

Selection and interpretation of clinical pathology indicators of hepatic injury in preclinical studies

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Abstract: This position paper delineates the expert recommendations of the Regulatory Affairs Committee of the American Society for Veterinary Clinical Pathology for the use of preclinical, clinical pathology endpoints in assessment of the potential for drug-induced hepatic injury in animals and humans. Development of these guidelines has been based on current recommendations in the relevant preclinical and human clinical trial literature; they are intended to provide a method for consistent and rigorous interpretation of liver-specific data for the identification of hepatic injury in preclinical studies and potential liability for hepatic injury in human patients. (*Vet Clin Pathol.* 2005;34:182–188)

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Key Words: ALT, hepatic injury, hepatotoxicity, liver, preclinical safety assessment

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Detection of potential human hepatic injury is a major challenge in preclinical pharmaceutical research and can present a severe impediment to the development of novel, efficacious, and safe drugs. The majority of drugs that cause hepatic injury in preclinical studies do not progress to clinical trials or are developed with substantial patient monitoring.^{1,2} Integrated evaluation of all study data, including the results of alanine aminotransferase (ALT) measurement and liver histology, has been shown to be crucial for the identification of hepatic injury in preclinical studies as an indicator of potential liability for hepatic injury in humans. However, a retrospective review of 548 compounds marketed between 1975 and 1999 indicated that 10.2% were withdrawn or acquired a black box warning,³ with hepatic injury cited as a predominant reason for withdrawal or addition of a warning to the label. Growing concerns about adverse hepatic drug reactions have led to commentaries⁴ concerning the adequacy of current practices for evaluating hepatic injury in preclinical studies. Similar concerns have resulted in the release of draft position papers regarding the detection of drug-induced liver injury from agencies responsible for the investigation of the safety and efficacy of human health products within the United States (Federal Drug Administration, FDA)⁵ and Canada (Health Canada).⁶ Recently, the agency responsible for evaluation of human health products in Europe, the European Medicines Agency (EMA), also indicated their intent to draft a guidance paper for detection of drug-induced liver injury for human health products.⁷

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The draft documents from the FDA and Health Canada and the notice from the EMEA suggest that the introduction of novel biomarkers and assays (eg, transcript profiling, proteomics, and metabonomics) may enhance detection of hepatic injury in preclinical studies. However, before these techniques can be incorporated into the assessment of hepatic injury in a preclinical study, they must undergo a robust validation process that encompasses traditional methods of assay validation (ie, sensitivity, specificity, accuracy, and precision) as well as characterization of biologic context, variability, species relevance, and linkage to a clinical endpoint. Therefore, the best method for identification of potential drug-induced hepatic injury in preclinical studies remains the integrated evaluation of clinical pathology parameters and results of histologic evaluation with other preclinical study data. As described above, ALT is a critical parameter for the identification of potential drug-induced hepatic injury in both preclinical studies and human patients. For this reason, the measurement and interpretation of serum ALT activity is a cornerstone of these updated recommendations for the identification of hepatic injury in preclinical studies.

Objective

This position paper was prepared by members of the Regulatory Affairs Committee of the American Society for Veterinary Clinical Pathology (ASVCP) for submission to the EMEA Committee for Medicinal Products for Human Use (CHMP) for use during the preparation of their hepatotoxicity position paper. The Committee's goal was to provide expert recommendations on the use of clinical pathology data for identification of potential drug-induced hepatic injury in preclinical toxicity studies.

The Regulatory Affairs Committee is composed of veterinary clinical pathologists with expertise in interpretation of clinical pathology data from preclinical studies in the safety assessment of xenobiotics (ie, food additives, chemicals, and drugs) for domestic and international pharmaceutical, chemical, and research companies. The committee's primary charge is to respond to regulatory guidance from national and international regulatory agencies regarding the use and interpretation of clinical pathology parameters in safety assessment studies.

Included within this article are recommendations for appropriate parameters for identification of hepatic injury in multiple laboratory animal species (rat, dog, and nonhuman primate [NHP]) and guidelines for interpretation of those parameters in preclinical studies to provide the best possible detection of potential adverse effects in humans. A review of previously published recommendations of clinical pathology testing in preclinical studies, current recommended parameters, and the use and limitations of additional parameters not routinely assessed (clinical chemistry parameters, novel biomarkers, and novel bioassays) also are provided.

Previously Published Recommendations for Clinical Pathology Testing in Toxicity and Safety Studies

Minimal recommendations for the panel of clinical chemistry analytes to be measured as part of the clinical pathology

evaluation of laboratory animals in toxicology and safety studies have been established and published.⁸ The recommendations include the times for sample collection and a list of parameters in preclinical repeat-dose toxicology or safety studies for identification of hepatic injury. Of note, the guidelines for timing and frequency of sample collection were minimal recommendations that could be modified based on species, study objectives and duration, and the biological activity of the compound. Following is a summary of these previously published recommendations:

- For studies with either rodent or nonrodent species (ie, dogs and NHPs), clinical pathology parameters should be evaluated at study termination. If a compound-free recovery group is included in the study design, clinical pathology evaluation should also occur at termination of the recovery period.
- In rats, interim evaluation in long-duration preclinical studies is generally considered unnecessary provided the clinical pathology evaluation is conducted in studies of shorter duration using doses that meet or exceed those used in the study of longer duration. Predose clinical pathology evaluations are not recommended in rats because of genetic homogeneity within laboratory rat strains and the relatively large number of animals per group in rat studies.
- In nonrodent species, predose and ≥ 1 interim evaluation of clinical pathology parameters are recommended because of the small number of animals per group and the interanimal variability. Additionally, in studies of < 6 weeks duration, evaluation of clinical pathology parameters is recommended within 7 days of the initiation of dosing.
- A minimum of 2 of the following serum parameters for the identification of hepatocellular injury should be measured in preclinical studies: ALT, aspartate aminotransferase (AST), sorbitol dehydrogenase (SDH), glutamate dehydrogenase (GDH), or total bile acids (TBA). Similarly, ≥ 2 of the following serum parameters for identification of hepatobiliary injury should be measured: alkaline phosphatase (ALP), γ -glutamyltransferase (GGT), 5'-nucleotidase (5'-NT), total bilirubin (TBIL), or TBA.

Updated Recommendations for Clinical Pathology Parameters of Hepatic Injury in Preclinical Studies

The recommendations of this committee for identification of hepatic injury in preclinical toxicology or safety studies in rats, dogs, and NHPs are presented below. All of these recommended parameters are included in the minimal recommendations for clinical pathology testing in preclinical studies that were published previously (as presented above).⁸ Furthermore, these recommended parameters are included within the list of parameters recommended by a committee within the National Academy of Clinical Biochemistry (NACB) and approved by the American Association for the

Study of Liver Diseases (AASLD) for identification of hepatic injury in human beings. This list comprises ALT, AST, and ALP activities, and TBILI, direct bilirubin, total protein, and albumin concentrations.⁹ The correlation of these recommended parameters with those recommended for identification of hepatic injury in humans makes them suitable and applicable parameters for the evaluation of potential hepatic injury in preclinical studies. It is important to note that this recommended list could be modified at the discretion of the veterinary clinical pathologist to include parameters that are optimized to the species of interest or to specific patterns of hepatic injury.

Indicators of hepatocellular injury: ALT and AST

The aminotransferase enzymes, ALT and AST, also termed transaminases, are recommended for the assessment of hepatocellular injury in rats, dogs, and NHPs in preclinical studies. ALT is considered a more specific and sensitive indicator of hepatocellular injury than AST in rats, dogs, and NHPs. The magnitude of ALT increase is usually greater than that of AST when both are increased due to hepatic injury, in part because of the longer half-life of ALT and the greater proportion of AST that is bound to mitochondria.¹⁰⁻¹³ Hepatic causes of increased serum ALT activity, with or without increased AST activity, include hepatocellular necrosis, injury, or regenerative/repairative activity.¹⁴⁻¹⁶ Decreases in ALT activity have been observed with concurrent hepatic microsomal enzyme induction in the rat.¹⁷ Increases in ALT activity also have been reported with concurrent hepatic microsomal induction in the dog and rat but were not considered indicative of hepatic injury because no substantive concurrent changes in liver histology or liver weight were observed.^{18,19} Increased serum ALT activity can also be affected by extrahepatic factors. Muscle injury can cause increases in serum transaminase activity, but AST is generally higher than ALT when both are concurrently increased.^{16,20} Procedure-related handling and type of restraint also can cause increases in AST activity with or without increases in ALT activity in mice and NHPs.^{21,22} Therefore, type of restraint and extent of handling should be considered in the evaluation of increased AST activity, with or without increased ALT activity.

ALT is considered a more specific and sensitive test of hepatocellular injury in humans, compared with other clinical pathology analytes.⁹ Guidelines have been published for interpretation of ALT activity in humans in the absence of histologic data (ie, results from autopsy or liver biopsy). Specifically, $>2\times$ increases in ALT activity alone above the upper limit of normal (ULN) are considered indicative of hepatocellular injury whereas $>3\times$ increases in ALT accompanied by $2\times$ increases above the ULN in TBILI concentration are indicative of more severe and potentially life-threatening hepatocellular injury.^{14,23}

Supplemental indicators of hepatocellular injury: SDH and GDH

Serum SDH and GDH activities have been shown to be specific for liver injury.^{13,24} The measurement of these supplemental

enzymes is useful when additional indicators of hepatic pathology are desired or in species where ALT activity is either low or nonspecific such as swine, guinea pigs, some strains of rats, and woodchucks.^{25,26} Of note, neither parameter is used routinely by physicians to evaluate human hepatic damage.

Indicators of hepatobiliary injury: ALP and TBILI

ALP and TBILI are recommended for the assessment of hepatobiliary injury in rats, dogs, and NHPs in preclinical studies. These recommended parameters are included within the group of parameters recommended for use in humans for identification of hepatic injury as listed above.⁹

In species used in preclinical studies, hepatobiliary pathology and bone growth/disease are the 2 most common causes of increased serum ALP activity. Increases in ALP activity due to hepatobiliary pathology generally precede increases in TBILI concentration in most species.^{16,27,28} Increased ALP activity from the liver in the absence of cholestasis has been reported in dogs with increased endogenous or administered glucocorticoids^{15,16,29} and in rats and dogs with concurrent microsomal enzyme induction.^{17,19} In rats, intestinal ALP is the major circulating isoenzyme, so a transient increase in serum ALP may occur postprandially, whereas fasting can result in a decrease in serum ALP.^{30,31} Decreases in ALP activity have been reported in rats with concurrent microsomal enzyme induction in the absence of changes in food consumption.¹⁷

In human beings, serum ALP activity increases with cholestasis but generally remains $<3\times$ the ULN.³² Extrahepatic isoforms of ALP are present in bone, intestine, and placenta. Differentiating between hepatic and extrahepatic isoforms in human medicine is performed rarely, as the hepatic origin of the increased ALP can be confirmed with concurrent increases in other indicators of cholestasis such as GGT.³³

Increases in the serum TBILI concentration are generally the result of bile retention subsequent to impairment of intrahepatic or extrahepatic bile flow (cholestasis), increased production associated with accelerated erythrocyte destruction, or altered bilirubin metabolism.^{16,26,34,35} The unconjugated (indirect-reacting) fraction of bilirubin generated from the degradation of erythrocytes and the conjugated (direct-reacting) fraction together comprise the TBILI concentration. Bilirubin fractionation is generally only of value for exploring drug-related inhibition of the bilirubin-conjugating enzyme, uridine diphosphate glucuronosyltransferase (UGT1A1).³⁶⁻³⁸ Concomitant bilirubinuria is supportive of an increase in the circulating conjugated bilirubin level due to cholestasis.

In human beings, consistent increases in TBILI $<2\times$ ULN or isolated increases $>2\times$ ULN are interpreted as a biochemical abnormality rather than as an indicator of hepatic injury. In contrast, repeated increases in conjugated bilirubin $>2\times$ ULN alone or with concurrent increases in AST and/or ALP activities are considered indicative of liver injury.²³

Supplemental indicator of hepatobiliary injury: GGT

GGT is a canalicular enzyme whose serum activity is increased in cholestatic liver disease. Increases in circulating GGT activity can arise from impaired bile flow and biliary epithelial

necrosis.^{15,26,39,40} An increase in circulating GGT without evidence of biliary pathology is associated with the use of some anticonvulsant medications in humans and dogs and an increase in circulating glucocorticoids in dogs and rats.^{18,41-43} In both dogs and rats, GGT is a less sensitive but more specific indicator of cholestasis when compared with ALP.^{31,43}

Concurrent increases in GGT and ALP activities are considered more indicative of a hepatic enzyme source but do not exclude concurrent bone disease.⁹ Currently, there are no established guidelines for interpretation of GGT activity in drug-induced hepatic injury in human beings.²³

Supplemental indicators of hepatic synthetic function

Total protein, albumin, triglycerides, cholesterol, glucose, urea, activated partial thromboplastin time, and prothrombin time are considered as supplemental indicators of hepatic synthetic function. In drug-induced hepatic injury, evaluation of these parameters may be instrumental in the identification of deleterious effects on glucose metabolism and hepatic synthesis of proteins, lipids, and coagulation factors.¹⁶ Furthermore, with the exception of triglycerides, these parameters are included in the minimal recommendations for clinical pathology testing in preclinical studies.⁸

Hepatic parameters not routinely assessed in preclinical studies

Several additional analytes are available that, for a number of reasons, are not routinely assessed in preclinical studies. These include: ALP isoenzymes, 5'NT, α -glutathione-(S)-transferase (α GST), lactate dehydrogenase (LDH), and TBA. In routine preclinical safety studies, the determination of tissue-specific ALP isoforms/isoenzymes (liver, bone, intestine, and in dogs, steroid-induced) generally provide no additional information beyond that provided by the measurement of total ALP activity. 5'-NT and α GST assays are used to a limited extent in toxicity and safety studies and are not widely used by physicians to evaluate human hepatic damage. 5'NT lacks additional value beyond that offered by the measurement of ALP and GGT activity.²⁶ The measurement of α GST does not offer additional information beyond the measurement of ALT.⁴⁴ LDH is an enzyme present in most tissues and lacks hepatic specificity. Serum TBA concentration is measured as an indicator of hepatic function because bile acids are synthesized in the liver, transported to the intestine in bile, and absorbed via the enterohepatic circulation. However, serum TBA concentration is generally less sensitive for the evaluation of hepatocellular injury and cholestasis than are routine enzyme activities.^{13,16,34}

Novel bioassays and biomarkers

Novel safety and efficacy biomarkers are needed to enhance clinical diagnostic capability as well as reduce drug development costs and late-stage attrition within the pharmaceutical industry.⁴⁵ The increased use of transcript profiling, proteomic, and metabonomic platforms in drug development will undoubtedly lead to the identification and greater implementation of novel bioassays in future preclinical and

clinical studies. These biomarkers may be highly varied technologically, ranging from microarray and differential gene or protein expression "signatures" based on polymerase chain reaction (PCR) assays to measurement of serum, plasma, or urinary proteins or metabolites. Regardless of the technologic platform, validation and implementation of each candidate biomarker or bioassay requires a robust validation process that encompasses traditional methods of assay validation (ie, sensitivity, specificity, accuracy, and precision) as well as characterization of biologic context, variability, species relevance, and linkage to a clinical endpoint.^{46,47}

Recommendations for the Interpretation of Clinical Pathology Data in the Identification of Hepatic Injury in Preclinical Studies in Rats, Dogs, and Non-human Primates

Expert recommendations were developed for the interpretation of clinical pathology data in the identification of potential drug-induced hepatic injury and in preclinical studies. Definitions for frequently used terms in preclinical toxicology studies are included below and, with the exception of the definition of "NOAEL," are adopted from those previously published.⁴⁸

It is important to note that criteria used for the interpretation of clinical pathology data in preclinical studies differ from those used in the clinical evaluation of humans exposed to xenobiotics. Specifically, interpretation of clinical pathology data in human clinical trials is contingent on the magnitude (or fold) increase of the value above the upper limit of the population reference interval or the known ULN for that individual. This is not an appropriate approach to the assessment of toxicity in preclinical trials. Rather, compound-related effects on clinical pathology values in preclinical trials in rats, dogs, and NHPs are determined by comparison with mean and individual animal data from concurrent controls⁸ using reference intervals developed from historic control data to place the changes in context. In dogs and NHP, comparison of interim, terminal, or reversibility data with pretreatment data is also useful for determining compound-related effects and their reversibility.

Terminology of effect level

No observed-effect level (NOEL). "The highest exposure level at which there are no effects (adverse or nonadverse) observed in the exposed population, when compared with its appropriate control."⁴⁸ Compound-related changes therefore define the NOEL.

Adverse. "A biochemical, morphological, or physiological change (in response to a stimuli) that either singly or in combination adversely affects the performance of the whole organism or reduces the organism's ability to respond to an additional environmental challenge."⁴⁸ An adverse hepatic effect identified within the confines of a study should be considered as an indicator of a potential liability for hepatic injury in humans.

No observed-adverse-effect level (NOAEL). For the purpose of identifying hepatic injury in preclinical studies, we propose the following definition of NOAEL: the level of exposure

within a preclinical study at which there are no adverse effects. Specifically, the NOAEL is the level of exposure at which there are no toxicologically relevant increases in the incidence or severity of effects between compound-treated and control populations. Compound-related findings must be identified as *adverse* to establish the NOAEL. Nonadverse changes are compound-related but of insufficient magnitude to be adverse and often consist of normal physiologic compensatory mechanisms or anticipated pharmacologic activity. The proposed definition of NOAEL has been modeled after a previous definition.⁴⁸ Importantly, this definition differs from that of Lewis et al, in that we propose that statistical significance alone does not indicate that a change in the value of a parameter is compound-related nor does it indicate toxicologic or biologic relevance.

General guidelines for clinical pathology data interpretation

Rendering an opinion regarding the classification and interpretation of the finding is the obligation of the veterinary clinical pathologist. General guidelines for interpretation of clinical pathology parameters are as follows:

- Determination of the relationship of a change in the value of a clinical pathology parameter to compound administration (ie, is it compound-related?) and interpretation of its biologic or toxicologic importance (ie, is it adverse?) are accomplished through comparison of individual animal and group mean data with concurrent controls in all preclinical studies, regardless of species. In studies using dogs and NHPs, it is essential that the determination of the relationship of the finding to the compound and interpretation of the magnitude of change also incorporate comparisons of post-treatment changes to pretreatment values.
- Statistical analyses can be a useful tool to aid in data interpretation; however, statistical significance alone does not indicate that the change in the value of a parameter is compound-related nor does it indicate toxicologic or biologic relevance.^{8,15} In dogs and NHPs, statistical analysis of changes in clinical pathology data is further complicated by interindividual and intra-individual variability and by the low number of animals per dose group. For these reasons, the use of statistical analysis in the evaluation of clinical pathology data augments but does not replace comparisons of individual data with concurrent controls in all species, nor does it replace comparisons of individual data with pretreatment values in dogs and NHPs. Therefore, the role of the veterinary clinical pathologist is to identify the relationship of the finding to the compound and its biologic relevance based on individual and group mean data compared with concurrent control data and, in dogs and NHPs, to pretreatment values.
- Clinical pathology parameters cannot be evaluated in isolation. The determination of whether drug-induced clinical pathology findings are adverse is based on the magnitude of the changes in all concurrently evaluated

pathology parameters, as well as in-life observations, metabolism data, and pharmacologic class effects. In-life observations and body weight and food consumption data provide additional indicators of compound-related and adverse effects on treated animals. Other compound-related information, such as potential interference, hepatic microsomal enzyme data, and previous experience with the compound or related compounds in other species may be useful in interpreting clinical pathology indicators of hepatic effects. Concurrent evaluation of clinical pathology data with absorption, distribution, metabolism, and elimination (ADME) data and the results of safety pharmacology studies can help determine the relevance of the findings to human metabolism and safety. Concurrent evaluation of toxicokinetic data is helpful for the evaluation of individual animals with incongruous changes in clinical pathology values. Determination of changes after a drug-free period provides important evidence about the potential for reversibility of compound-related findings.

- Ultimately, concurrent evaluation of all data from the study results in the determination of the number of affected animals per dose group, dose-specific and/or gender-specific responses, and the reversibility of the changes. These data then are integrated to identify compound-related changes (NOEL) and compound-related adverse changes (NOAEL). Correlation of these study-specific interpretations with data from other studies in the same or alternative preclinical species provides important information for assessment of the risk profile for the human population.

Guidelines for interpreting changes in the values of specific hepatic parameters

As mentioned previously, guidelines have been published for the interpretation of ALT activity in humans when concurrent histologic data (ie, results from autopsy or liver biopsy) are not available. In contrast, numeric guidelines for the integrated interpretation of increases in ALT activity using all preclinical data have not been established in rats, dogs, and NHPs. However, general guidelines for the integrated interpretation of ALT with concurrent histologic changes or increases in other serum indicators of hepatocellular injury have been published.⁴⁹ Specifically, those guidelines recommend that increases in ALT activity that correlate with detrimental histologic changes should be considered adverse. Additionally, concurrent increases in the activity of ≥ 2 enzymatic indicators of hepatic injury also should be considered adverse. This document, however, does not provide guidance regarding the interpretation of increases in ALT activity alone in interim clinical pathology evaluations, when histologic evaluations are not routinely conducted, or at study termination in the absence of concurrent histologic changes. Therefore, the members of the Regulatory Affairs Committee, in consultation with colleagues from domestic and international companies with expertise in preclinical safety assessment, propose the following guidelines for the integrated assessment and interpretation of indicators of

hepatic injury in preclinical studies, to augment the previously-published general guidelines.

Serum ALT activity

An increase in serum ALT activity in the range of 2–4× or higher in individual or group mean data when compared with concurrent controls should raise concern as an indicator of potential hepatic injury unless a clear alternative explanation is found. When an increase in ALT activity of this magnitude is identified, it is the role of the veterinary clinical pathologist to determine whether the increase is adverse based on the integrated evaluation of all preclinical data. The data for this integrated evaluation consist of other clinical pathology indicators of hepatocellular and hepatobiliary injury and diminished hepatic function, organ weight, macroscopic and microscopic observations in liver and other tissues, enzyme induction data, and relationship to dose, duration, gender, species, and reversibility. This recommendation for evaluation of ALT data is presented as a range based on the slight variability of the concentration gradients between hepatic tissue and serum ALT enzyme activities for preclinical animal species and human beings. The magnitude of ALT enzyme activity in the liver of humans, dogs, and rats is approximately 35 U/g, 32 U/g, and 24 U/g, respectively, whereas the respective mean serum levels are approximately 16 U/L, 23 U/L, and 38 U/L. These data suggest that increases in serum ALT levels of 2–4× in the dog and rat are of sufficient magnitude to indicate extensive hepatocellular injury and serve as a harbinger of potential hepatic injury in humans based on the similar concentration gradients. Although a value for ALT activity in the liver of NHPs was not found for this comparison, the approximate mean serum value of 40 U/L in cynomolgus and rhesus monkeys suggests that a similar concentration gradient is likely for these species.^{50–52}

Serum TBILI concentration

Increases in serum TBILI concentration in individual or group mean data when compared with concurrent control values should be critically evaluated and compared with other study data to exclude other factors that can influence serum bilirubin concentrations. Causes for increased bilirubin in the absence of cholestasis consist of hemolysis (in vivo or in vitro), sepsis, and drug-related inhibition of UGT1A1.^{16,36–38} Once these causes have been eliminated, concurrent evaluation of bilirubin with other hepatic parameters is necessary to determine the relationship of the changes to the compound and their toxicologic or biologic significance. In the absence of changes in other hepatic parameters or hemolysis, increases in TBILI concentration alone are unlikely to be adverse. Additionally, the pattern of concurrent increases in ALT and bilirubin values should be critically evaluated in preclinical toxicity studies given the increased number of serious outcomes associated with this pattern of changes in humans.^{14,23}

Serum ALP and GGT activities

Specific guidelines for interpretation of the magnitude of changes in ALP activity in preclinical studies are not provided

because of the potential contribution of extrahepatic factors. These contributing factors include increases in intestinal or bone isoenzyme activity, primary compound-related effects (ie, glucocorticoid-induced ALP production in the dog), and increases in ALP activity with concurrent hepatic microsomal enzyme induction in dogs and rodents.^{16,17,19,29}

Specific guidelines for interpretation of changes in GGT activity in preclinical studies are not provided because this parameter is recommended only as a supplemental test in certain species, particularly when evaluated with changes in ALP activity.

Summary

These recommendations for the selection of clinical pathology parameters and interpretation of liver-specific clinical pathology data provide a consistent and rigorous approach to the use of preclinical, clinical pathology endpoints in the identification and assessment of drug-induced hepatic injury in animals and the potential for hepatic injury in humans. These guidelines are based on the best available knowledge in the relevant preclinical and human clinical trial literature and the consensus expertise of veterinary clinical pathologists, who are responsible for and trained in the integrated assessment of clinical pathology data.

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