JWH-018 Tox Results Summary:

	CYPs	hERG	Cytotox	Genotox	Rat Repeat Tox	Rat PK
JWH-018	4/6 +ve	Negative	Negative	Negative	Lethargy, Catatonia, Tachyphylaxis, No Organ Pathology	Bi-phasic, Well Distributed

*Highlighted denotes positive results and indicates further testing needed to define the issue.

CYPs Background:

- This assays determines whether a test agent inhibits specific CYP450 enzymes.
- CYPs are the major enzymes involved in drug metabolism, accounting for ~75% of the total metabolism with CYP3A4 accounting for about half of that and CYPs 3A4, 1A2, 2C9, 2C19, and 2D6 accounting for ~95% of the total dependent metabolism of approved drugs. (F. Peter Guengerich (2008). "Cytochrome P450 and Chemical Toxicology". Chemical Research in Toxicology 21: 70–83 and The AAPS Journal 2006; 8 (1) Article 12.)

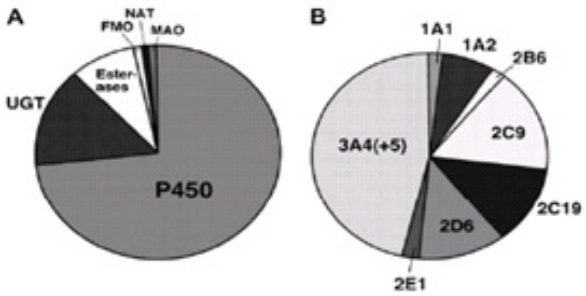

Figure 4. (A) Contribution of individual enzyme systems to metabolism of marketed drugs; (B) contribution of individual P450s in metabolism of drugs. UGT indicates uridine dinucleotide phosphate (UDP) glucuronosyl transferase; FMO, flavin-containing monooxygenase; NAT, *N*-acetyltransferase; MAO, monoamine oxidase; P450, cytochrome P450.[5,6]

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- Quote from FDA Guidance: "It is important that metabolic drug-drug interaction studies explore whether an investigational agent is likely to significantly affect the metabolic elimination of drugs already in the marketplace and likely in medical practice to be taken concomitantly and, conversely, whether drugs in the marketplace are likely to affect the metabolic elimination of the investigational drug. Even drugs that are not substantially metabolized can have important effects on the metabolism of concomitant drugs. For this reason, metabolic drug-drug interactions should be explored, even for an investigational compound that is not eliminated significantly by metabolism" (FDA Draft Guidance "Drug Interaction Studies — Study Design, Data Analysis, and Implications for Dosing and Labeling")

CPYs Results Commentary:

- The likelihood of an *in vivo* interaction is projected based on the [I]/Ki ratio where [I] represents the mean steady-state Cmax value for total drug (bound plus unbound) following administration of the highest proposed clinical dose. As the ratio increases, the likelihood of an interaction increases. The following table suggests the likelihood of in vivo interaction based on estimated [I]/Ki ratios. An estimated [I]/Ki ratio of greater than 0.1 is considered positive and a follow-up in vivo evaluation is recommended.

Table 2. Prediction of clinical relevance of competitive CYP inhibition:

[I]/Ki	Prediction
[I]/Ki > 1	Likely
1 > [I]/Ki > 0.1	Possible
0.1 > [I]/Ki	Remote

	IC_{50} (μM)					
Client ID	Cyp1A2 / CEC	Cyp2C9 / DBF	Cyp2C19 / DBF	Cyp2D6 / AMMC	Cyp3A4 / BFC	Cyp3A4 / DBF
Controls	0.62 α-naphtho-flavone	0.10 sulpha-phenazole	10 tranyl-cypromine	0.15 quinindine	0.18 ketoconazole	0.050 ketoconazole
JWH-018	3.0	1.4	2.8	>100	>100	8.0

- If dose is 5 mg then I = 3 μM (theoretical max that assumes 5 mg by IV injection and 5 L blood vol.)
  Ki = 0.5 times IC50
  IC50s that pose a potential for drug-drug interaction are highlighted above (<60 μM)

- The inhibition of cytochrome P450 (CYP) enzymes may cause severe interactions with other drugs if co-administered. This interaction is caused by the increase of plasma drug levels due to inhibition of the key metabolic breakdown of the co-administered drugs and perhaps the drug itself. JWH-018 was shown to inhibit some of these key CYPs.

- The FDA has issued a draft guidance in 2006 outlining the studies needed to look at drug interactions. Further studies will be needed to be performed to further characterize the drug interaction properties of JWH-018. However, it should be noted that if the human Cmax level is found to be near the Ki for inhibition of the recommended CYPs a drug interaction is likely.

- Many drugs inhibit CYPs. Classic examples include the bioactive compounds found in grapefruit juice and anti-epileptic drugs such as Phenytoin.

hERG Background:

- In the late 1990s a number of drugs, approved by the FDA (the U.S. Department of Health and Human Services Food and Drug Administration) and available on the market, had to be withdrawn from sale in the US when it was discovered they were implicated in deaths caused by heart malfunction. It is now known that a side effect of these drugs was the blocking of hERG channels in heart cells. This caused prolongation of "action potentials"—the electrical pulses responsible for controlling heart muscle cells. With proper control of the rate of heartbeat lost, dangerous arrhythmias could develop, which lead in some cases to death. The hERG test is used to screen drug candidates for possible cardiac issues.

hERG Results Commentary:

- The IC50 for JWH-018 was found to be >100 µM.
- If we use the same I/Ki ratio as the FDA prescribes for CYPs then the IC50 needs to be >30 µM for the potential for there to be an issue with hERG to exist, therefore JWH-018 is negative for hERG.
- Solubility was also poor at 30 and 100 µM potentially underestimating the inhibition at 30 and 100 µM.

Cytotoxicity Background:

- Cytotoxicity is the quality of being toxic to cells**.** Cytotoxicity assays are widely used by the pharmaceutical industry to screen for cytotoxicity in new drug candidates.

Cytotoxicity Results Commentary:

- JWH-018 showed no cytotoxicity up to 250 µM in HepG2
- The cytotoxicity IC50 has been shown to correlate with the LD50 in rodent toxicity.
- We performed a cytotoxicity assay in human HepG2 hepatocellular carcinoma cells. It was found that JWH-018 showed little cytotoxicity with an IC50 >250 >µM. These results are consistent with the other cell based assays.

GreenScreen HC S9 Background:

- Genotoxicity is the toxic affect on genetic material (ie. DNA). Genotoxic substances are known to be potentially mutagenic or carcinogenic, specifically those capable of causing genetic mutation and of contributing to the development of tumors.
- The GreenScreen HC with and without S9 (metabolism) gentoxicity assay is an assay that measures the potential of a compound to be genotoxic and be potentially mutagenic and/or carcinogenic. Genotoxic compounds are compounds which in various ways damage the information stored in chromosomes. Since cancer can arise as a consequence of exposure to genotoxins all new chemicals such as pharmaceuticals and consumer products that come into contact with humans have to be tested for genotoxicity.
- The accuracy of GreenScreen HC reflects its unique combination of high sensitivity (ability to correctly identify genotoxins) and high specificity (ability to correctly identify non-genotoxins). Existing *in vitro* mammalian tests have low specificity, i.e. their false positive rate is high whilst bacterial tests suffer from poor sensitivity, i.e. their false negative rate is high.
- Validation studies have shown that GreenScreen HC identifies all mechanistic classes of direct acting genotoxins including aneugens, topoisomerase inhibitors and DNA synthesis inhibitors. It can significantly reduce the number of ‘problem compounds’ in preclinical development, thus minimizing the need for expensive and time consuming mechanistic studies.
- The GreenScreen HC can also be run with S9 fraction from liver hepatocytes which metabolizes compounds and looks for genotoxic metabolites.

GreenScreen HC S9 Results Commentary:

- JWH-018 along with its metabolites was found to be negative in both GreenScreen HC and GreenScreen HC S9 (metabolism) genotoxic assays at concentrations up to 75 µg/ml.

- The positive cytotoxicity indicated in the GreenScreen HC S9 is likely an anomaly due to the S9 fraction causing interference. It was noted only at the highest concentration. Any cell permeability will show the dye indicating false positive and does not necessarily indicate cell death. This test is not best marker for cytoxicity.

Rat Repeat Toxicity Background:

- Repeat dose rat tolerability studies are performed to determine the potential for toxicities of drugs dosed repeatedly (chronically) over 7 days. Repeated dose toxicity testing is used to evaluate chronic toxic effects, primarily effects on various organ systems. Doses are selected to be sub-lethal but still high enough to cause toxic effects.

- Animals are euthanized after 7 days and organs are checked for visual signs of toxicity.

Rat Repeat Toxicity Results Commentary:

- In the repeat dose study the animals given JWH-018 appeared lethargic and non-ambulatory up to 2 hrs post-dose on day 1, day 2, and day 3. At day 4 and day 5 all animals dosed with JWH-018 appeared active, alert, and responsive. On days 6 and 7 JWH-018 rats appeared slightly lethargic for up to 1 hour post dose. This is a typical tachyphylatic (rapidly decreasing response to a drug following administration of the initial doses) response seen.

- Animal weights compared to the control group were also seen to be decreased probably due to the lethargy of the animals.

- The drugs levels for both compounds monitored over the 7 days seem to be pretty stable with little or no accumulation.

- The largest concern from these studies is the potency of the compound as well as the tachyphylaxis. It is suggested that *in vivo* efficacy studies in animals be performed to both identify the lowest efficacious dose but also to see if a repeat dose study causes the need for the efficacious dose to rise due to their tachyphylatic properties. If simple scaling is applied using the FDA guidance on suggested human clinical doses, a HED, or human equivalent dose, based upon only the rat tolerability data and using the 0.1 mg/kg dose as a potentially acceptable dose then the HED is 0.016 mg/kg or for a 68 kg human a 1.1 mg dose. (see http://www.fda.gov/cber/gdlns/dose.pdf for conversion table used to determine HED from various animal species). Please note that this dose is usually determined by performing these tox studies on multiple species including a non-rodent species.

- The main observations from these studies was that JWH-018 caused a severe lethargic, unresponsive catatonic state at all doses tested from 0.1mg/kg to 10 mg/kg in the rats. At the upper drug concentration of 10mg/kg there was decreased breathing, and some animal deaths occurred. There is also the observation that only male rats died. It seemed the deaths were related to the catatonia and decreased breathing rather than organ toxicicity.

- Male rats were observed to be significantly more sensitive to the effects of JWH-018 than females. Two out of the three male rats died during the high dose 5mg/kg PK study (equivalent HED is .8mg/kg or for a 68kg human a 54.4mg dose). Perhaps rodents are not the best species to test. This discrepancy of potency between male and female rats has been noted in the research of other cb1/cb2 cannabinoid agonist compounds such as CP-55,940. In one such study they found male rats to require 10 times less than female rats for an effective dose. (See: "Cannabinoid agonist, CP 55,940, facilitates intake of palatable foods when injected into the hindbrain" Physiology & Behavior, Volume 80, Issue 5, February 2004, Pages 611-616 Cheryl C. Miller , Thomas F. Murray , Kimberly G. Freeman and Gaylen L. Edwards

- No tissue abnormalities were observed at time of necropsy. There was no serious acute toxicities observed in the main organs.

Rat Pharmacokinetics Background:

- Pharmacokinetics is the study of the way the body deals with the absorption, distribution, metabolism, and excretion of drugs under investigation expressed in mathematical terms. The effects and the duration of action of the drug are also taken into account. The data obtained from such studies are useful for the design and conduct of subsequent clinical trials.

- Pharmacokinetic studies are performed to observe how long the parent drug remains in the blood system after administration, how the drug is absorbed into the tissues and how it is eliminated.

- Absorption is the process whereby a substance entering the body is assimilated by it. For proper pharmacokinetics study, it is necessary to know both the rate and the extent to which the active substance or therapeutic moiety are absorbed. They include substances intended to produce / not produce systematic effects.

- Distribution is the dispersion or dissemination of substances throughout the fluids and tissues of the body.

- Metabolism is the process whereby a substance is irreversibly transformed into metabolites.

- Excretion is the elimination of the substance from the body. In rare cases, not all substances are eliminated; some drugs irreversibly accumulate in a tissue in the body.

Rat Pharmacokinetics Results Commentary:

- A rat pharmacokinetic study was performed by bolus IV injection at 5 mg/kg and blood was collected at various time points for 24 hours post-dose. JWH-018 showed a bi-phasic distribution suggesting both distribution and elimination phases. The clearance was consistent of hepatic blood flow rates in a rat of (55 ml/min/kg). The volume of distribution suggests that the drug is well distributed. No accumulation was observed.

- JWH-018 distributes well throughout the rat. It is metabolized and eliminated normally and shows a half life of ~2 hours.