has a C_{max} of approximately 3 h. Dextroamphetamine is rapidly absorbed by the bowel, needs no conversion to act on peripheral or CNS tissue and has a half-life of approximately 9.8 h, and is metabolized by cytochrome P450 2D6. On the other hand, cocaine has an almost immediate C_{max} , a half-life of approximately 15 min and is metabolized rapidly by pseudo-cholinesterases. In spite of these pharmacokinetic and biodistributional differences all 3 psychomotor stimulant substances are in the same stratified schedule of drug control under the CSA-CII.

With these facts in mind, we suggest that the automatic inclusion of both sexes in abuse liability studies, based on a potential for pharmacokinetic differences between male and female rats, may be inconsistent with current views on the issue appearing in peer-reviewed scientific journals and potentially poses a conflict with the statutory (legal) requirements of the Animal Welfare Act and the NIH Revitalization Act. These two federal mandates direct NIH and FDA to reduce the number of animal subjects used in preclinical study designs. They don't have a choice—they have a statutory mandate to do so. Even if there are sex-based differential pharmacokinetics or bioavailability data defined in preclinical studies, their relevance to schedule control actions activated by the NDA represent a minimal standard of evidence to initiate differential schedule control placement under the CSA. As detailed by the US Congress, Office of Technology Assessment [30]:

No one Federal agency policy on animal care and use has all the characteristics needed to address all issues adequately. Combining certain aspects from each would produce an effective uniform Federal policy.

Drug control status and sex: A review of the published literature reveals that the majority of all animal abuse liability studies have been conducted with one sex, males. As detailed above, drug control policy is sex-neutral—an abuse potential liability discovered in either sex will

activate the two independent 8-factor analyses required by FDA and DEA. A prime example of the major issues regarding preclinical data reviewed for NDA approval is the sedative/hypnotic, zolpidem. The immediate released formulation of zolpidem (Ambien™) was approved for use in the United States in April 1992, under NDA#19-908. In the NDA overview dated November 19, 1991, a number of indices of abuse potential were reported in clinical trials that are listed in Table 2:

Characteristic	Male	Female
Amnesia	12	18
Euphoria	28	1
Depression	6	8
Hallucinations	9	1
All Others	25	5

Table 2: Unexpected adverse events reported in clinical trials related to zolpidem NDA suggestive of an elevated potential for abuse.

On page 79 of that NDA review, FDA proceeded to summarize the “variations in pharmacokinetics” known at the time of NDA approval:

Females given zolpidem had a C_{max} and AUC approximately 25% higher than males when corrected for weight.

Under general circumstances, the abuse liability studies would be conducted in the most sensitive sex—females. However, the following two clinical abuse liability studies were completed: 1) LSH-14 was conducted by Dr. D. Jasinski in 12 inpatient males, and 2) LSH-16 was conducted by Dr. Roland Griffiths in 25 outpatient male volunteers, of which 15 completed the full cross-over design. Zolpidem was approved in the European Union (OECD approval) prior to FDA approval in the US, for which several relevant preclinical studies were conducted [31-34]. All of these studies were also conducted in male experimental subjects (DD: rats; SA, DD, Dependence: baboons). At the time, zolpidem was placed into Schedule IV. The placement into Schedule IV was in full agreement with international and national drug control policies, irrespective of the fact that FDA was aware that there were higher pharmacokinetic profiles in females and that all abuse liability studies were conducted in males only. If the least sensitive sex (males) demonstrated positive preclinical biomarkers for abuse liability it would be generally assumed, based on the preponderance of similar reports in peer-reviewed scientific journals, that females would show similar biomarkers, albeit possibly at lower doses. Surprisingly, experimental studies of sex differences in the pharmacokinetics and pharmacodynamics of zolpidem in human men and women found that body weight, not sex, was the critical factor. Women clear zolpidem from their system more slowly than men, but body weight eliminates the statistical significance of sex as a variable in clearance of zolpidem [35,36]. Because body weight, not sex, is the independent biological variable, sex-based preclinical research protocols would likely not have predicted sex differences in rates of adverse events with zolpidem.

As recently as 2013 the approval process for the sublingual formulation of zolpidem (Intermezzo®), acknowledged the rate (measured by the peak plasma concentration or C_{max}) and extent (measured by the area under the plasma concentration time curve or AUC) of absorption of zolpidem following oral absorption were both approximately 45% higher in women compared to men for immediate-

release zolpidem and approximately 50% and 75% higher, respectively, for controlled release zolpidem (Intermezzo®). Zolpidem, the substance, and all available formulations of zolpidem are scheduling IV non-narcotics-regardless of these PK differences.

The sex-specific labelling recommendations reflect an evidence-based approach to risk management and dose individualization but are not critical for Schedule Control actions. These examples suggest that both the exposure differences as well as the corresponding response changes are considered when dosing adjustments are recommended in FDA’s regulatory role in establishing “labelling”. However, these facts have no regulatory weight to change schedule control actions on the drug substance, zolpidem.

To extend the point recently made by Bevins and Charntikov [37], there is a vast literature base demonstrating the increased sensitivity of female subjects to the rewarding effects of drugs of abuse [38]. Carroll, Craft, and colleagues have been interested in the role that gender/sex play on drug effects for some time, and have published extensively on the varying role of sex in the effects of numerous drugs and experimental preparations [39-47].

There have been differences noted in some of the neurological pathways relevant to drug abuse [48]; however, it is unclear how these differences are expressed behaviourally. Furthermore, these differences have primarily been discussed in the context of psychomotor stimulants, which only constitute a portion of the demonstrated drugs of abuse. Clearly, there are measurable differences that express themselves in applied research, but the results are inconsistent across studies and differ substantially across substances and pharmacological classes [49,50].

Identified sex-differences in common drugs of abuse: Dr. Rebecca Craft and colleagues [51-55] and Dr. Ted Cicero and colleagues [56-58] have highlighted the sex differences in opioid antinociception in mice, rats, monkeys, and humans. In these models, mu opioid agonists have been shown to be more potent or efficacious in males than in females, although this is not always the case. One possible explanation for sex differences posed by Craft and colleagues is the structural or functional differences of the descending pain modulatory system, including the midbrain periaqueductal gray (PAG). According to Craft, female rats have more PAG to rostral ventromedial medulla (RVM) output neurons than do males, and persistent pain activates more output neurons in males than in females. Furthermore, mu opioid receptor expression in the ventral PAG (vPAG) is two-fold higher in male than in female rats. Sex differences in antinociception have been observed after opioid administration to the vPAG and RVM; however, whereas several studies have shown that supraspinal administration of morphine or DAMGO produces greater antinociception in male rats [59-61], other studies have reported greater antinociception in female rats [62] or no sex difference [60,63]. Disagreement among such studies may result from differences in the type/intensity of the nociceptive stimulus, efficacy of the mu agonist, use of awake vs. anesthetized animals, estrous phase of females, and genotype (strain/vendor) of the rodent. In regard to estrous phase, previous studies of the antinociceptive effects of systemically administered mu agonists show that female rats in estrus typically are less sensitive than females in other stages [54,64,65].

Sex-based differences in pain thresholds, opiate pharmacokinetic profiles, biodistributional differences or the magnitude of the qualitative and quantitative expression of withdrawal of the opiate type are interesting academic questions that may have FDA’s interest with

respect to labelling. However, none of these characteristics of a new opiate medication under NDA review has significant regulatory weight in determining what specific schedule of control it will be placed. The rate of onset from an immediate release formulation (i.e., Oxy-IR™) the duration of action of an opiate for use in chronic pain syndromes (i.e., OxyContin™) or the differential doses recommended for treatment in males and female patients have minimal regulatory

weight in deciding that the new opiate compound will be placed in Schedule II of the CSA.

How relevant are the preclinical differences between males and female rats in standard abuse liability assays in regards to regulatory decisions on schedule control actions? Some comparisons are made, below.

Training (citations above) in Drug text	Training Dose mg/kg	Sex Training Days?	Diff	DD Met?	Criteria	ED ₅₀ Diff?	Cross General Test?	Full General. to Training Stimulus?		Meet Analysis Criteria for SC?	8-Factor Criteria for
								Males	Females		
Cocaine	5.6	No		Yes		No	Amphetamine	Yes	Yes	Yes	
Morphine	3	Yes		Yes		Yes	Fentanyl	Complete	Complete	Yes	
		F<M			F<M	BW373U86	Partial	Partial	Yes		
U69,593	0.13	Yes		Yes		Yes	Bremazocine	Complete	Complete	Yes	
		F>M			F>M	Ethylketazocine	Complete	Complete	Yes		
Relevant to Schedule Control?	No	No		Yes		No	Yes	Yes	Yes	Yes	

Table 3: Sex-dependent differences in parameters established in drug discrimination studies that provide valid, reliable, and statutory defensible data for schedule control actions at the NDA review.

Table 3 shows the comparative data between male and female rats in three different drug discrimination assays from one laboratory. Craft et al. have reported the results from male and female rats trained to discriminate between cocaine and saline [66], the kappa opioid agonist U69, 593 and vehicle [67] and the prototypic mu opioid agonist, morphine and saline [53].

While Craft et al. have shown subtle differences between males and females in drug discrimination assays with regard to the length of time to reach training criteria and threshold doses (ED₅₀) of the training drug, these two findings do not rise to the level of 8-factor analysis exclusion with respect to regulatory drug control policy decisions. In the 1998 report on U69, 593, Craft et al. [67], concluded,

Considering all κ, μ and δ agonist substitution tests together, the fact that there were only a few sex differences in maximal substitution or slope of the substitution curve suggests that the U69, 593 discriminative stimuli is qualitatively similar in male and female rats.

Therefore, preclinical data from these males OR female data sets would most likely initiate 8-factor analysis by both FDA and DEA.

To present a set of common findings when male and female rats are used in drug discrimination studies, we direct the reader to two different studies conducted in male and female Wistar rats by Anderson and van Haaren [68,69]. These two studies provide some illustrative data to show the impact of sex on a short-acting Schedule II drug, cocaine, with respect to generalization gradients of the training drug and the malleability of the cocaine cue by concomitant administration of SCH-23390 and raclopride.

Anderson and van Haaren [68] first trained male and female Wistar rats to discriminate the presence/absence of 10 mg/kg cocaine (ip) training cue, using a 10-min pre-session injection interval (PSII). Once trained, various doses of cocaine were tested in “non-reinforced” test

sessions. During Phase A, two consecutive test sessions were conducted on a single day both 10 min and 30 min after administering the dose of cocaine. This allowed for the completion of two tests for each dose of cocaine and the generation of a two point time-effect function for every single test day. The full dose-effect function was generated over multiple test days. When these tests were completed the laboratory retested the rats following a single 30 min pretreatment interval for comparative purposes.

Sex of Trained Wistar Rats	Test 1, 10 min postdose, Phase A	Test 2, 30 min postdose, Phase A	Test 3, 30 min postdose, Phase B
Females	3.07 mg/kg (2.75-3.43 mg/kg)	6.07 mg/kg (5.37-6.85 mg/kg)	6.09 mg/kg (2.71-13.70 mg/kg)
Males	3.16 mg/kg (1.76-5.68 mg/kg)	8.23 mg/kg (7.63-8.89 mg/kg)	5.37 mg/kg (0.86-33.63 mg/kg)

Table 4: Calculated ED₅₀ and 95% Confidence Limits for the cocaine response-choice generalization functions for 10 mg/kg cocaine training cue in male and female Wistar rats. Complete dose response functions were generated in two phases. In Phase A, a series of two non-reinforced test sessions were conducted for each dose of cocaine on single days, at 10 min and 30 min postdose intervals to generate a full dose-response function. Phase B conducted as series of one-test-per-day at 30 min pre-treatment intervals for comparisons. Study data from Anderson and van Haaren [68].

There were no sex-differences in the shape or distribution of the response-choice measure and no significant differences in the ED₅₀ estimates from Phase A, 30-min post-dose discriminative functions and Phase B, 30 min post-dose discriminative functions (Table 4). Sex

did not provide any significant qualitative or quantitative difference in cocaine dose-effect functions in this study.

Anderson and van Haaren [69] replicated the cocaine dose-effect functions for 10 mg/kg cocaine training stimulus under identical experimental contingencies and tested for sex-dependent differences in the blockade of the cocaine cue using the D1 selective dopamine antagonist, SCH-23390, and the D2 selective dopamine antagonist, raclopride. As shown in Figure 1, there were no sex-differences in cocaine training cue established with 10 mg/kg cocaine (top panel), and found no differential response to SCH-23390 (middle panel) or raclopride (bottom panel) blockade in either male or female rats.

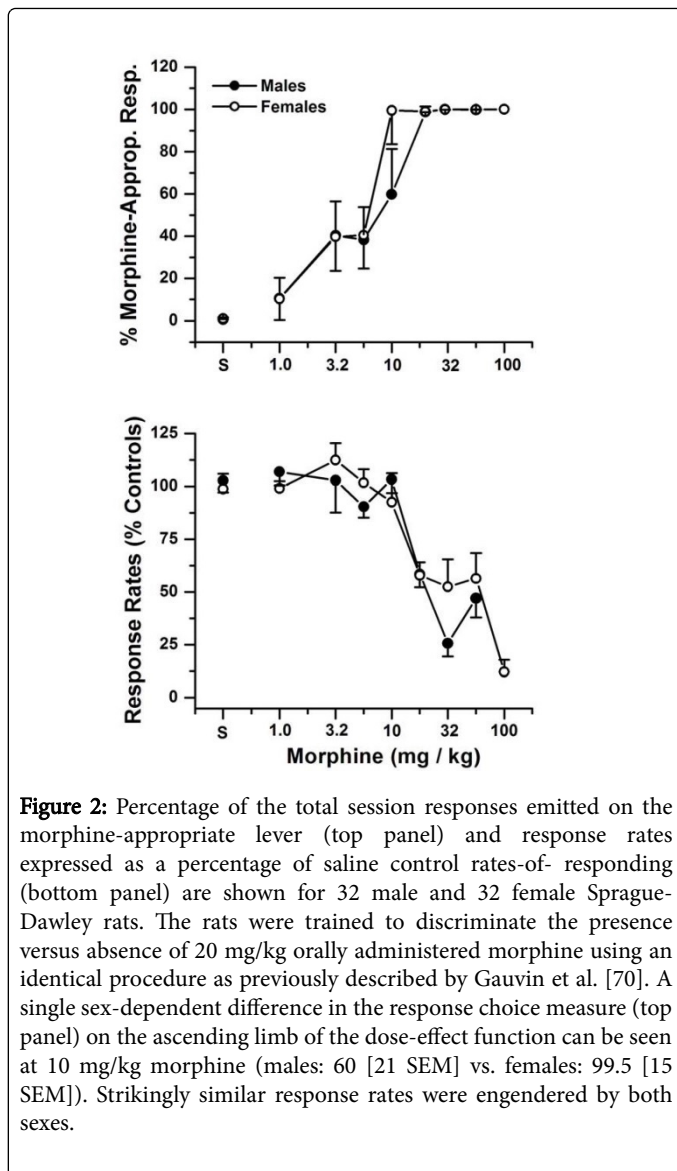
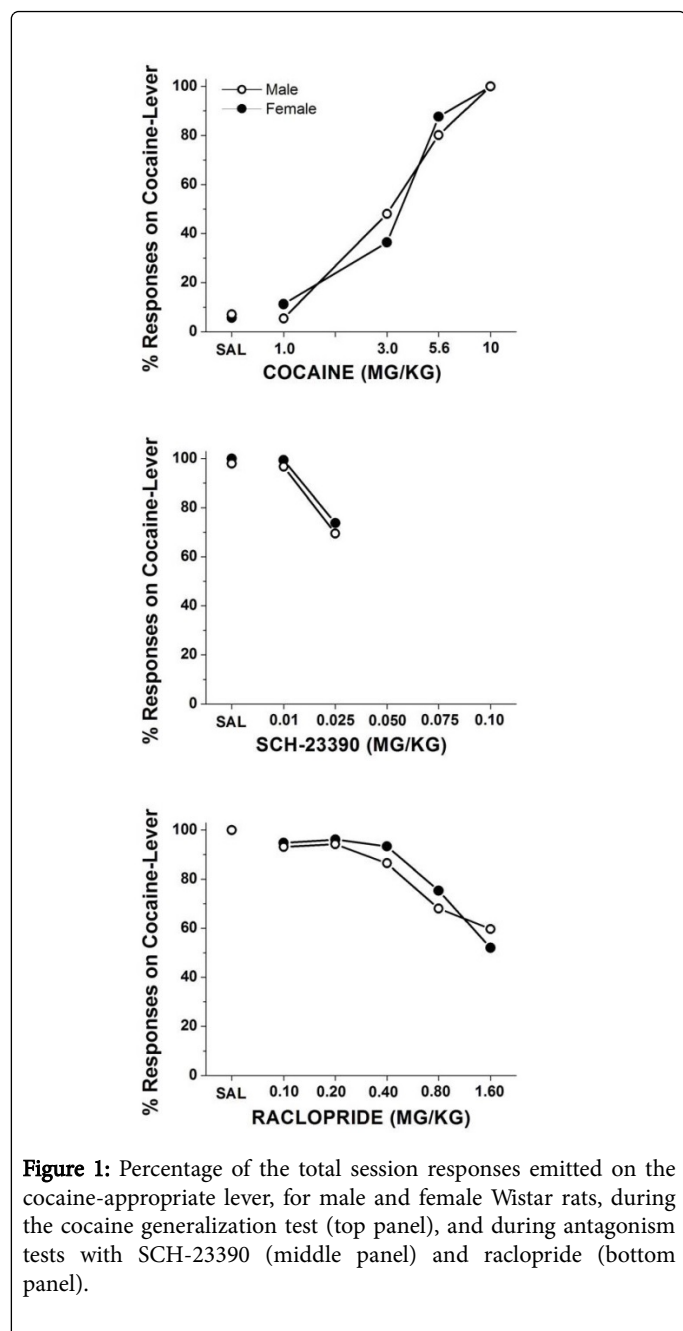


Figure 2: Percentage of the total session responses emitted on the morphine-appropriate lever (top panel) and response rates expressed as a percentage of saline control rates-of-responding (bottom panel) are shown for 32 male and 32 female Sprague-Dawley rats. The rats were trained to discriminate the presence versus absence of 20 mg/kg orally administered morphine using an identical procedure as previously described by Gauvin et al. [70]. A single sex-dependent difference in the response choice measure (top panel) on the ascending limb of the dose-effect function can be seen at 10 mg/kg morphine (males: 60 [21 SEM] vs. females: 99.5 [15 SEM]). Strikingly similar response rates were engendered by both sexes.

Using a standard drug discrimination training and testing procedure previously described by Gauvin et al. [70], male and female Sprague-Dawley rats were trained to discriminate the presence versus absence of 20 mg/kg orally administered morphine (Figure 2) or 3 mg/kg orally administered zolpidem (Figure 3) or 10 mg/kg IP administered cocaine (Figure 4) under a standard FR10 schedule of food reward (full details of the procedures are described in Gauvin et al. [70]).

As can be clearly seen in these data, there are subtle sex-related differences in the response choice measure at a single dose on the ascending limb of the dose-effect function, 10 mg/kg morphine, 1.8 mg/kg zolpidem and 3.2 mg/kg cocaine for Figures 2-4, respectively.

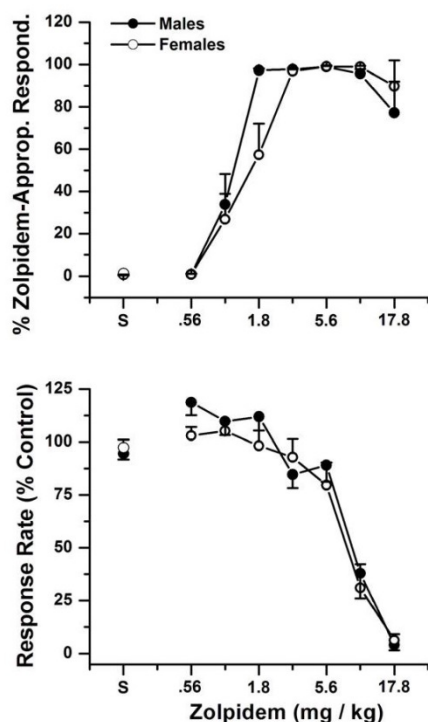


Figure 3: Percentage of the total session responses emitted on the zolpidem-appropriate lever (top panel) and response rates expressed as a percentage of saline control rates-of-responding (bottom panel) are shown for 32 male and 32 female Sprague-Dawley rats. The rats were trained to discriminate the presence versus absence of 3 mg/kg orally administered zolpidem using an identical procedure as previously described by Gauvin et al. [70]. A single sex-dependent difference in the response choice measure (top panel) on the ascending limb of the dose-effect function can be seen at 1.8 mg/kg zolpidem (males: 97.7 [0.8 SEM] vs. females: 57.3 [15 SEM]). Strikingly similar response rates were engendered by both sexes.

The response rates were remarkably similar across the full dose range tested for both drugs. While there clearly is a single sex-dependent response-choice difference in the discriminative stimulus effects of all 3 of these common positive comparators using the regulatory-recommended species for this abuse liability assay, the magnitude of the difference and the range of doses that represent the ascending limb of the dose-effect function has been previously referred to as the “interval of ambiguity” that represents the normal variation in perceptual thresholds common to all exteroceptive and interoceptive stimuli [71].

This single difference between males and females are well within the expected variations in dose-effect functions generated in two different groups at two different times within the same laboratory.

Figure 5 shows the dose effect functions for three separate training drug stimuli trained in two different groups of male rats in our laboratory.

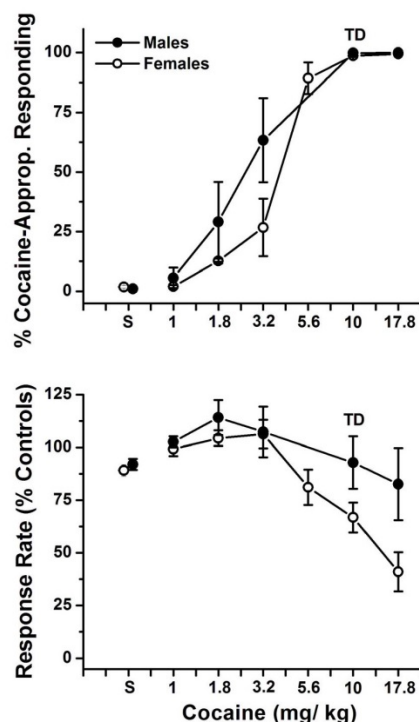


Figure 4: Percentage of the total session responses emitted on the cocaine-appropriate lever (top panel) and response rates expressed as a percentage of saline control rates-of-responding (bottom panel) are shown for 32 male and 32 female Sprague-Dawley rats. The rats were trained to discriminate the presence versus absence of 10.0 mg/kg IP administered cocaine using an identical procedure as previously described by Gauvin et al. [70]. A single sex-dependent difference in the response choice measure (top panel) on the ascending limb of the dose-effect function can be seen at 3.2 mg/kg cocaine (males: 63.30 [17.63 SEM] vs. females: 26.7 [12.06 SEM]). Strikingly similar response rates were engendered by both sexes.

The response-choice function for nicotine (0.32 mg/kg s.c., top panel), cocaine (10 mg/kg, i.p., middle panel), and ketamine (7.5 mg/kg, i.p., bottom panel) are shown. The “interval of ambiguity” between doses that engendered consistent saline-appropriate (default) responding to doses that engendered only drug-appropriate responding varied between the groups trained at two different intervals over the last 3 years.

These differences on the slope of the ascending limb of the response-choice dose effect function do not rise to the level of legally-defensible evidence required by the CSA for differential schedule control review. Both males and females (Figures 2-4) and between males at two different times of year (Figure 5) met testing criteria for the demonstration of stimulus control by the selected drugs and generalized to lower and higher doses similarly.

The demonstrated sex-differences in the dose-effect functions of drug discrimination studies do not provide sufficient regulatory weight in the determination of schedule control actions by FDA or DEA.

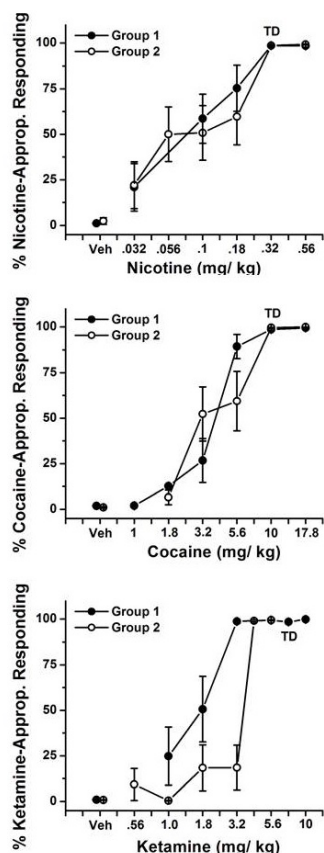


Figure 5: The percentage of the total session responses emitted on the nicotine- (top panel), cocaine- (middle panel) and ketamine-appropriate levers (bottom panel) for groups of male rats trained to discriminate the presence of 0.32 mg/kg nicotine (sc), or 10 mg/kg cocaine (ip), or 7.5 mg/kg ketamine in a 2 choice drug discrimination task (cf) [70]. The three dose-effect functions for the "response-choice" measure were generated in two different groups of male rats for each training drug over the course of 2 years in the same laboratory using the same procedures, equipment, and technical staff. Variations in the response choice measure on the ascending limb of the dose-effect functions between two equivalently trained groups of male rats diminish the meaningfulness of variations based on sex alone.

So what other preclinical assay must be included for CSS review? The acquisition of self-administration of common drugs of abuse like cocaine, methamphetamine and heroin [46,47,71-73] have demonstrated sex differences with respect to the acquisition of stable intakes, with females showing faster and greater intakes of the maintenance dose of the selected drug-of-abuse [74]. With respect to NDA review for schedule control actions, the speed of acquisition has minimal regulatory weight for schedule control actions. If either sex or both male and female rats acquire and maintain self-administration of these prototypic drugs of abuse, the drugs are said to have reinforcing effects predictive of human abuse potential. If the test article initiates and maintains self-administration (for 3-5 days) during test sessions with either male or female experimental subjects, the test article is considered to be a risk for abuse, once approved for distribution. This

conclusion also extends to self-administration of nicotine, caffeine, and ethanol that are not Controlled Substances. In contrast, most serotonergic hallucinogens (5HT2 agonists) are Schedule I controlled substances with limited evidence that they will initiate or maintain self-administration in male or female rats [22].

Drug	Rat Self Administration: Yes / No	
	Collins [75]	O'Connor [76]
	Females	Males
Codeine	Yes	Yes
Etonitazene	Yes	Yes
Morphine	Yes	Yes
Propoxyphene	Yes	Yes
Butorphanol	Yes	Yes
Nalbuphine	Yes	Yes
Nalorphine	Yes	Yes/No
Pentazocine	Yes	Yes
Cyclazocine	No	No
Ethylketazocine	Yes	-
Naloxone	No	No
Cocaine	Yes	Yes
Amphetamine	Yes	Yes
Methylphenidate	Yes	Yes
Diazepam	No	Yes/No
Flurazepam	Yes	Yes/No
Methohexital	Yes	Yes
Pentobarbital	Yes	Yes
Phenobarbital	No	Yes/No
Imipramine	No	-
Chlorpromazine	No	-
Haloperidol	No	Yes
Ketamine	Yes	Yes
Nicotine	Yes	Yes
Phencyclidine	Yes	Yes
Procaine	Yes	-

Table 5: Drugs self-administered in female (Collins [75]) or male (O'Connor [76]) rats-the preferred regulatory species.

In 1984, Collins et al. [75] published a self-administration summary of studies conducted over a 7 year period at The Upjohn Company (Kalamazoo, MI). In it they report the abuse liability of 31 drugs tested using 788 female Sprague-Dawley rats, only. In 2011, O'Connor, Chapman, Butler, and Mead [76] conducted a literature search and

summarized 71 drugs that had high concordance between preclinical self-administration study data and actual abuse of the drugs in clinical populations. Studies conducted in male rats, only, were extracted from O'Connor et al. [76].

As shown in Table 5, using males and females provide consistent results from self-administration study designs conducted in the standard regulatory species-the rat. The minimal sex-dependent differences in preclinical self-administration study data do not provide sufficient legally-defensible, valid or reliable evidence supportive of differential schedule control actions by either FDA or DEA.

As summarized by Back et al. (2009), both national and international data indicate high rates of chronic pain (i.e., pain that persists for three to six months or more), ranging from 15% to 50% in community samples in the general population. In the 2000 Danish National Health and Morbidity Survey, women had 1.2-1.6 higher odds of reporting chronic pain than did men. In comparison to men, women typically report more frequent and more intense pain, pain in a greater number of locations throughout the body, longer lasting pain, and more interference with daily activities as a result of pain. Women also have higher rates of chronic pain conditions as compared to men including musculoskeletal pain, osteoarthritis, rheumatoid arthritis, irritable bowel syndrome, and fibromyalgia. Back et al., [77] admonish the fact that these higher rates of pain and pain syndromes among women warrant careful attention among health care providers, but do not necessarily represent a “red flag” for nonmedical use.

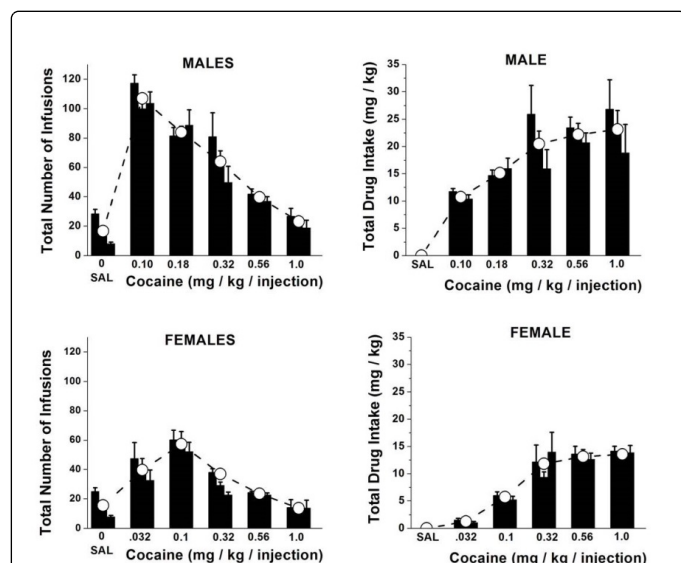


Figure 6: The total number of injections of cocaine (left panel) and the total amount of cocaine self-administered (right panel) over 3-consecutive days of 1 h operant sessions. Rats lever-pressed under a fixed ratio (10) schedule of drug deliveries to earn a single bolus of cocaine. Substitution tests were conducted under unlimited drug access contingencies in both male (top panels) and female (bottom panels) Sprague-Dawle rats in this laboratory. Each bar at saline and 0.56 mg/kg/injection represents the means of 32 trained rats. Each of the other selected doses of cocaine used to generate a full dose effect function was conducted in 6 rats.

Historical control self-administration data for male and female rats conditioned to lever press for drug deliveries under a fixed ratio-10

schedule in daily one-hour sessions are shown in Figure 6. The training and test procedures were identical to those previously described by Gauvin et al. [70]. Both male and female rats engendered an inverted U-shaped dose-effect function. While males were more robust responders and had higher intakes for the deliveries of the maintenance drug, cocaine, the conclusions drawn from the two sexes were the same. Cocaine initiated and maintained self-administration in these studies. There was a shift-to-the-left in the dose of cocaine that elicited the highest intakes when compared to the maintenance dose of 0.56 mg/kg/injection of cocaine–this “peak shift” is a common feature of all drugs of abuse in this rat assay. If this were a novel drug being tested for abuse liability, similar conclusions would be drawn and these data would initiate the 8-factor analysis for schedule control review.

Considering the sex differences described in animals by both Craft and colleagues [51-55] and Cicero and colleagues [56-58], as well as the clinical data from humans by Back et al., [77] what meaningful sex differences exist with respect to drug control policies? The non-medical use of opiates and the use of illicit opiates leading to admissions into drug treatment programs (TEDS) and emergency departments (DAWN) around the country clearly identify a preponderance of male drug abuse patterns when compared to females, indicating that the clinical relevance of any demonstrable sex differences remains unclear.

We acknowledge that the preponderance of all preclinical abuse liability screening assays have been conducted in male experimental animals (rats, and NHPs), though we would contest that this is necessarily a limitation. The pharmacokinetic profiles in animal subjects that establish sex differences in C_{max} , T_{max} , AUC, or elimination half-lives have not yet convincingly established a differential, sex-based preclinical abuse liability profile using the required three core battery of tests. Furthermore, there are no known drugs-of-abuse that are selectively misused or abused by only males or only females. The historical epidemiological control data reviewed here from reports appearing in peer-reviewed scientific journals, as well as NIH (SAMHSA) treatment-related data sets, clearly support the selective and continued use of single sex subjects in the preclinical abuse liability screening required for NDA submissions.

Conclusions

The schedule control actions dictated by international and national drug control statutes are sex-neutral. The 8-factor analysis determinative of schedule control does not require both sexes for determination of abuse liability. Though sex differences may be revealed in both preclinical and clinical research findings, no drug has been shown, through actual abuse, diversion, or treatment-related hospitalizations, to be abused by one sex and not the other. Furthermore, a potential or actual pharmacokinetic difference, based on sex, is not a determinative factor in schedule control actions or in the three core assays required for abuse liability assessments. Pharmacokinetic differences may alter subjective thresholds (ED_{50} values), but the quantal responses such as “did the test article completely generalize with the training drug” in a drug discrimination assay (yes or no), or “did the test article initiate or maintain self-administration in rats conditioned to self-administer a known drug-of-abuse” (yes or no) are not substantially altered by sex-dependent differences in C_{max} , AUC, or T_{max} parameters. Any significant difference in sensitivity to a new drug candidate seems relevant for labelling purposes under the Food, Drug and Cosmetic Act; however, it is not a determinative factor in drug control scheduling. We believe

the recent FDA decisions to require inclusion of both male and female subjects in every abuse liability study, are inconsistent with the spirit and intent of the AWA [15,16], NIH Revitalization Act of 1993 [23] and the “least burdensome principles” established by Congress [78]. The ICH Guidelines have established that a simple identification of a

sex-difference in any of the required safety assessment endpoints listed under ICH M3 (R2) [5] is not the *sin qua non* for the automatic inclusion of both sexes in preclinical safety assessments (e.g. cardiovascular QT prolongation, CNS, respiratory safety, etc.).

Drug	Approval Date	NDA#	DEA Schedule	Therapeutic Target	Were there dependent differences?	sex-PK	Was specifically targeting sex?	Sex of Experimental Animals Used in Abuse Liability Studies
Lorcaserin HCl Belviq™	2012	22529	IV	Weight management	Yes Females>Males		No	DD-Males Depend- Males and Females
Suvorexant Belsomra™	2014	204569	IV	Enhance Sleep Onset	Yes Females>Males		No	Females, only
Eluxadoline Viberzi™	2015	206940	IV (N) not completed	Irritable bowel syndrome	Yes Females>Males		No	Males, only
Rolapitant Varubi™	2015	206500	IV	Antiemetic; nausea and vomiting associated with chemotherapy	Yes Females>Males		No	Males, only
Brivacetam Briviact™	2016	205836	IV	Adjunctive therapy in the treatment of partial onset seizures in epilepsy	Yes Females>Males		No	Males, only

Table 6: Recent NDA-related schedule control actions by FDA and DEA.

For comparison purposes, Table 6 shows that since the 1992 NDA approval of zolpidem by FDA, five different new drugs submitted for NDA approval and subsequently controlled under the CSA were based solely on male drug discrimination (5/5) and self-administration (4/5) studies. All self-administration and drug discrimination studies of the most recently approved CNS-active drugs were conducted in only one sex. Dependence liability assessments with Belviq™ were incorporated with standard toxicology program study plans and thus included male and female animals. Review of the approval notices demonstrated that all five drugs showed greater female sensitivity to the drug when compared to male cohorts. Belsomra™ abuse liability studies were conducted in only one sex, the more sensitive females. Abuse liability studies for Viberzi™, Briviact™, and Varubi™ were all conducted in male subjects. The FDA recommended schedule control actions based on male only abuse liability data (including the 1992 approval of zolpidem, discussed above).

Just like randomization, blinding, sample size calculations, and other basic design elements, consideration of sex is a critical component of rigorous experimental design. Failure to account for SABV may undermine the rigor, transparency and generalizability of research findings in the clinical arena, but it has minimal regulatory weight with respect to drug control decisions. The CSS staff at FDA expects researchers to study both male and female vertebrate animals and NIH expects male and female human subjects where applicable, thereby improving our understanding of health and disease in men and women. (http://orwh.od.nih.gov/sexinscience/overview/pdf/NOT-OD-15-102_Guidance.pdf). The expectation of the NIH for the researcher is to “explain how relevant biological variables, such as sex, are factored into research designs and analyses for studies in vertebrate animals and humans” in NIH (public) funded research grant endeavours.

Furthermore, “strong justification from the scientific literature, preliminary data, or other relevant considerations, must be provided for applications proposing to study only one sex.” In its reliance on scientific literature, the NIH adopted the view that if there are differences between males and females in previous preclinical studies, this would provide a strong rationale for building consideration or exclusion of sex into the research design and analyses of data. FDA has generalized these requirements to all privately funded research conducted by FDA registrants.

The choice of research design depends on a variety of considerations, including preliminary data, past studies, scientific literature review, scope of the work, and specific questions and hypotheses to be addressed. Where little or no sex-specific data are available, sex-specific hypotheses may not be possible, whereas previously observed sex differences may prompt sex-specific hypotheses. Researchers working with animal models should consider if and how the female estrous cycle is relevant for experimental design and analysis; it may be relevant for some research questions and not others.

We have reviewed the extant literature of CNS-active drugs that have been scheduled under the CSA. While subtle sex-differences exist with respect to drug sensitivities, there are no clear evidentiary examples that dissociate controlled vs. non-controlled drug status based on sex under the CSA. Furthermore, while statistically significant sex differences can present in abuse liability assessments, there are no current drugs of abuse that show differential sex-dependent criteria for schedule control actions under the 8 factors determinative of schedule control under national and international drug control policies. Drugs that are self-administered by male animals are also self-administered by female animals. Drugs that generalize

partially or completely to a known drug-of-abuse in males will generate similar profiles in females. Additionally, drugs that produce a discernable discontinuation syndrome following abrupt cessation of sub-chronic, repeat dose administrations in males will also be characterized in female animals.

In summary, administrative precedence by FDA has been established by the approval of such drugs as Ambien™, Belviiq™, Belsomra™, Viberzi™, Briviact™, and Varubi™, which have a clear sex-based differential pharmacokinetic profile demonstrating females were more sensitive than males, yet drug approval was based solely on data collected using male subjects.

We believe these data are sufficient to meet the expectations of what McCullough et al., [19] referred to as “reasonable exclusion” criteria to conduct the core abuse liability studies in only one sex (<https://www.niaid.nih.gov/grants-contracts/decision-tree-inclusion-women-part-2>). According to Clayton and Collins [20], the NIH and FDA are now developing policies that require applicants to report their plans for the balance of male and female cells and animals in preclinical studies in all future applications, unless sex-specific inclusion is unwarranted, based on rigorously defined exceptions.

We believe there is sufficient credible data regarding the validity and reliability of using one sex in the required drug discrimination, self-administration, and dependence liability studies that are conducted to address drug control policy 8-factor reviews at the time of the NDA review. The use of one sex in the regulatory required preclinical assays remains in full compliance within the ICH S7A Safety Pharmacology [4] requirements for NDA submissions with respect to drug scheduling by DEA. The reduction in animal use in these study designs ensures that:

1. The process of testing remains grounded in the spirit of the UN Drug Control Conventions [8-10];
2. Allows for full compliance with the United States Code (USCA, Title 21, Chapter 13, §801; the CSA) of the Comprehensive Drug Abuse and Control Act, 1970) [6];
3. Offers sufficient flexibility to allow for the application of the most advanced science, at all times;
4. Meets the “best practices” model for the “quality of evidence” required for risk assessment analysis espoused by the Center for Disease Control ([79]);
5. Is in full compliance with the spirit and intent of the NIH policy for “Consideration of Sex as a Biological Variable in NIH-funded Research” (http://orwh.od.nih.gov/sexinscience/overview/pdf/NOT-OD-15-102_Guidance.pdf);
6. Achieves the spirit and intent of the 3 R’s- Replacement, Reduction, and Refinement [80]; and
7. Meets the spirit and intent of the Congressionally-mandated policies enacted through the AWA [15] and the NIH Revitalization Act of 1993 [23] relative to the reduction of the number of animals used in research.

Understanding the mechanisms of sex differences in drug therapy is critical for optimal dosing in both sexes when FDA is reviewing the NDA for label development. Evaluation of sex differences in PK of drugs certainly can enhance our understanding of sex-based differences in the safety and efficacy of drugs and minimize therapeutic adverse events. We acknowledge that PK differences are the most common sex differences and early detection of these differences during drug development can lead to clinical trial design

that will use sex-based dosing and better individualization of therapy as suggested by the NIH policies. However, the establishment of regulatory required abuse liability studies based on FDA’s role in labelling or dosing recommendations cannot substantiate the need to increase the number of animals used in preclinical study designs under the AWA [15] or the NIH Revitalization Act [23].

FDA has established a process of developing tools to be used by both Agency staff and its stakeholders to periodically assess the implementation of the least burdensome principles to determine the type of valid scientific evidence needed to support marketing approval [78]. FDA has taken the opportunity to encourage its stakeholders (i.e., the pharmaceutical industry) to use these assessment tools to accurately assess the Agency’s incorporation of the least burdensome principles into its various regulatory activities. The Agency encourages industry evaluation of its efforts to determine whether the least burdensome approach is being successfully implemented and to accurately assess its impact on the public health. In establishing the statute, Congress added sections 513 (i) (1) (D) and 513 (a) (3) (D) (ii) to the Federal Food, Drug, and Cosmetic Act. This is the objectives of this review. These provisions capture both of the ideas expressed in the legislative history: FDA should eliminate unnecessary burdens that may delay the marketing of beneficial new products.

Any determination of schedule control actions must be legally-defensible, and based on valid and reliable science-based data. This, by definition, requires a minimum number of male and female subjects determined by standard statistical power analyses, sufficient to determine reliability of any sex-based differences above those expected by chance. With the current public sentiment driving the “zeitgeist” of public policy regarding the reduction in use of animals in research, and the lack of any credible data to suggest a differential outcome of preclinical abuse liability testing that would alter the course of schedule control actions on the NME, we believe that the recent CSS/CDER recommendations to include both sexes in all abuse liability assessments are in direct opposition to these goals and places an additional regulatory burden on FDA registrants whose intent is to bring NME’s to market.

Highlights

- Federal statutes require the minimization of laboratory animals used in research.
- Recent interactions between pharmaceutical industry and FDA have required the inclusion of both sexes in abuse liability testing.
- There are sufficient exclusionary criteria to sustain the standard of single sex study designs in abuse liability screening.

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