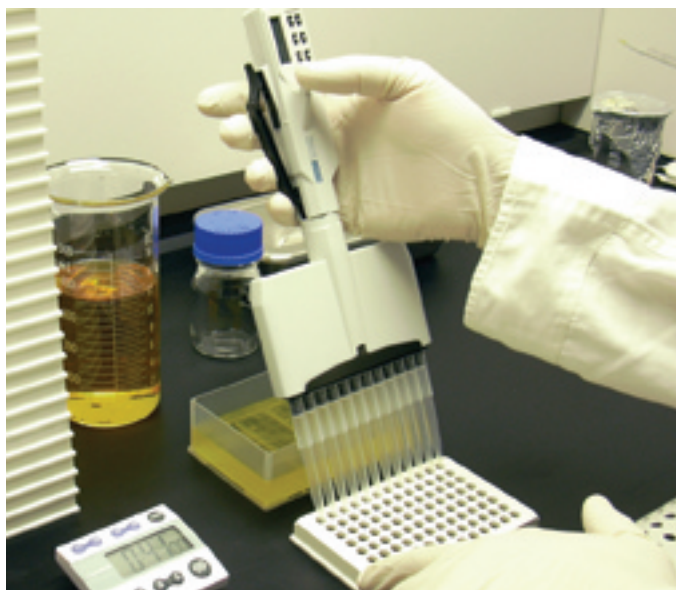


Drug-drug Interaction Tox Screen



For identifying potential toxicity related to cytochrome P450 induction, inhibition, and metabolic activation.

This very affordable screen requires only 2 mg of compound and provides data in less than 2 weeks.

Problem:

Drug-drug interactions (DDIs) occur when one drug alters the pharmacokinetics of a co-administered drug. This effect is most often the result of drug-induced induction or inhibition of cytochrome P450 (CYP) enzymes.

Induction of specific P450 enzymes may alter the metabolic profile of a drug by increasing metabolism or by creating an alternate pathway of metabolism. These changes can have profound effects on the pharmacology and toxicology of drugs. The effects of new chemical entities on the induction of CYP enzymes can be done in multiple species. An understanding of species-specific changes in these important drug-metabolizing enzymes can provide important information for predicting how a drug is handled in animals versus humans. Moreover, these data may also provide an explanation for why a test compound that produces adverse effects in rodent studies, may not be relevant when the drug is administered to humans.

Inhibition of CYP enzymes can have significant effects on the safety profile of drugs that are co-administered. Drugs such as cisapride, terfenadine, and mibefradil are examples of drugs that have been withdrawn from the market because of DDIs associated with CYP induction or inhibition. The United States Food and Drug Administration encourages pharmaceutical companies to investigate potential DDI reactions prior to regulatory submission. The type of inhibition and relative potency of inhibition are important pieces of data for assessing the potential of a new drug to produce a biologically significant DDI in humans treated with therapeutic doses.

Metabolic activation involves the formation of reactive (electrophilic) metabolites. These intermediates can deplete cellular levels of important thiols such as glutathione, form reversible protein modifications, and irreversible protein-adducts all of which can lead to an increased risk of adverse effects. The development of new drugs should limit chemical moieties known as toxicophores which can undergo metabolic activation.

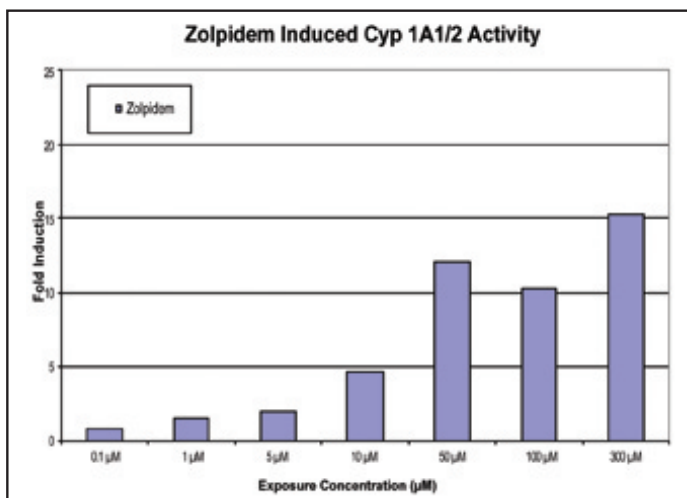
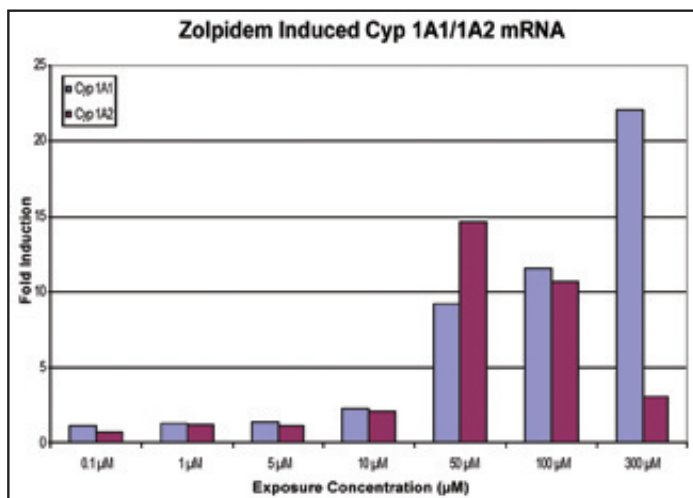
Clearly, the ability to evaluate new chemical entities (NCEs) early in development for their ability to induce or inhibit CYP enzymes, as well as their potential to undergo metabolic activation, would provide an important criterion for selecting compounds for further development.

Solution:

CeeTox offers a high-throughput system to evaluate enzyme induction, enzyme inhibition and metabolic activation. We provide more than a set of numbers; our extensive database of non-proprietary drugs and chemicals that can be used to put unknown DDI profiles into perspective. Our systems can evaluate DDI parameters in multiple species. CeeTox is a specialty research organization that focuses on problem solving. That means we can become an extension of our customers' research and development programs and can therefore increase their throughput. Our scientific staff has more than 100 years of combined experience in large-pharmaceutical drug-discovery research, and development and we use that expertise to help our customers solve problems.

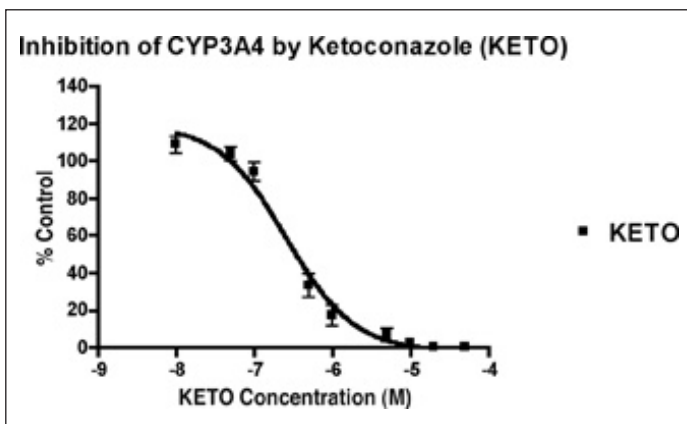
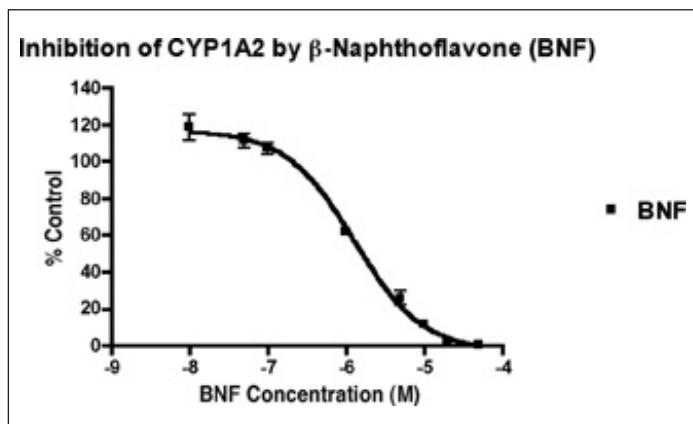
CYP Induction Assays:

The ability of an NCE to induce the production of CYP enzymes is determined by measuring changes in the relative abundance of mRNAs, encoding CYPs 1A, 2B, 2C, 2D, 3A, 4A, and, wherever possible, enzyme activity. The magnitude of induction is compared to known inducers. Changes in CYP mRNA levels are determined using branched DNA technology (bDNA, Genospectra). This analysis amplifies signal, not target, and enables measurements to be done in high-throughput format. Activity assays are based on known substrates for each CYP enzyme. The ability of an NCE to induce CYP enzymes can be evaluated in rat and human primary hepatocytes. Specialized cell systems designed to evaluate organ-specific induction, such as lung or kidney are also available.



CYP Inhibition Assays:

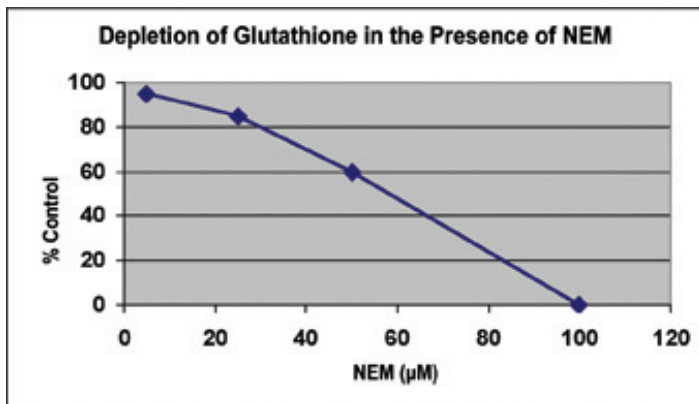
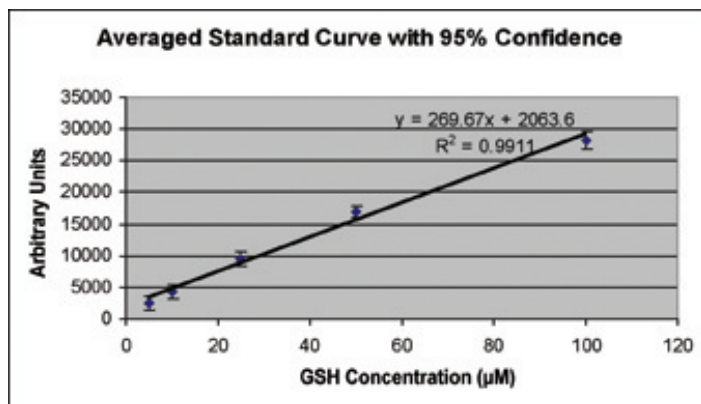
CeeTox uses recombinant P450 enzymes and substrates that generate a luminescent signal upon metabolism. The data generated can be used to determine the type of inhibition, which can be compared to CeeTox database of CYP inhibitors. IC50 or Ki values can be determined and then used to evaluate NCEs for potential DDIs. The CeeTox human CYP inhibition panel consists of CYPs 1A2, 3A4, 2C9, 2C19, and 2D6.



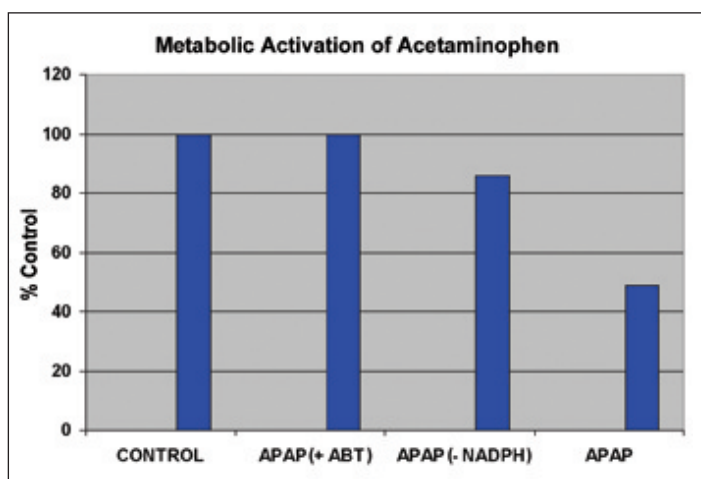
Metabolic Activation Assays:

CeeTox has developed and validated a system that evaluates the formation of reactive metabolites by monitoring the loss of reduced glutathione in a cell-free system. This assay is sensitive and has been optimized for linearity of response. Detection of GSH is accomplished with a probe that fluoresces upon binding with GSH.

N-ethylmaleimide (NEM) binds directly with GSH in a 1-to-1 molar ratio and therefore provides a means of evaluating the sensitivity of the assay. A 5 μM depletion of GSH resulted in a 5% reduction in signal while a 25 μM depletion of GSH produced a 15% reduction in signal. These data indicate that the system can accurately measure a loss of about 25 μM of GSH, approximately a 15% change.



Each data point depicts the mean of 3 standard curves.



The bar graph above depicts P450-mediated metabolic activation of acetaminophen (APAP) and subsequent depletion of glutathione (GSH). Rat microsomes were used as the metabolizing system. P450 involvement was verified by including a potent inhibitor of P450 (ABT) and an incubation mixture where the co-factor NADPH was not added.