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Assessment of Dependence Liability of New Molecular Entities under the Current FDA Draft Guidance Document: "Seeking Best Practices"

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Abstract

In a recent series of unprecedented collaborative meetings between U.S. FDA members of the Controlled Substances Staff (CSS) within the Center for Drug Evaluation and Research (CDER) and the pharmaceutical industry (pharmaceutical research and manufacturers association–PhRMA) members have delineated a "standard" for conducting preclinical abuse liability screening of all new molecular entities that affect the CNS. We argue for a "mind shift" in policies and methodologies used to quantify the potential discontinuation syndrome that may be engendered following abrupt cessation of repeated dose administrations of all NMEs under this new model. We argue against the use of the current "fixed dose" strategy to the more informative "escalating dose" or "equivalent dose" strategies that more accurately predicts the dependence potential of drug substances regardless of expected therapeutic doses.

Keywords: Drug dependence; Dependence liability; Abuse liability testing; Preclinical development; Discontinuation syndrome; Methods; Review

Introduction

In 2010, the Controlled Substance Staff (CSS) of the Center for Drug Evaluation and Research (CDER) of the United States Food and Drug Administration (FDA) issued a draft guidance document titled, "Assessment of Abuse Potential of Drugs" (CSS) [1]. Over the last decade the CSS of CDER of the FDA has worked diligently with the Pharmaceutical Research and Manufacturing Association (PhRMA) to establish a dialogue on the development of standards for the preclinical screening of NMEs for schedule control actions required as part of every New Drug Application (NDA) process. This has been an unprecedented and unique collaboration between government and industry to develop strategies that align nonclinical drug safety, toxicology, and pharmacokinetic (PK) evaluations related to the preclinical screening of New Molecular Entities for their potential to be diverted, misused and abused once approved for human consumption.

Risk assessment plans for abuse potential are reduced through knowledge and best scientific practices. It was the intent of both industry and government regulators to set forth an action plan that was clear, concise, and in full accordance with national and international drug control policies. The current thinking within the U.S. Federal Public Health Policy (National Research Council [2] and the European Monitoring Centre for Drugs and Drug Addiction [3] in regards 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 and the risk assessment must consider how people actually use drug substances outside the scope of medical practice which includes consideration of cognitive and behavioral factors affecting human judgment and decision-making [4].

The purpose of this review is to highlight the specifics of the methodologies detailed in the dialogue sessions promulgated by the agency as "best practice" to assess the dependence potential for all NMEs. We intend to highlight specific operational details that may influence the reliability of these assays to accurately predict the liability of NMEs and, even if completed per these "guideline-directed practices", the data may not provide sufficient support for adequate schedule control actions.

The Methods of Dependence Liability Assessments Based on the CSS-PhRMA Dialogue Sessions:

A summary of discussion points regarding preclinical dependence liability assessments that were covered during the 2006 to 20010 PhRMA and CSS dialogue session are listed in Table 1. The full presentation from the dialogue sessions can be accessed at: http://www.fda.gov/AboutFDA/CentersOffices/OfficeofMedicalProductsandTobacco/CDER/ucm180766.htm.

Both parties were aware that these sessions were not legally-binding on the agency and the CSS staff did not represent or speak on behalf of the FDA. During these sessions, PhRMA proposed four preclinical study scenarios, supportive hypothetical data, and a set of questions regarding the interpretation and level of evidentiary status for conducting drug control reviews by the CSS. The CSS staff of CDER then reviewed and addressed each scenario with their view and "current thinking" on the subject, in this case, drug dependence liability (Table 1).

A	The presence of physical dependence (withdrawal behaviors following drug discontinuation) is not sufficient to indicate abuse potential. However, full characterization of a drug's abuse potential does require assessment of physical dependence;	PhRMA Question 10: CSS Slide 29
	Even if the Sponsor were to accept Schedule II status, the highest control schedule for a safe and effective drug, abuse	PhRMA Question

	potential studies are still necessary in order to write an accurate label.	47: CSS slide 103
B	The preclinical dependence liability studies should not be conducted until Phase 2 or Phase 3 clinical trials have selected a proposed clinical therapeutic dose.	PhRMA Question 48: CSS Slide 105
C	The purpose of assessing physical dependence is to determine whether the drug produces withdrawal behaviors following discontinuation of the drug.	PhRMA Question 37: CSS slide 91
C	It is necessary to fully characterize the abuse potential of a centrally-active NME in order to write an accurate Drug Abuse and Dependence section of the product label and for CSS staff to be able to prepare recommendations in support of scheduling, if necessary.	PhRMA Question 47: CSS slide 103
D	Observations for a "discontinuation syndrome" following abrupt cessation of treatment of the NME should:	
D	1. Be based on the pharmacokinetic parameters of the drug for the species used.	PhRMA Question 36: CSS Slide 90
D	2. A compendium of behavioral and physiological observations should be identified in advance of this study based on those behaviors related to known withdrawal signs and symptoms in animals and humans associated with drugs from the same pharmacological class appearing in the peer reviewed scientific literature (if possible);	PhRMA Question 36: CSS Slide 90
D	3. Include frequent observation periods during the first 8 hours following drug discontinuation and should extend for a long enough duration as to detect all behaviors.	PhRMA Slide 11: CSS Slide 27
D	4. Document all behaviors observed and should not be limited to a given set or lists of behaviors of interest. All unexpected findings should be documented to help to understand the drug's actions.	PhRMA Slide 11: CSS Slide 27
D	5. Behaviors of interest include not only changes in body weights, but also changes in feeding, locomotor behavior, as well as unusual or unexpected behaviors	PhRMA slide 34: CSS slide 86
D	6. Video recording may be helpful during the study that would allow for peer review by multiple raters, and	PhRMA Slide 11: CSS Slide 27
D	7. If the therapeutic indication for the NME is for females, only, then abuse potential should include female animals.	PhRMA Slide 11: CSS Slide 27
D	8. The duration of observation for a discontinuation syndrome should extend to at least 3 to 7 days, and it may be necessary to extend the observational period further if the drug is known to be eliminated slowly	PhRMA Question 13: CSS slide 32
D	9. Body weights should be taken daily throughout the study. Since animals respond differently with handling, the amount of "social interaction" with the animals should be maintained through both dosing and withdrawal assessment periods of the study.	PhRMA Comments on Slide 35: CSS slide 88
E	The functional observational battery (FOB) should be used for documenting behavioral changes on study. It should be "open-minded". Do not limit observations to a pre-specified list of possible behaviors (i.e., based on similar drugs in the same pharmacological class)	PhRMA Comments on Slide 35:
		CSS Slide 88)
F	The duration of the repeat-dose phase of the study should be based on the derived elimination half-life of the NME established in the IND phase of experimental history. For most drugs, 14-day duration of drug administration should be sufficient.	PhRMA Question 38: CSS slide 92, and PhRMA Question 12: CSS Slide 31
F	However, with drug with half-lives that are relatively long, additional drug dosing may be necessary prior to discontinuation.	PhRMA Question 12: CSS Slide 31
G	In regards to pharmacological exposures during the repeat-dose phase of the study, should the plasma concentrations be variable (qd, bid, tid, or qid dosing) or fixed (IV infusion or osmotic mini-pump)?	PhRMA Comments on Slide 35: CSS Slide 88; And PhRMA Question 38: CSS slide 92
G	1. The drug exposures should parallel the targeted exposure in clinical populations. The dosing strategy may depend on the intended use of the drug and the PK parameters of the drug. (CSS response to PhRMA Comments on Slide 35: CSS Slide 88; and CSS response to PhRMA Question 38: CSS slide 92).	
G	2. For example, a drug may be intended for chronic use if steady-state levels of the drug are needed for optimal clinical effects	PhRMA Question 14: CSS Slide 33).
G	3. If the clinical drug formulation is to be a "controlled-release" or "sustained-release" oral medication, then a mini-pump may be an appropriate method for delivering drug to animals in this study design.	PhRMA Question 14: CSS Slide 33
G	4.Continuous infusion may be appropriate if an extended release formulation is being developed, while single-dosing may be justified if the plasma levels parallel those observed in humans with once-daily dosing	PhRMA Question 39: CSS slide 93
G	5. If the drug produces PK profiles that peak and trough across the day, then the animal drug administrations should attempt to produce a PK profile that is similar as possible	PhRMA Question 14: CSS Slide 33; and PhRMA Question 36: CSS Slide 90
G	6. The dose of the drugs used should remain stable over the course of the dosing period (fixed dose strategy; (that is, the dose should not be increased over time: e.g., no escalating dose or equivalent dosing strategies	PhRMA Question 38: CSS slide 92).
G	7. The specific fixed doses selected for dosing (i.e., 1.5 X Ceff) should be based on the clinically therapeutic dose, safety profile, and PK of the drug. A reasonable estimation of the clinically therapeutic dose should be known before undertaking some studies.	PhRMA Question 39: CSS slide 93).
H	Positive Control Article:	
H	1. The positive control article selected can produce beneficial information that can be used in comparison with the test drug, but it is not required.	PhRMA Question 40: CSS Slide 94)

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2. Justification is needed for the choice of any positive control article selected for the study.	PhRMA Comments on Slide 35: CSS Slide 88	
3. If a positive control is chosen it should have a similar mechanism of action as the test drug. If the test drug is a novel mechanism of action, then the choice of positive control may be justified on the basis of similarity in therapeutic indication.	PhRMA Question 40: CSS slide 94	
As stated, above, the purpose of the physical dependence study in animals is to predict what behaviors may be observed in humans during the drug discontinuation process. Thus, a "naturalistic withdrawal period", that is also known as "abrupt withdrawal", or as stated above as, "direct addiction" is the preferred design. In contrast, the precipitated withdrawal test, in which a known pharmacological antagonist is administered to induce withdrawal, is NOT the preferred design.	PhRMA Question 41: CSS Slide 95).	
1. Precipitate withdrawal may not be available for new mechanisms of action. It is noted that the antagonist-induced precipitated withdrawal can be useful in delineating the mechanism of action of the withdrawal process and providing useful information related to overdose and complications related to Emergency Room treatments. If an antagonist exists clinically for the NME, it is important to characterize the withdrawal syndrome that will emerge if the antagonist is utilized under Emergency Room conditions – i.e. overdose,	PhRMA Question 41: CSS Slide 95	

Table 1: Summary of Study Design Issues Delineated During CSS-PhRMA Dialogue Sessions (2006-2010).

Issues of "Standard Study Designs", "Best Practices" and Drug Control Policies

National and international drug control policy requires the best, most accurate, valid and reliable data to base control decisions that best serve its function of preventing and reducing the diversion of drugs from medical practice to "the streets". Almost 70 years of WHO drug control policies have been developed and implemented based upon actual abuse patterns and the collective preclinical animal data from laboratories from the numerous member states of the drug control treaties. For decades the generally held belief was that diversion of pharmaceutical grade opiates was led by illicit heroin users during times of "drought" or short supply to avoid withdrawal or anticipatory anxiety about impending withdrawal (albeit real or imagined). In 2014 NIDA [5] revealed the stunning and recent trend in the U.S. of just the reverse—a switch from prescription opioids to heroin when the cost and availability of legitimate pharmaceuticals (e.g., Vicodin® or OxyContin®) becomes too high or the immediate supply chain is broken. Such information is especially relevant to researchers and policy makers given that drug control policies are based on the premise of study conduct employing industries' best practices.

What is known about the scope and definitions of best practices? In a 2013 paper from the Center for Disease Control and Prevention (CDC), Spencer et al. [6] attempted to define the term "best practices" with respect to regulatory-based practice of science and public policy. Spencer et al. confessed that a consensus definition of "best practice" was not found, however, some common elements were. In particular, Spencer and colleagues found that "best practice" and related terms do not refer to a static assessment or activity; rather, they refer to where, on a continuum, a particular practice falls at a given time. The review identified multiple ways to characterize this continuum or hierarchy,

along with considerable variability in the number of stages or levels and in the rigor of methods used for identifying best practices.
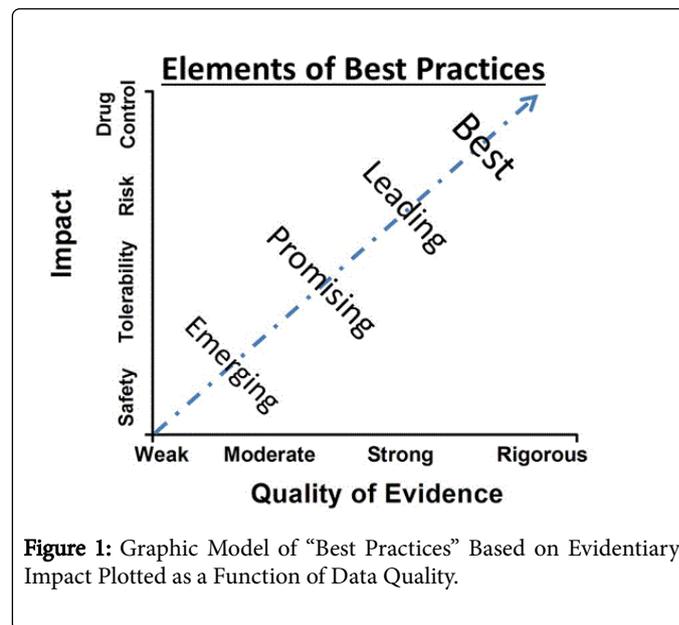
Figure 1: Graphic Model of "Best Practices" Based on Evidentiary Impact Plotted as a Function of Data Quality.

The "best practices" model used by the CDC wing of the Department of Health and Human Services (HHS), the same Executive Branch Department as the FDA, is shown in Figure 1. The "quality of evidence" refers to where a practice lies on an evidence-based practice continuum. These elements represent four levels of evidentiary quality: weak, moderate, strong, and rigorous. For example, general receptor binding assays, with possible but not direct impact on predicting abuse liability, may be considered "weak" or "initial" evaluations (as already stated in the draft guidance document).

Drug Dependence Liability Assessments: What currently defines Best Practices?

As detailed above in Section 2, above, the PhRMA-CSS dialogue sessions clearly identified that antagonist-induced (precipitated) withdrawal paradigms are not preferred and should not be conducted as part of the NDA approval abuse liability testing. This suggestion is most likely based on:

The fact that pharmacological antagonists are not benign substances – they have stimulus properties of their own.

the physical signs of the precipitated withdrawal syndrome is likely to be more intense and qualitatively different from the syndrome that presents following abrupt cessation of treatment – this may effectively over-exaggerate the severity score and unduly place the NME in higher schedule under the CSA, and

There may not be a known pharmacological antagonist for NMEs with novel mechanisms of action.

The interested reader is directed to Bläsig and Herz, Harris, Frenois, Cador, Callé, Stinus, and Le Moine [7-9], for further review of opioid-related precipitated withdrawal procedures and effects.

Best Practices: Fixed versus Escalating Dose Strategies

As summarized by Langerman, Piscoun et al. [10] the lack of uniformity in the drug delivery methods, dosages, techniques, and

indicators used for the evaluation of withdrawal intensity substantially complicates the comparison between studies. We posit a premise that the position of exclusive use of "fixed dose strategies" in conjunction with the stated objection to the use of "escalating dose strategies" in the current thinking of the draft guidance document does not appear to be consistent with the published, peer-reviewed scientific literature and does not represent "Industry Best Practices" under International and National Drug Control Policies.

Physiological dependence is an altered biological condition caused by repeated drug intake that must be continued to prevent the appearance of specific illness known as the withdrawal syndrome. As suggested by Essig [11,12] since dependence varies with the drug involved it is scientifically prudent that the term be coupled with the drug involved (e.g., dependence of the opiate type).

Chronic drug treatments of longer duration and/or greater cumulative dose are associated with both greater frequency and intensity of withdrawal symptomatology [13]. However, Goldstein [14] has pointed out that the degree of withdrawal reactions tends to level off after weeks of high dose exposures. There appears to be a maximum that would not be exceeded even with prolonged exposures of higher and higher doses. The plateau for each effect is dose-related. Based on theoretical grounds, the time for approach of the withdrawal intensity plateau would be a property of the drug receptor or target system and therefore all drugs that elicit physical dependence by the same mechanism should have the same time course even though they might differ in potency. Differential time courses to the plateau by two drugs may suggest that the drugs elicit physical dependence by different mechanisms [15].

In his review of the dependence liability literature, Aceto [16] concluded that if the objective of the study is to determine the "inherent" physical dependence liability of a substance, then the "dose is raised progressively and maintained at the highest tolerable level". This strategy is more likely to induce dependence than any other procedures reported in the literature. There are at least two methodologies used within the "escalating dose" strategy of dependence induction: 1) the dose schedule is adjusted as tolerance develops to selected actions of the drug preselected by the Sponsor, (such as analgesia, or rate-of-responding in a standard operant lever press response) or 2) the use of a dose equivalent to that of a reference standard or behavioral endpoint, such as [13,17-19]. For example, Martin, Wikler, Eades, and Pescor [20] used an escalating dosing procedure first described by Sloan, Brooks et al. [21] because it had been found not to cause loss of body weights in the experimental rats over 43 days of dosing.

As described above, multiple factors are involved in selecting the doses for dependence liability studies. Okamoto [13], Hollister [18] and Goldstein [14] have all suggested that the severity of withdrawal reaction is related to the rate of disappearance of drugs from plasma. Drugs that have short half-lives have little potential for producing physical dependence because their residual CNS concentrations at the time of successive doses are small. There is insufficient drug to produce and maintain functional dependence. According to these authors, drugs that have long half-lives are less likely to result in severe withdrawal signs and symptoms despite the fact that they produce severe physical dependence. That is, the CNS can gradually re-adapt, when the drug is no longer administered, due to the slow elimination of the drug from the CNS.

During protocol development it is important that the dose of drugs be selected as to insure that the CNS is chronically exposed to the drug across the full dosing intervals (14 or 30 days). If drug plasma concentrations approach zero, withdrawal reactions will be expressed. If the half-life of the compound is short, then the animal will be exposed to many small episodes of withdrawal over the course of dosing. If two groups of animals have achieved the same magnitude of physical dependence with two different drugs, Goldstein [15] predicted that the shorter-acting drug would produce the stronger withdrawal reactions. Van der Laan and de Groot [22] van der Laan et al. [23] included that studying spontaneous morphine withdrawal requires regular administration of morphine to take place over a period of several weeks to induce symptoms which can be used to evaluate the effectiveness of therapeutic treatments.

From a practical point of view with respect to FDA's Physician Labeling Rule (PLR; Requirements on Content and Format of Labeling for Human Prescription Drug and Biological Products, 71 FR 3922, January 24, 2006 and 21 CFR §201.56(d) and §201.57) requirements, there is little concern whether dependence exists as long as there is no withdrawal reaction. In contrast, international drug control policies require the evidence that a dependency state exists or can be induced, regardless of whether or not this state is overly displayed as a withdrawal reaction. This legally-binding requirement is based on differentiating between drug control reviews conducted under the 1961 Single Convention on Narcotic Drugs – those NMEs that do show drug dependence potential, and reviews conducted under the 1971 Convention on Psychotropic Substances – those psychoactive substances that do not show dependence liability. International treaty commitments to the other member states of the U.N. are based, in part, on differentiating CNS-active NMEs based on the property of dependence liability. This requirement is codified in the U.S. under the Comprehensive Drug Abuse and Control Act of 1973 (aka Controlled Substances Act) [24].

Industry Best Practices for dependence liability assessments require the use of protocol methodologies that provide the highest degree of predictive validity to best answer two agencies' objectives: 1) the FDA's concern for safety assessment and labeling requirements, and 2) the DEA's concern for national and international drug control policy compliance. The stringent criteria for drug control for all NMEs are the foundation of the 8-factor analysis conducted independently by both regulatory agencies. Based on this primary premise of drug control (DEA) and health safety policies (FDA) and based on multiple literature reviews by many of the world's most prominent scientific leaders in drug abuse testing, the most reliable dosing methodology in dependence liability screening appears to be the "escalating dose strategy".

Cases - in – Point: Fixed Dose Strategy

Case I: Varenicline is a nicotinic acetylcholine receptor partial agonist used to aid smoking cessation and nicotine addiction. Varenicline was approved under NDA 21-928 on May 10, 2006 as Chantix®. It was approved in two strengths, a 0.5 mg capsule containing 0.85 mg of the varenicline tartrate salt and a 1.0 mg capsule containing 1.71 mg of the salt. In the "Pharmacology Review(s)" available on the FDA website at: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2006/021928_s000_Chantix_PharmR.pdf the results of Study Reference Number 2004-63943 (page 29) was summarized as a standard operant lever-press food-reinforced (FR-10) rat study in which each rat had the opportunity to earn 50 reinforcer deliveries in

repeated daily sessions. An acute pre-session dose administration study of varenicline was used to generate a behavioral response-rate dose-response function (CP-526,555-18). An ED-50 for response rate suppression was determined to be 1.7 mg/kg. Based on these findings a "dependence/tolerance liability study" was conducted by repeated pretreatments of 1.7 mg/kg of varenicline prior to the food-motivated operant task for 14 days. To assess tolerance and/or signs of discontinuation syndrome, rats were observed for clinical signs prior to each daily dose, for a minimum of 5 minutes post-dose, and once again "several hours later". Each rat received daily "practice sessions" in the lever-press operant task to document the development of behavioral tolerance to varenicline's behaviorally-disruptive effects over the 14 day repeated dose exposure. The study results were summarized, as follows:

Tolerance, defined by a return to day 0 or basal response rates, developed to the 1.7 mg/kg varenicline treatment after 10 days of dosing. Discontinuation of varenicline after 14 days and substitution with sterile water on Days 15 through 21 resulted in no change in response rate, and no observable behavioral effects. Withdrawal from varenicline did not result in any nicotinic withdrawal behaviors such as teeth chattering, chewing, gasping, writhing, head shakes, body shakes, tremors and ptosis.

Interesting to note, the half-life of 3.0 mg/kg orally administered varenicline in the rat was reported to be 4.0 to 4.1 hours (Table, Single Dose, Section 2.6.5, page 72) and the No-Observable-Adverse-Effect (NOAEL) level in the rat was reported to be 3.0 mg/kg in a 6 week, and 10 mg/kg in a 3-month and 10-month standard toxicity studies (Section 2.6.6.10, page 226).

The neurological squeal of chlordiazepoxide withdrawal in the rat resemble that seen in the "dependence syndrome of the barbiturate type" previously described.

Based on the 4.0 hour half-life, is it reasonable to predict an adequate assessment of a NME's dependence liability in study designs using once-a-day fixed dosing of the drug with such a short half-life? With a demonstrated NOAEL of 10 mg/kg orally administered drug, is it reasonable to predict an adequate assessment of a NME's dependence liability using a 5 to 6 fold lower fixed dose strategy of 1.7 mg/kg in rats? While the current draft guidance document proposes the use of FOBs that are "open ended" and admonishes against limiting observations to a pre-specified list of possible behaviors (i.e., based on nicotine, in this case; See Item D, Table 1), is it reasonable to predict sensitivity to a discontinuation syndrome by a NME that the FDA acknowledged had primary pharmacodynamic effects through partial dopamine agonist activity (NDA 21-928, page 18)? And finally, is it reasonable to predict the best sensitivity of standard clinical observations conducted pre-dose, 5 minute postose, and "several hours" following sterile water administrations on Day 15 through 21 to adequately identify signs of a discontinuation syndrome?

Recently, Toffey, Rabin, and Kurlan [25] reported two cases of Chantix® related withdrawal convergent dyskinesias. In their report, these authors report that of 60,675 patients reporting "side effects" to FDAs "eHealthMe" databasein 2013, 25 had tardive dyskinesia. Tardive dyskinesias have been linked to chronic dopamine blockade.

Case 2: Pregabalin was approved on June 24, 2004 under NDA #21-446. The proprietary name of Lyrica™ was used for the treatment of diabetic neuropathy. In the NDA pharmacological reviews a 4 week repeated oral dose cardiovascular study (page 78) was described as administering a maximal dose of 500 mg/kg (bid, with 4 hour

interdose intervals) in cynomolgus monkeys. In that study 3 animals died. In another repeat IV dose administration study (Report #250-01675) 300 mg/kg/day continuous intravenous infusion dose was administered for 14 days (10 mL/kg) with no deaths, and was used to support the requisite dependence potential aspects of the "abuse liability' section of the NDA (page 33, of the "Pharmacology/Toxicology Review and Evaluation" section, dated May24, 2004).

Abuse liability: The Sponsor conducted multiple nonclinical studies to examine the potential abuse liability of pregabalin…….Pregabalin also did not maintain IV self-administration studies in rhesus monkeys. However, there was some evidence of withdrawal signs in rats upon cessation of treatment, although this effect was not statistically significant. Overall, the preclinical data would suggest the pregabalin has a low abuse liability….(page 33).

Lyrica™ was subsequently placed into Schedule V of the CSA, based on the conclusion that it had less liability than Scheduel IV, benzodiazepines.

Recently, Aldemir, Altintopprak, and Coskunol [26] reported on a case of pregabalin abuse and the presentation of a discontinuation syndrome. At the time of admission, the patient had escalated his initial dose of 150 mg per day upward to a daily consumption of 15,600 mg (15.6 grams) by taking 104 (150 mg) capsules a day, and frequently used 7,800 mg daily dose. With such lenient control status afforded by the WHO on pregabalin, it is not surprising to understand the "ease of access" a patient would have under these conditions. At the lowest consumption to avoid withdrawal he was consuming 1,950 mg (1.95 grams) by oral administration of 13 capsules containing 150 mg of API per capsule a day. Using the fixed dose strategies of the draft guidance document recommendations, one has to question the validity or reliability of this dosing strategy compared to actual drug use by patients consuming the product outside the scope of medical practice.

Dependence Liability "Best Practices": The Critically-Relevant Literature:

In their comprehensive review of the abuse liability of benzodiazepines, Woods, Katz, and Winger [27] described the results of a series of previously published primary dependence studies. Since dependence is presumably more likely to develop or to be of greater magnitude with greater exposure to drug, attempts often are made to administer the highest tolerable dose of the drug. In a later review, these same authors [27] further summarized that withdrawal signs are more frequent or of greater magnitude (a) following administration of higher doses or doses with greater effects, (b) following longer duration of treatment, or (c) following continuous rather than intermittent drug administrations [28].

In his review, Aceto [16] concluded:

"The dose is raised progressively and maintained at the highest tolerable dose"

Tomoji Yanagita [29] highlighted the general findings that using fixed dose strategies allow for tolerance to develop to the drug which lessens the intensity or shortens the duration of these effects (p. 51). He then concludes:

"Therefore the dosing schedule in the physical dependence producing test has to be determined according to the gross behavior manifestation of the drug effects, so that the intensity and duration of the effects at a certain level are maintained……..a dosing schedule that

allows the maintenance of the plateau effect level be used, what is sought in this test is the physical dependence potential of a drug when the drug is used non medically, rather than when it is used medically under a doctor's auspices."

In her evaluation of dependence liability of CNS depressants, Michiko Okamoto posits the premise that if physical dependence production results primarily from the chronic depression of the nervous system, then the measurement of the degree of CNS depression produced during dependence production is as important as the measurement of chronic drug load and be best achieved by an experimental design that permits production of equi-effective CNS depression across the repeat-dose phase of the study. According to Okamoto, the "maximally tolerable" dose method, in which each animal is treated with escalating doses of drug to produce equi-effective CNS depression throughout the chronic or sub chronic dosing regimen. Under this methodology, Okamoto suggests:

The sequentially adjusted drug [sic] doses are given…to produce a chronic depression of 10-11 according to our standard CNS depression rating scale.

On three separate occasions, the Committee on Problems of Drug Dependence (CPDD), in collaboration with the NIDA, have published their "state-of-the-art" reviews on standardized methodologies and terms associated with the "Testing Drugs for Physical Dependence Potential and Abuse Liability" [30-32]. The CPDD and NIDA clearly delineated the procedures for assessing physical dependence potential in animals. In the 1984 Monograph, under the heading of "Withdrawal from Chronic Drug Administration" the consensus of CPDD and NIDA was:

"…the test compound is administered on a chronic basis to drug-naïve animals; the initial dose is usually low, and as tolerance to toxic effects develops, the dose is increased" (p. 19).

In the same document, the consensus of the CPDD and NIDA for the assessment of "primary physical dependence" of CNS depressants was described as:

"Ideally, evenly spaced injections should be used, but dependence can also easily be produced with two injections per day: 1 in the morning and 1 in the later afternoon or evening. As tolerance to the sedative and depressant effects develops, the maintenance dose is increased."(p. 37)

As described in Table 1, above, the CSS-PhRMA dialogue sessions delineated the exclusive use of "fixed dose strategies", and specifically stated the position that "escalating dose strategies" should not be used to assess the relative drug dependence liability of NMEs. Based on the half-life (elimination rate) the divided doses have to be selected to be high enough to maintain test article blood levels above zero before the next scheduled dose, but be low enough to not interfere with cardio-pulmonary function, or the ability to drink and eat. Based on the preponderance of valid and reliable data (described, in part, above), we take the position that a fixed dose strategy is not "Industry Best Practices".

Best Practices for the Selection of Chronic Doses (dose range)

It is most interesting to note that none of the three major International Drug Control Treaties [33-35] as well as the W.H.O.'s Guidelines for the W.H.O. Review of Dependence-Producing Psychoactive Substances for International Control [36] make a single reference to, or mention, the term "therapeutic dose". Additionally, not a single reference to "therapeutic dose" can be found in the U.S. Controlled Substances Act [24]. Risk assessment analysis for schedule control action is unique in its purview. A presumptive factor in schedule control action is based on the premise that the liability assessments be viewed as behavioral patterns outside the scope of medical practice. That is, determination is NOT solely based on the therapeutic target, the relative therapeutic plasma concentration of the NME, or on a belief that the NME will be taken as prescribed. The targeted therapeutic dose is minimally relevant with respect to existing drug control policies. The current thinking within the US Federal Public Health Policy [2] and the European Monitoring Centre for Drugs and Drug Addiction [3] in regards 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 behavioral factors affecting human judgment and decision-making [4]. In the clinical population the progression from prescription use, to misuse, to abuse follows the voluntary and intentional progressive increase of self-administered doses that is more consistent with the escalating dose strategy in animals. We would posit the premise that modelling such dosing strategies in preclinical dependence liability studies seems intuitively more logical than fixed dose strategies.

It is well known that small withdrawal symptoms have the potential to serve as sub-threshold chemical stimuli in a process called "chemical kindling". Both clinical and experimental evidence support the existence of a kindling mechanism during withdrawal. Withdrawal symptoms result from neurochemical imbalances in the brain when drug administration is discontinued. These imbalances may be exacerbated after repeated withdrawal experiences [37-39]. If over the weeks of drug exposure an animal is exposed to repeated sub-threshold withdrawal symptoms, the degree and magnitude of the final withdrawal symptoms following termination of the repeat-dose phase will be potentiated or larger than expected due to chemical kindling of CNS effects.

Dose selection for dependence liability studies is the most critical and data-based decision that needs to be made in any of the three abuse liability paradigms (drug discrimination, self-administration, and dependence liability). Dose selection is based on the pharmacokinetic information available at the time of dependence liability testing in rats. Doses have to be carefully selected to be high enough to provide sufficient plasma drug concentrations from the first dose of the day at the time of the next scheduled dose (i.e., bid, tid, etc.) to avoid withdrawal. However, the individual doses have to be low enough as to not threaten the life of the rat by such direct effects as respiratory depression, single dose lethality, or motor control impairments that hinder access to food and water. These are further complicated by the need to ensure doses of "several fold" higher than the targeted therapeutic plasma concentrations "Therapeutic C_{max}".

Shown in Table 2 (below), is the list of descriptors related to the discontinuation or withdrawal syndrome in rats expressed following 30 days of 75 mg/kg b.i.d. dosing with chlordiazepoxide by Boisse, Ryan and Guarino [17]. The signs of dependence of the benzodiazepine-type are compared to the withdrawal abstinence signs associated with dependence of the morphine type reported by Aceto [16] (Table 2).

Clinical Signs	Boisse, Ryan and Guarino [17]	Aceto [16]
	Chlordiazepoxide	Morphine
	Schedule IV*	Schedule II
Increased Activity	Yes	Yes
Wet Dog Shakes	Yes	Yes
Bodyweight loss	Yes	Yes
Food and Water Intake	Yes	Yes
Motor Disturbance	Yes (Jerking)	Yes (Jumping, Rearing)
Increased Startle	Yes	Yes
Decreased seizure threshold	Yes	Yes

*The neurological sequelae of chlordiazepoxide withdrawal in the rat resemble that seen in the "dependence syndrome of the barbiturate type" previously described Crossland & Leonard and Essig

Table 2: Clinical signs of withdrawal following lower dose administrations for schedule II and IV drugs.

Since the publication of the FDA draft guidance document we have conducted a number of dependence liability studies and have established historical control data in the hopes to minimize the need to repeatedly replicate study-after-study the inclusion of a positive control group of rats. In the CSS-PhRMA dialogue sessions (Table 1, above), it was clearly encouraged to use historical positive control data to reduce the use of animals in these study designs. To comply with the FDA draft guidance document and to replicate historically traditional escalating dose strategies appearing in peer-reviewed scientific journals we set forth to establish in-house historical control data. We used the "fixed dose" and escalating, equivalent, dosing strategies with CDP in male Sprague-Dawley rats to assess the relative discontinuation syndromes following 30 days of twice daily administrations. The fixed dose strategy used 100 mg/kg/day (50 mg/kg bid) and the escalating equivalent dose strategy was initiated using 32 mg/kg (bid) dosing up to a final daily dose of 700 mg/kg on Day 30 of the study plan. The specific escalating dose schedule used is shown in Table 3.

Daily Chlordiazepoxide Escalating Dose Schedule		
Day	Morning Dose (mg/kg/dose)	Afternoon Dose (mg/kg/dose)	Total Daily Intake (mg/kg/day)
Day 1	32	32	64
Day 2	56	56	112
Day 3	100	100	200
Day 4	200	100	300
Day 5	200	200	400
Day 6	200	200	400
Day 7	200	200	400
Day 8	225	200	425
Day 9	250	200	450
Day 10	250	200	450
Day 11	250	225	475
Day 12	250	225	475
Day 13	250	250	500
Day 14	275	250	525
Day 15	275	250	525
Day 16	300	250	550
Day 17	300	250	550
Day 18	300	250	550
Day 19	300	275	575
Day 20	300	300	600
Day 21	300	300	600
Day 22	300	300	600
Day 23	300	300	600
Day 24	325	300	625
Day 25	325	325	650
Day 26	325	325	650
Day 27	325	325	650
Day 28	325	325	650
Day 29	350	325	675
Day 30	350	350	700

Animals were dosed at volumes from approximately 0.8 to 8.75 mL/kg to achieve the required dose level.

Table 3: Dosing schedule for escalating dose strategy with chlordiazepoxide.

The initial dose of CDP was 32 mg/kg bid. This dose was in effect a loading dose to achieve chronic equivalence from the start of treatment in order to provide a reference point to evaluate chronic tolerance development. Based on the acute time course of action of this dose previously reported by Boisse, Ryan and Guarino [17], the dosing times of (7:00 am and 5:00 pm) were selected to conveniently give a 12 hr average dose interval. These dose intervals (10 and 14 hrs) were selected to ensure continuity of CNS depression with adequate recovery of self-sufficient health between drug administrations.

In order to design a dosing schedule that achieved both equivalency of peak responses as well as continuity of drug effect between doses, a behavioral scoring system, first developed to measure CNS depression by Ryan and Boisse [19], was used here in our laboratory to escalate the dose of CDP from day to day up to a maximum of 700 mg/kg/day (350 mg/kg bid). Using the Ryan and Boisse depression scores, each animal was observed at 1:00 pm (approximately 6 hours following the early morning dose). The lowest possible score of "0" implies no CNS impairment or depression. The highest score of "11" implies severely depressed. On scores of 6 or less the daily dose CDP were increased to

the next incremental level. Scores of 7 or greater would require Study Director Approval prior to the next incremented increase in dose. Based on this strategy of maintaining an equivalent measure of behavioral disruption over the entire course of the 30 day dosing plan, the animal's homeostatic physiology would be challenged to a degree that would ensure the presentation of a discontinuation syndrome following abrupt withdrawal of treatments on the evening of Day 30.

Yanagita [29] concluded that the development of physical dependence on sedative-hypnotic drugs is greatly influenced by the depth and duration of drug effects that are manifested in the animal's gross behavior during the chronic or repeated drug administration delivery period. According to Schuster and Villareal, Tatum, Seevers and Collins, Seevers, Kolb [40-43] and others, continued drug administration is not simply preventing the manifestations of abstinence; the dependent organism is in the peculiar condition of being subject to the influence of the drug both in its presence and when it is removed. During the latter stages of chronic drug administrations, it is invariable that tolerance to the escalating doses of the positive control article (CDP) has developed to some aspects of normal druginduced functioning such that withdrawal signs are expressed in the presence of drug administrations. According to these authors (cited above), a complete physiological account of the CDP-dependent organism must therefore include descriptions of its conditions during the state of chronic intoxication as well as during abstinence. As found in the present study (see below), using a more traditional escalating dose strategy (CDP) a greater withdrawal syndrome developed when compared to fixed 100 mg/kg CDP dose strategy (Table 4).

Signs of Benzodiazepine Withdrawal-Escalating Dose Group					
Behavioral Signs	**Day 31**	**Day 32**	**Day 33**	**Day 37**	**Day 45**
Motor					
Twitches					
Tremors					
Increase muscle tone					
Tail Erection					
Alterations in posture					
Alterations in gait	↓	↓	↓		
Righting Reflex	↓	↓	↓	↓	
Decreased motor activity	↓				
Teeth chatter					
Grip Strength	↓	↓	↓		
Autonomic					
General Arousal	↑	↑	↑		
Piloerection					
Blanched ears					
Exopthalmus					
Pupillary dilatation			↓		
Palpebral Closure	↑	↑	↑		
Behavioral					
Struggle Response					
Handling Reactivity					
Irritability					
Increased startle response (ease of removal)					
Auditory evoked					
Tactile evoked					
Paw Lick Latency	↑	↑	↑	↑	
Other					
Bodyweight Change	↓	↓	↓		
Defecation	↓	↓			
Urination					
Body Temperature	↓	↓↓↓↓			
↓ Decreased	↑	Increased			

As shown in Table 5, the 30 day escalating dose treatments of CDP, up to 700 mg/kg/day, produced a pronounced withdrawal syndrome in rats. The withdrawal syndrome was demonstrated within all domains assessed in the standard functional observational battery (FOB). Most notable were the weight loss, motor deficits, and hypothermia. Autonomic excitation was clearly evident with increases in responses to handling, approach, tactile and auditory stimuli, as well as the presence of stereotypic patterns of sniffing. As would be expected, the direct acute effects as well as the magnitude of withdrawal signs documented were greater in the escalating CDP treatment group (Table 5) when compared to the fixed 100 mg/kg treatment group on measures of: palpebral closure, gait, righting reflex, general arousal, handling reactivity, and posture. Clinically significant changes in the FOB were evident on Day 1 of withdrawal following the escalating dosing strategy (Table 5). In contrast, the fixed dose strategy had minimal changes until Day 2.

Signs of Benzodiazepine Withdrawal-Fixed Dose Group					
Behavioral Signs	**Day 31**	**Day 32**	**Day 33**	**Day 37**	**Day 45**
Motor					
Twitches					
Tremors					
Increase muscle tone					
Tail Erection					
Alterations in posture					
Alterations in gait		↑	↑		
Righting Reflex					

Decreased motor activity					
Teeth chatter					
Grip Strength		↓	↓	↓	
Autonomic					
General Arousal	↑	↑	↑		
Piloerection					
Blanched ears					
Exopthalmus					
Pupillary dilatation					
Palpebral Closure		↓			
Behavioral					
Struggle Response		↑			
Handling Reactivity and Ease of Removal	↑	↑	↑		
Irritability		↑			
Increased startle response			↑		
Auditory evoked					
Tactile evoked					
Paw Lick Latency				↑	
Other					
Bodyweight Change		↓	↓	↓	
Defecation					
Urination					
Body Temperature					
Decreased		Increased			

Table 5: FOB findings of chlordiazepoxide discontinuation syndrome following fixed dose strategy.

It should be noted that the 8-factor analysis that is determinative for schedule control action by the FDA and DEA is based on the relative differences in abuse potential between classes of scheduled drugs. If the data in Tables 3, 5, and 6 were submitted for review as NME's, is there sufficient data to make a judgment as to what schedule should these drugs be controlled under the CSA? If based on relative dependence liability assessments, should morphine be a CIV drug or should CDP be a CII drug based on this similar behavioral profile. If one or both of these drugs were an NME – these data provide little support in determining schedule control status. It is most likely that a higher schedule would be selected if such a decision had to be made by International or national drug control policy makers.

Dose Selections Based on Best Practices from Other IND-Enabling Study

Designs

To strike a balance between the dosing strategies from other high-dose regulatory-based preclinical toxicology study designs and the dependence liability study protocols we would propose one of two ICH Guideline directives as a starting point for negotiating the general principles for "best practices" in dose selections for future study designs.

The ICH S7A guidelines [44] regarding general safety pharmacology studies are defined as those studies that investigate the potential undesirable pharmacodynamic (PD) effects of a substance on physiological functions in relation to exposure in the therapeutic range and above. The objectives of the FDA-approved ICH guidelines are 1) to identify undesirable PD properties of a substance that may have relevance to its human safety; 2) to evaluate adverse PD and/or pathophysiological effects of a substance observed in toxicology and/or clinical studies; and 3) to investigate the mechanism of the adverse PD effects observed and/or suspected. The investigational plan delineated by the ICH S7A guidelines seems to be consistent with the intent of standard abuse liability study designs.

Under these safety guidelines the dose selection is set by the acute dose that elicits an adverse effect. It is recognized that there are species differences in sensitivity. Therefore, doses should include and exceed the primary PD or therapeutic range. This is likely the "starting point" for the current FDA draft guidance document target of "several fold higher than therapeutic target". However, the S7A guidance [44] further advised that in the absence of an adverse effect on the safety pharmacology parameter(s) evaluated in the study, the highest tested dose should be a dose that produces moderate adverse effects in this or in other studies of similar route and durations. Since abuse liability testing is expected to occur during Phase II or Phase III clinical trials the dose selection should be based on any and all preclinical toxicology studies conducted with the test article. Under the ICH M3 (R2) guidelines [45], this includes those doses that achieve large exposure multiples or saturation of exposure or use of the maximum feasible dose (MFD) to set the upper limits of the dosing regimen. The MFD is determined by an escalating dose strategy. Limit doses for acute, sub chronic, and chronic toxicity studies of 1000 mg/kg/day for rodents and non-rodents are considered appropriate in all cases under the ICH guidelines [44,45]. However, doses providing a 50-fold margin of exposure (usually based on group mean AUC values of the parent drug or the pharmacologically active molecule of a pro-drug) to the clinical systemic exposure generally are also considered acceptable as the maximum dose for acute and repeated-dose toxicity studies in any species. These limits could be adopted to be the foundation for dependence liability testing, at a minimum, based on the stated purpose of assessing the relative safety of "real life" drug escalations expected based on current knowledge of "street use" of known drugs-of-abuse outside the scope of medical practice.

Another regulatory-based dose selection strategy that might address the industry's best practices to achieve the objectives of dependence liability study designs is those listed in the ICH guidance S1C, "Dose Selection for Carcinogenicity Studies of Pharmaceuticals" [46]. Under this guidance document, the doses selected for rodent bioassays for pharmaceuticals are based on an exposure to the test article that (1) allows an adequate margin of safety over the human therapeutic

exposure, (2) is tolerated without significant chronic physiological dysfunction and is compatible with good survival, (3) is guided by a comprehensive set of animal and human data that focus broadly on the properties of the test article and the suitability of the animal, and (4) permits data interpretation in the context of clinical use. The S1C guidance document (ICH, 2008) proposes that any one of several approaches could be useful for dose selection. These include: (1) toxicity-based endpoints; (2) pharmacokinetic endpoints; (3) saturation of absorption; (4) PD endpoints; (5) maximum feasible dose; (6) limit dose; and (7) additional endpoints. Under these dose selection criteria, consideration of all relevant animal data and integration with available human data would be paramount in determining the most appropriate endpoint for selecting the high dose in dependence liability studies.

Similar to the admonishment stated in the S1C guidance, the use of the rodent to predict human dependence liability has inherent limitations, although this approach is the best available option at this time. Thus, while the use of plasma levels of test article represents an important attempt at improving the design of the rodent assay, progress in this field calls for continuing examination of the best method to detect human risk. The selected dosing strategy is therefore intended to serve as guidance in this difficult and complex area. Under the ICH 1SC guideline model dose selection for dependence liability studies would generally be determined from the 90-day toxicity studies using the route and method of administration that was used. The ICH Expert Working Group on Safety has agreed to continue use of the maximum tolerated dose as a useful toxicity-based endpoint for high dose selections [44-46]. The following definition of the MTD is considered consistent with those published previously by international regulatory authorities:

The top dose or maximum tolerated dose is that which is predicted to produce a minimum toxic effect over the course of the study. Such an effect can be predicted from a 90-day dose range-finding study in which minimal toxicity is observed. Factors to consider are alterations in physiological function that would be predicted to alter the animal's normal life span or interfere with interpretation of the study. Such factors include: no more than 10% decrease in body weight gain relative to controls; target organ toxicity; significant alterations in clinical pathological parameters.

Using the ICH S1C guidelines [46], to help determine the high dose for dependence liability studies using the "best practices" approach outlined in that guidance it may be appropriate to limit the dose to 1500 mg/kg/day. This limit dose applies where the maximum recommended human dose does not exceed 500 mg/day. The rodent systemic exposure at 1500 mg/kg/day should be greater by at least an order of magnitude than human exposure measured at the intended human therapeutic dose. (If this is not the case, efforts should be made to increase the rodent exposure or to reconsider the animal model in a case-by-case approach.) If the human dose exceeds 500 mg/day the high dose can be increased up to the maximum feasible dose.

As detailed in Note 11, of the ICH 1SC guidelines [46], it has been shown that systemic exposure comparisons between rodents and humans are better estimated by a dose using mg/m2 than using mg/kg. Therefore, the human dose should be at least 25-fold lower on a mg/m2 basis than the high dose in the study. The factor 6-7 (6.5) is used to convert rat doses from mg/kg to mg/m2 and the factor 40 is used to convert human doses from mg/kg to mg/m2. Thus, the estimated systemic exposure ratio of 25-fold rodent/human is equal to about a 25-fold mg/m2 ratio or a 150-fold mg/kg ratio (150 ≈ 25 x

40/6.5). Therefore a human dose below 10 mg/kg/day (about 500 mg/day or less) could be tested in rats at 1500 mg/kg as the high dose.

All relevant information should be considered for dose and species/strain selection for the dependence liability study. This information should include knowledge of 1) the actual patterns of human use outside the scope of medical practice for the specific pharmacological class of compound or therapeutic target being studied, 2) the relative exposure patterns known to exist in the actual drug abuse patient community, and 3) the metabolic pathways of the test article of interest. The availability of multiple criteria for dose selection will provide greater flexibility in optimizing the design of carcinogenicity studies for therapeutic agents.

Conclusion

It is intuitively obvious that there are a variety of methodologies that have been used to induce states of dependence in laboratory animals. However, setting the standards by which the pharmaceutical industry conducts preclinical screening of NMEs for dependence liability must be tempered with the knowledge of the characteristic features of the eight known discontinuation syndromes from prototypic drugs from each of the pharmacological classes first described by Eddy, Halbach et al. [47]. These well-known and prototypic syndromes have historically characterized by using escalating dose strategies and not the fixed dose strategies as described in the current FDA's draft guidance document. Fixed dose strategies do not engage the requisite homeostatic physiological mechanisms that develop the full qualitative or quantitative

"Cluster of physiological, behavioural and cognitive phenomena of variable intensity in which the use of a psychoactive drug (or drugs) takes on high priority" [48].

It is most likely that the resulting profile of the NME will not adequately address comparative review of known drugs of abuse under the international review process mandated for schedule control by treaty requirements as well as the CSA.

It is imperative to acknowledge and accept that the critical requirement for testing abuse liability in all NMEs that interact with the CNS springs forth from legally-binding commitments to international standards set forth in the three major drug control treaties [33-35] as well as U.S. drug control policies delineated in the Comprehensive Drug Abuse and Control Act [31]. Sufficient, valid, and reliable data that can stand up to scientific rigor and legal scrutiny addressing the 8-factors determinative of schedule control placement should be based on "industry best practices". Since all known examplars of withdrawal syndrome typologies of the well-characterized standard drugs-of-abuse have been identified using escalating dose strategies, it would seem intuitive that the fixed dose strategy proposed during the CSS-PhRMA dialogue sessions should be modified. We would propose that the current draft guidance document should be amended to include similar "industry best practices" methodologies for dependence potential studies in the upcoming finalized guidance document.

Highlights

We summarize the methodologies to assess dependence potential by the CSS staff.

There may be discrepancies between CSS recommendations and the literature base.

Escalating dose strategies may be needed to ensure drug control policy compliance.

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