Health Canada Santé Canada

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Notice

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Release of Guidance Document: Pre-market Evaluation of Hepatotoxicity in Health Products

Health Canada is pleased to announce the release of the guidance document *Pre-market Evaluation of Hepatotoxicity in Health Products*. A draft version of this guidance document was first released for consultation on February 16, 2011. There were 125 comments received and changes were incorporated where appropriate.

The purpose of this document is to provide guidance to sponsors to facilitate the detection, assessment, mitigation and reporting of hepatoxicity induced by human health products prior to issuance of market authorization pursuant to the *Food and Drug Regulations*. This guidance is expected to support the safe and effective use of health products by health care professionals, patients, and consumers.

This guidance document is applicable to pharmaceutical drug products for human use, health products regulated solely as natural health products subject to the provisions of the *Natural Health Products Regulations*, and radiopharmaceuticals and biological drugs as listed in schedules C and D of the *Food and Drugs Act*. It is not applicable to disinfectants or drugs for veterinary use.

The development of this guidance document is the result of a thorough survey of the scientific literature, current clinical practice and other regulator's approaches for the assessment of hepatotoxicity.

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GUIDANCE DOCUMENT

Pre-market Evaluation of Hepatotoxicity in Health Products

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Products and Food Branch

Canada

Our mission is to help the people of Canada maintain and improve their health. *Health Canada*	The Health Products and Food Branch's mandate is to take an integrated approach to the management of the risks and benefits to health related products and food by: • minimizing health risk factors to Canadians while maximizing the safety provided by the regulatory system for products and food; and • promoting conditions that enable Canadians to make healthy choices and providing information so that they can make informed decisions about their health. *Products and Food Branch*

Également disponible en français sous le titre : *Ligne directrice : Évaluation pré-commercialisation de l'hépatotoxicité des produits de santé*

FOREWORD

Guidance documents are meant to provide assistance to industry and health care professionals on **how** to comply with governing statutes and regulations. Guidance documents also provide assistance to staff on how Health Canada mandates and objectives should be implemented in a manner that is fair, consistent and effective.

Guidance documents are administrative instruments not having force of law and, as such, allow for flexibility in approach. Alternate approaches to the principles and practices described in this document ***may be*** acceptable provided they are supported by adequate justification. Alternate approaches should be discussed in advance with the relevant program area to avoid the possible finding that applicable statutory or regulatory requirements have not been met.

As a corollary to the above, it is equally important to note that Health Canada reserves the right to request information or material, or define conditions not specifically described in this document, in order to allow the Department to adequately assess the safety, efficacy or quality of a product. Health Canada is committed to ensuring that such requests are justifiable and that decisions are clearly documented.

This document should be read in conjunction with the accompanying notice and the relevant sections of other applicable guidance documents.

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1. INTRODUCTION

Hepatotoxicity caused by exposure to a drug or non-infectious agent is injury or damage to the liver that may be associated with impaired liver function (Navarro and Senior 2006). Drug-induced hepatotoxicity is one of the most common causes of termination of drug development, a major reason for refusal of market authorization and for restricted use, and an important cause of the withdrawal of market authorization for products. Thus, drug-induced hepatotoxicity is a major concern during the discovery, development to post-authorization phases of the product life cycle.

1.1 Objectives

This document is intended to provide basic considerations for the detection, assessment, mitigation and reporting requirements of hepatotoxicity caused by human health products [biologics, medical devices, natural health products, pharmaceuticals and radiopharmaceuticals], both alone and in the presence of other health products, foods or xenobiotics, prior to market authorization by Health Canada.

1.2 Policy Statements

This guidance should be used in conjunction with other non-clinical and clinical guidances in the development and execution of risk assessments and the preparation of reports for product-induced hepatotoxicity prior to market authorization. The guidance provides suggestions, not requirements, for those involved in the research, reporting and regulatory assessment of health products.

Health Canada recognizes that the investigational/risk assessment approach used for a particular product will depend on multiple factors, including the pharmacodynamic and pharmacokinetic characteristics of the product, the indications sought for authorisation, and the dosage(s) and route(s) of administration. The methods potentially used for the assessment of hepatotoxicity are undergoing rapid evolution, therefore regular consultation of the literature is recommended to determine the status of research/risk assessment best practices in this field.

1.3 Scope and Application

The scope of this document is limited to hepatotoxicity induced by human health products regulated under the *Food and Drugs Act*, both alone and in the presence of other products, foods or other xenobiotics prior to market authorization.

The external Scientific Advisory Panel on Hepatotoxicity determined that the general principles used for evaluation of hepatotoxicity in drugs can be extended to all health products regulated under the *Food and Drugs Act*.

1.4 Abbreviations and Definitions

1.4.1 Common Abbreviations

ALT	alanine aminotransferase (formerly known as serum glutamic-pyruvic transaminase, SGPT)
ALP	alkaline phosphatase
AST	aspartate aminotransferase (formerly known as serum glutamic-oxaloacetic aminotransferases, SGOT)
CB	conjugated (direct) bilirubin
GGT	γ-glutamyltransferase (also known as γ-glutamyltranspeptidase, GGTP)
ICH	International Conference on Harmonisation
INR	International Normalized Ratio
TB	Total Bilirubin (summation of conjugated and non-conjugated serum bilirubin)
ULN	Upper Limit of the Normal reference range (or N)

1.4.2 Definitions

Abnormal liver test - any hepatic test value greater than the population-defined upper limit of the normal reference range (ULN).

Adverse event - any untoward medical occurrence in a patient administered a health product and which does not necessarily have a causal relationship with this treatment. An adverse event can therefore be any unfavourable and unintended sign (for example, an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to this medicinal product.

Adverse reaction - a noxious and unintended response to a product and includes "adverse drug reaction" as defined in the *Food and Drug Regulations* and "adverse reaction" as defined in the *Natural Health Products Regulations* (Canadian *Food and Drug Regulations,* Part C: Drugs; and C.01.001. *Natural Health Products Regulations*, Interpretation, C.R.C., SOR/2003-196.

Enzymes - in the context of this guidance, this refers to alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transpeptidase (GGT).

Idiosyncratic - individualistic response where the individual is unable to adapt to or tolerate ordinary doses of a product that may be safe in others, and not predicted by the known pharmacokinetic or pharmacodynamic properties of the stimulus.

Liver failure - clinical manifestation of sudden and severe liver injury; a broad term that encompasses both fulminant (within 8 weeks of symptoms) and subfulminant (late-onset) hepatic failure in a person with a previously healthy liver.

Serious adverse reaction - is a noxious and unintended response to a health product that occurs at any dose and that requires in-patient hospitalization or a prolongation of existing hospitalization; that causes congenital malformation; that results in persistent or significant disability or incapacity that is life-threatening, or that results in death, or is a medically significant event (ICH E2A). Seriousness, not severity, serves as the guide for defining regulatory reporting obligations.

Severity - defines the intensity from mild, moderate to severe (ICH E2A); grades 1 to 5 in the Common Terminology Criteria for Adverse Events (CTCAE), Version 4.0, Published: May 28, 2009 (v4.03: June 14, 2010), United States Department of Health and Human Services, National Institutes of Health, National Cancer Institute.

Xenobiotic - a foreign chemical compound that is not produced in the human body; this includes environmental contaminants, health product and their metabolites.

2. GUIDANCE FOR IMPLEMENTATION

2.1 Background

There are no definitive diagnostic criteria for health product-induced hepatotoxicity, defined as hepatic injury caused by any health product, as it may simulate any known hepatic disease (Lee and Senior 2005). Hepatotoxicity may result from direct action of the xenobiotic, or indirectly from interactions with other foods or xenobiotics. Intrinsic and extrinsic factors may affect the pharmacokinetic and pharmacodynamic properties of this stimulus, thereby affecting the safety profile (ICH E5(R1), www.ich.org/LOB/media/MEDIA481.pdf). As a result of these factors, there may be considerable geographic variability in causation and frequency of injury because of differences in the intrinsic and extrinsic factors, and the availability and prescribing patterns of the health products. Genetic polymorphisms affecting metabolic and transport pathways may affect the local concentration of the product or reactive metabolite at the cellular level, which in some instances may either form a covalent complex or trigger damage directly (Andrade et al. 2009; Daly 2010). Susceptibility may also be increased by the presence of another condition that impairs function in one or more metabolic or regulatory pathways (see section 2.2.2). Product-induced hepatotoxicity may occur as an expected dose-dependent hepatic toxicity or as an unexpected idiosyncratic reaction. Hence, there is a complex relationship between the patient, stimulus and disease which may affect the individual response and risk of hepatotoxicity. Diagnosis of health product-induced hepatotoxicity relies on the exclusion of multiple elements such as the medical history (specific risk factors, exclusion of other diagnoses), presentation

(time to onset of symptoms, jaundice or objective laboratory abnormality; and course of recovery), laboratory results, and subsequent course of the disease (clinical features).

Detection of health product-induced liver injury usually depends on valid causality assessment and a sufficient number of trial subjects. The number of subjects studied in clinical trials must be approximately three times the incidence to achieve 95% confidence that the event did not happen by chance; detecting a single reaction at a frequency of 1 in 10,000 (very rare) would require testing 30,000 subjects. As phase 3 studies typically involve less than 3,000 patients, many products complete phase 3 testing and are approved before a single case of liver injury is identified. Absence of hepatotoxicity in clinical trials may only provide a limited predictive value on whether a product is hepatotoxic. Biochemical properties, metabolism and transport, including *in vitro* and preclinical *in vivo* liver data, should be taken into account in assessing a drug's potential for causing hepatotoxicity.

2.1.1 Hepatic Injury

The liver is a complex, multifunctional organ. Injury may result from direct damage to the hepatocytes, or from damage to bile canalicular cells, sinusoidal epithelial, stellate or Kupffer cells which alters function or indirectly damages the hepatocytes (Lee and Senior 2005). The liver has significant resilience and regenerative properties as an adaptive response to many agents; hence, hepatic injury may not always lead to clinically decreased function. However, hepatic injury causing functional change is of significant concern.

Liver injury encompasses a wide range of clinical and pathological manifestations, and a variety of mechanisms may cause presentations ranging from asymptomatic elevations of enzymes to severe dysfunction (Assis and Navarro 2009). Liver enzyme elevations above the ULN or in case of liver diseases, above the baseline may be indicative of liver injury or further liver injury, respectively. The causes of these elevations should be investigated. Elevations above an individual's baseline activity (values prior to drug treatment) should also be considered, particularly in subjects with underlying liver disease. Further guidance on frequency and duration of monitoring under such circumstances are discussed in Section 2.4.2. The pathophysiology of health product induced liver injury varies depending on the drug and, in many cases, is not entirely understood.

Liver injury is broadly classed as hepatocellular, cholestatic, mixed (cholestatic and hepatocellular), immunologic and mitochondrial (Table 1). The mechanisms of hepatic injury may include: disruption of intracellular calcium homeostasis (membrane); disruption of actin filaments (canaliculus); covalent binding of a substance to cellular proteins resulting in immune injury, inhibition of cell metabolic pathways, blockage of cellular transport pumps, induction of apoptosis and interference with mitochondrial function.

Liver injury may develop at any time point along the continuum of exposure from within days to after several weeks, and less commonly after several months. The injury pattern may be consistent for a particular product or class of products, but not all products or product classes have a characteristic time to onset, pattern of abnormal biochemical values, clinical course, degree of severity, or speed of recovery (adapted from Fontana et al. 2010).

Hepatocellular injury can also result from damage caused by bacterial or viral infections, parasitic infestation, or exposure to health products or other xenobiotics. Hepatocellular injury leading to hepatic necrosis is the most common pathogenic process that leads to life-threatening reaction, and is normally detected by increases in activity of serum aminotransferases, alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The increase in ALT is usually greater than the increase in AST, although in the absence of changes in creatine kinase or other muscle markers an increase in AST greater than the increase in ALT may occur with alcohol consumption.

Cholestatic injury is manifested by stoppage or suppression of bile flow. This may be due to disease or bile duct blockage or stricture among other reasons. Cholestasis may be intrahepatic or extrahepatic. The common intrahepatic causes include drugs, toxins, viral hepatitis; less common causes include alcoholic liver disease, hemochromatosis, primary biliary cirrhosis, primary sclerosing cholangitis, steatohepatitis, Wilson's disease. The common extrahepatic causes include common bile duct stone, pancreatic cancer; less common causes include acute cholangitis, pancreatic pseudocyst, primary sclerosing cholangitis, common duct strictures caused by previous surgery or other tumours. Cholestatic injury is commonly associated with increases in alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (GGT) activity, and bilirubin level. Cholestasis is usually reversible and has lower mortality and morbidity, therefore, it is a less severe problem. However, cholestasis due to specific agents like terbinafine appears to be more chronic, possibly in the case of terbinafine because it has a very long terminal half-life. Since ALT, AST, ALP and GGT are not exclusively specific for hepatocytes and/or the biliary epithelium, interpretation of the entire panel of liver parameters (including bilirubin) is necessary for the diagnosis of hepatic damage. Furthermore, increased serum/plasma activities of ALP and GGT may not be related with cellular damage, but can be the result of a health product (for example [e.g.], phenobarbital) related enzyme induction.

Occasionally, the liver injury pattern is mixed. The wide variability in response may result in a single product exhibiting all three patterns (Fontana et al. 2010). Clinical phenotype is not necessarily class-specific as products with no structural homology may share a similar phenotype.

Determining what predominant pathogenic process is responsible for the apparent hepatic injury by evaluating the relative ratio of different biochemical marker tests is challenging as the reliability of the ranges of ratios for establishing patterns has not been assessed prospectively, particularly as the ratios may change based on whether early (onset) or peak elevation values are used for the determination.

The immunologic mechanism of hepatotoxicity may involve formation of a covalent complex between the health product or its reactive metabolite and cellular protein. This complex may then be presented to T-cells through action of human leukocyte antigen (HLA) and result in an inappropriate local T-cell response. Mitochondrial injury may develop through one or more pathways such as oxidative phosphorylation, severe mitochondrial adenosine triphosphate (ATP) depletion, interference of normal lipid metabolism, and may be identified by the presence of lactic acidosis and microvesicular steatosis; and altered enzymatic activities of respiratory chain complexes II-IV in peripheral white blood cells, manganese superoxide dismutase (SOD2) and glutathione peroxidase (GPX1) which are involved in mitochondrial oxidative stress management (Apostolova et al. 2010; Berger et al. 2010; De Bus et al. 2010; Jones et al. 2010; Lucena et al. 2010; Pessayre et al. 2010). Mitochondrial injury may result in liver cell necrosis or apoptosis. Necrosis and apoptosis can trigger cytolytic hepatitis resulting in lethal fulminant hepatitis in some patients. Other responses include extensive microvesicular steatosis, hypoglycaemia, coma, and death. Milder and more prolonged forms of drug-induced mitochondrial dysfunction can also cause macrovacuolar steatosis which can progress to steatohepatitis and then to cirrhosis.

2.1.2 Hepatic Function

Proper hepatic function is vital to the majority of homeostatic processes in the body. The status of these functions can be determined by measurement of biochemical parameters such as total bilirubin (TB), conjugated (direct) bilirubin (CB), serum albumin (Zimmerman 1999) and prolonged blood prothrombin time. Because the liver is a multi-functional organ, other functional biomarkers are possible. Although abnormal biochemistries are often the first indication of hepatic disease, normal or minimally abnormal tests do not preclude the development of significant disease or cirrhosis.

Clinically, acute health product induced liver failure is a syndrome of rapidly evolving hepatic dysfunction complicated by coagulopathy and, in advanced stages, hepatic encephalopathy. Acute liver failure is commonly divided into two subgroups: (1) fulminant hepatic failure, with hepatic encephalopathy developing within 8 weeks of the onset of illness (or 2 weeks after the onset of jaundice); and (2) subfulminant hepatic failure, with hepatic encephalopathy developing 8 weeks to 6 months after the onset of illness (or 2 weeks to 3 months after the onset of jaundice). Health product induced fulminant hepatic failure in about 50% of the cases is caused by acetaminophen overdose.

Subfulminant hepatic failure is more often caused by product-induced hepatotoxicity or unknown factors (Friedman and Keeffe 2004).

Acute liver failure occurs in approximately 10% of patients with hepatocellular injury accompanied by jaundice. Cholestatic injury is less likely to result in acute liver failure. Any product that causes hepatocellular injury with jaundice during product development and in pre-market clinical trials will probably result in the detection of acute liver failure after marketing, thus additional scrutiny is required before approval (Lee 2003). It is estimated that adverse product reactions account for 2-5% of all cases of jaundice in hospitalized patients, 40% of cases of hepatitis in patients over age 50, and up to 25% of cases of fulminant hepatic failure (Friedman and Keeffe 2004).

In chronic liver failure, there is typically progression of the hepatic injury leading to end stage signs and symptoms like advanced cirrhosis and clinical features including: intractable ascites, malnutrition, variceal bleeding, refractory encephalopathy, refractory coagulopathy, malaise and fatigue, with increased bilirubin, decreased albumin, and increased International normalized ratio (INR).

2.1.3 Hy's Law

Hy's Law or rule can be used to estimate severity and the likelihood that a health product will cause increased incidence of severe hepatotoxicity. Hy's Law is based on the combined evidence of hepatic injury, decreased hepatic function, and the absence of disease-induced damage (Kaplowitz and DeLeve 2003, Zimmerman 1999). The original Hy's Law parameters for treated patients relative to comparator subjects have been broadened over time to include additional parameters, but still require that these 3 criteria be met:

i) injury: elevation of >3 x ULN ALT or AST activity; and
ii) function: >2 x ULN TB (another clinical marker for function, such as > 1.5 x ULN INR may be acceptable if the change is clinically significant in the absence of obstruction) without >2 x ULN ALP; and
iii) clinical verification to ensure effect is health product-induced and not induced by disease or another cause of injury.

Limitations: Elevation of serum ALT activity is sensitive but not entirely specific for liver injury and TB is more specific but rather insensitive for determining liver dysfunction (Senior 2006); but together have been useful for predicting that some subjects will develop severe hepatotoxicity. The degree of ALT elevation most predictive of serious liver injury has not been fully established, nor is it clear whether the rate of change of these levels may be useful as a predictor of hepatotoxicity. It has been noted that ALP >2 x ULN occurs in one third of potential Hy's Law cases and can be

associated with subsequent liver failure. Sometimes a combination of R (that is [i.e.], ALT [x ULN]/ALP [x ULN]) ≥ 5 with total bilirubin ≥ 2 x ULN at time of peak ALT may be considered a better and more predictive definition of Hy's Law. Whether the appearance of clinical jaundice or elevations of serum bilirubin should be included as part of the predictive combination of abnormal findings has yet to be validated (Lewis 2006).

Despite these limitations, a single case of health product-induced hepatotoxicity meeting Hy's Law from clinical trial study should be considered as a potential signal of hepatotoxicity for the product; more than one case should trigger a careful review of the risk-benefit ratio.

2.2 Detecting Hepatotoxicity

Non-clinical animal testing is essential for the early detection of the hepatotoxic potential of products in development. These non-clinical studies along with controlled clinical trials in humans can detect and eliminate most of the obviously hepatotoxic products. There is no reliable method to predict or mitigate idiosyncratic risk. The assessment of chemical structure and biotransformation pathways for the potential to form reactive intermediates or metabolites is recommended, as is enhanced liver chemistry monitoring if a liver signal is detected. Consideration should be given to dose, temporal, inter-species and model specific differences to assess potential effect on metabolic enzyme or transporter expression and function. Animal toxicity studies have been successful in detecting dose dependent hepatic injury but have limited value in predicting human idiosyncratic hepatic injury.

The objective of non-clinical studies should be to:

i) identify signals indicating liver toxicity in standard toxicological studies that detect changes and their magnitude, with gross and histopathological assessment and *in vivo* observations;
ii) determine safety margins for human exposure by providing a No Observed Adverse Effect level (NOAEL) for liver toxicity for either the dose level or systemic exposure and provide information to support monitorability and reversibility; and
iii) where possible, determine a mechanism/pathogenesis for the toxicity to improve prediction of clinical safety.

Species differences in disposition, target and pathobiology must be taken into account in the interpretation of non-clinical findings and in assessing the relevance to humans. The general assumption is that the higher the animal species (rodent < non-rodent < non-human primate) that demonstrates signs of liver toxicity or histopathological adverse responses, the greater the relevance of clarifying the mechanism(s) responsible for liver toxicity. Overall, there may be < 60% concordance between health product-induced hepatotoxicity in humans and animals, and this could approach levels as low as 40% (Greaves et al., 2004, Olson et al., 2000). In a

comprehensive retrospective examination of published data, non-clinical concordance to a clinical hepatotoxic event was lowest with a rodent only, increased when the finding was in a non-rodent only, and greatest when a finding was found in both models (EMA 2010).

Standard histopathology and clinical pathology as described in effective guidelines (i.e., ICH M3(R2), 2009), are the most reliable methods for the assessment of hepatotoxicity in conventional *in vivo* toxicological studies. The clinical chemistry panel for hepatocellular injury should include ALT and AST, and in some species alcohol dehydrogenase (ADH), glutamate dehydrogenase (GLDH) or sorbitol dehydrogenase (SD) because metabolic pathways may differ among species. Also at least two of the following parameters for hepatobiliary injury should be determined: ALP, GGT, 5'-nucleotidase (5NT) and total bilirubin. Knowledge of the limitations of each parameter is necessary for the interpretation of changes.

Clinical signs, changes in clinical chemistry and microscopic changes are often transient, and assessments should be made at multiple time intervals to determine the effect of different durations of exposure. When clinical chemistry or histologic evaluations indicate hepatic changes, studies on the mechanism of action should be conducted with serial specimens of blood, urine or tissues, including samples from matched asymptomatic treated individuals, to determine the mechanism of action and the potential risk of hepatotoxicity in humans. Clinical signs, clinical chemistry and histology for *in vivo* studies should be evaluated in accordance with the appropriate pre-market guidances to extend the mechanistic understanding and examination of potential hepatotoxicity detected in *in vitro* studies.

To assist in the identification of mechanisms and characterization of sub-population differences that result in hepatotoxicity, *in vitro* studies may include analyses of relevant pharmacodynamic and pharmacokinetic genetic polymorphisms; expression and functionality of the relevant major Phase I and II metabolic and transportation pathways and nuclear regulators; proteomics; and metabonomics (includes clinical chemistry, and analysis of parent substance(s) and metabolites). These studies may determine if the pharmacokinetic profile of the parent substances and its metabolites, alone and in the presence of other health products or other xenobiotics, has changed; especially if there is the formation of new or unique metabolites. Receptor, immunologic and inflammatory pathways may also be considered for analysis.

2.3 Assessment of Hepatotoxicity

The complex nature of product-induced hepatotoxicity requires an awareness of factors such as timing, concomitant and/or pre-existing liver disease, concomitant health products, the exclusion of alternative causes of liver damage, the response to dechallenge, and where appropriate, rechallenge of the treatment.

Health product-induced liver disease is not necessarily a single disease, but may be a multifactorial response to a wide range of chemical entities, alone or in combination. The risk profile may also be equally broad, and vary with factors including age, gender, ethnicity and concomitant diseases.

The initial clinical manifestations of product-related injury can often be nonspecific. Constitutional symptoms such as malaise, anorexia and fatigue may be severe, and may occur much earlier than jaundice. Diagnosis of product-related hepatotoxicity is one of exclusion, as other causes of liver disease must be considered and then excluded through clinical, radiological, histological, biochemical and/or serological findings.

There is no diagnostic gold standard for the assessment of hepatotoxicity, and the reliability of the assessment often depends on the knowledge and experience of the investigators involved. Assessment of hepatotoxicity requires a thorough clinical review of the patient and a systematic exclusion of other potential causes for the hepatic abnormalities as outlined below. Whenever possible, the patient should be seen by an experienced gastroenterologist/hepatologist since these patients may be very challenging. A number of methods have been proposed for the assessment of hepatotoxicity in individual subjects, including but not limited to: Clinical Diagnostic Scale (Aithal et al. 2000), Council for International Organizations of Medical Sciences (CIOMS)/RUCAM scale (Danan and Benichou 1993), Maria and Victorino Scales (Maria and Victorino 1997), the Naranjo Adverse Drug Reactions Probability Scale (Naranjo et al. 1981), and World Health Organization (WHO) causality algorithm (http://www.who-umc.org/graphics/4409.pdf). *See also* 2.0.3. *Hy's Law*.

The assessment of product-induced hepatotoxicity can be confounded by other factors and diseases that may mimic or increase sensitivity towards health product-induced liver disease. Testing may be required to eliminate confounding factors including, but not necessarily limited to:

- non-alcoholic steatotic hepatitis (NASH);
- Gilbert's syndrome;
- co-morbidity (including Human Immunodeficiency Virus [HIV], paraneoplastic phenomena, metastases, viral hepatitis A, B, C or E);
- alcohol (a significant confounding factor with respect to the risk and severity of hepatotoxicity) and street drug use;
- biliary abnormalities (including obstruction or infection);
- autoimmune disease or immunosuppression;
- haemodynamic abnormalities (pulmonary hypertension, heart failure, cardiovascular shock, coronary artery disease, and renal failure);
- genetic and metabolic disorders;
- diabetes and obesity;
- pregnancy;

- concurrent and previous therapy with other health products (such as anti-infectives, anti-convulsants, and anti-inflammatory drugs (particularly acetaminophen), and NHPs;
- food-drug interaction that may alter pharmacokinetics;
- non-compliance with treatment regimen;
- environmental and occupational exposures to xenobiotics including pollutants; and
- specific toxic insults such as *Amanita sp.* (mushroom).

2.3.1 Morphologic Pathology

Nonspecific histologic lesions may be suggestive of product injury but other aetiologies should also be considered (e.g., viral hepatitis or autoimmune hepatitis). Nonspecific histologic lesions typically include: hepatitis, hepatocellular necrosis, granulomas, inflammatory cell infiltrates, zonal distribution of lesions, hepatocellular degenerative effects, apoptosis, cholestasis, steatosis, vascular lesions and neoplasia. These changes are not mutually exclusive, and more than one type of injury may be encountered. In addition, the same drug may cause different patterns of liver damage in different patients, which may result in a disproportionately severe degree of damage in relation to the patient's clinical condition and the extent of liver enzyme abnormalities. If histologic changes cannot be attributed to a disease process or an adaptive response, product-induced liver injury should be considered.

Changes in liver size or gross changes in colour, texture or anatomy may indicate hepatic injury; however, microscopic evaluation, that is, liver biopsy, is required to assess structural changes (Table 1). Additional assessments may include ultra-structural pathology, morphometrics, special histological stains, or antibody detection. The pattern of cellular injury, the presence of cellular infiltrates, and the presence of necrotic and/or apoptotic cells should all be assessed. Changes in morphologic pathology at or above the 'minimal to slight' level are normally considered adverse, but may result from adaptive responses or other causes of hepatic injury (e.g., alcohol) or disease (e.g., viral infection). No morphological pattern in a liver biopsy is specific to a health product induced liver injury.

2.3.2 Clinical Evaluation

In clinical trials, the occurrence of an increased frequency of one or more adverse events in a treatment arm may provide a signal of potential hepatotoxicity, even if the adverse event was considered unrelated to the product in individual patients (unless a clear adjudication reason was determined such as viral hepatitis sero-conversion). Careful comparisons of clinical signs and laboratory evaluations should be made between the control and treatment arms, as well as across doses within the treatment arms. As well as group means, the frequency and magnitude of shifts between pre-treatment and during therapy should also be compared in the treatment arms.

A higher frequency of elevated enzyme or liver function values may suggest a greater risk of serious hepatotoxicity. Any trend towards higher levels with successive testing over time denoting liver injury or altered function relative to the control is more important than the actual value or frequency. However, any evidence of increased frequency should trigger further review to determine the clinical significance. This was evident with troglitazone where the frequency of increases in ALT above 3 x ULN was 1.9% in treated patients and 0.6% in controls. Thus, there is no predetermined point for concern (i.e., 2%) or reporting. It is sometimes difficult to differentiate this increase from reversible, asymptomatic elevations in ALT and AST (tolerance) (Lewis 2006). Tolerance in the absence of any hyperbilirubinemia is not a predictor of severe hepatotoxicity; however, it is important not to ignore the transient injury signal in clinical studies.

Rechallenge in a subject or patient who has been withdrawn from a study raises the risk of creating a serious adverse reaction. Rechallenge should not be considered if there are signs of an immunological reaction. Rechallenge may be considered with close monitoring of the patient if no other product is available for treatment, or if the accumulated evidence does not indicate a potential for severe injury. After consultation and approval of the institutional review board, the patient may be rechallenged after being informed of the potential risk and consents to the rechallenge. Re-administration may fail to elicit a response possibly due to differences in dose, presence or absence of other health products, or intrinsic or extrinsic factors including health status at time of rechallenge; hence a negative finding may have a limited value in the causality determination.

2.3.2.1 Reporting

Hepatotoxicity reports should include key data elements and information on:

- history of previous exposure to the product or a similar product;
- information on potential contamination or adulteration of products;
- intrinsic and extrinsic patient characteristics (e.g., nutrition and diet including recently used seasonal foods, other health products, smoking and alcohol use, medical history, ethnicity/race, pharmacogenomic information, concomitant illness and autoimmunity, etc.);
- the frequency of monitoring, the time interval between product administration, the initial abnormal laboratory result and subsequent serial values;
- the number of subjects or patients who exceeded the test threshold, the number of subjects who returned to normal; any case report (national or international) of hepatotoxicity identified during clinical trials (regardless of the investigators' causality evaluations) including severity, treatment, course of the toxic liver disease and sequelae; and

- the results from dechallenge and any rechallenge with details on time and dose.

2.4 Clinical Trial Risk Mitigation Strategies

Hepatotoxicity risk mitigation strategies throughout clinical development may include, but are not limited to:

- appropriate inclusion and exclusion criteria;
- monitoring;
- predetermined dose interruption, or reduction, and/or withdrawal; and
- informing investigators and subjects as information becomes available.

These strategies, particularly with effective monitoring and early withdrawal, may prevent the detection of hepatotoxicity in the clinical trial, thereby precluding determining whether a product may have the potential to meet Hy's Law. Further clinical assessment should be undertaken to determine progression of hepatotoxicity in patients who have been withdrawn prematurely; this may include increased clinical monitoring (e.g., serum hepatic enzyme measurements at more frequent intervals).

2.4.1 Subject inclusion and exclusion

Inclusion or exclusion of healthy subjects or patients with existing liver disease should be based on the risk profile and potential benefit of the product, and justified for each individual trial.

For products not suspected of causing liver damage but which depend on normal liver function for maintaining the area-under-the time-concentration curve (AUC) within a desirable range, ALT and AST for healthy subjects should be < 2 x ULN and TB < ULN at entry. For products with known or suspected hepatotoxicity a desirable range of the entry values for ALT and AST would be < 1.5 x ULN and TB < ULN.

Inclusion of subjects with mild-to-moderate hepatic impairment (ALT < 3 x ULN and TB < 1.5 x ULN) in some Phase 2 and Phase 3 trials is prudent to assess drug tolerance in this special population prior to marketing approval. For patients with known liver metastases, clinical judgement is acceptable. Seriously ill patients are not routinely included in Phase II studies but may be included if they are part of the target population for the product. The entry values in these populations must be assessed individually, as limits to their transamininase levels would be arbitrary. A flare or complication of the underlying disease may make monitoring and causality assessment more difficult in these subjects.

2.4.2 Monitoring

The frequency and duration of monitoring during a clinical trial should depend on the length, risk profile of the product, and population of the study. Less frequent monitoring would be satisfactory if systemic exposure is extremely low as with products such as eye drops, ointments, bronchodilators, etc. or if previous large trials have established that there is less risk.

A conservative starting point for a product with no suspected hepatotoxicity may be to monitor hepatic chemistries every 2 to 4 weeks in the initial Phase 1 and 2 trials; subsequently every 2 to 3 months in longer clinical trials unless there is a suspicion of hepatotoxicity.

In subjects with known liver disease, or with a product or class of product of known or suspected hepatotoxicity, monitoring should be specifically tailored for each trial based on the nature of the disease and the health product.

Any test showing an increase of serum ALT > 3 x ULN or > 2 x ULN TB should be repeated within 48 to 72 hours for ALT, AST, ALP and TB. If the repeat value for ALT or TB is unchanged or indicates decreasing activity, monitoring should continue at weekly intervals until the results are acceptable or normalized. If any value has increased further, immediate close observation is required. If close monitoring is not possible, the drug should be discontinued.

2.4.3 Withdrawal

The decision to withdraw medication in a clinical trial may be based upon a conservative approach where the absolute value of the enzyme or TB is less important than the rate and direction of change as the activities of serum enzymes change much faster than functional indicators, such as TB or INR. Withdrawal should be considered if:

- ALT, AST > 5 x ULN and rising;
- ALT, AST remains > 5 x ULN with no change in TB for more than 2 weeks;
- a worsening of clinical symptoms with no other acceptable explanation; or
- the product meets Hy's Law (section 2.0.3).

Additional hepatic criteria or evolving biomarkers may be used for considering withdrawal.

2.5. Other Risk Minimization Activities

In cases where the risk-to-benefit is unclear or where the benefit of a product with hepatotoxicity outweighs this risk, authorization may require risk-minimization activities in the product monograph. This could include:

- additional educational material for both the patient and health care professional;
- restricting who may prescribe or dispense a product;
- specific conditions under which it may be used;
- required informed consent; and/or
- patient registries.

Risk communications in the relevant sections of Canadian product monograph prior to authorization should be considered. Risk communication may also involve issuance of a specific risk communication document. Additional details on these options are available in Annex B to the European Medicines Agency Guidelines on Risk Management Systems for Medicinal Products for Human Use (EMEA/CHMP/96268/2005).

3. ADDITIONAL INFORMATION

Hepatotoxicity remains an emerging field. Health Canada may update its guidance in response to new scientific knowledge, best practices, and/or on experience gained by the Department.

Questions concerning the submission of hepatotoxicity information to Health Canada should be directed to:

For pharmaceutical products:
Health Canada
Therapeutic Products Directorate
Regulatory Project Management Division
Office of Business Transformation
101 Tunney's Pasture Driveway
Ottawa, Ontario K1A 0K9
Address Locator: 0202D2
Telephone: (613) 954-6481
Fax: (613) 952-9310
E-mail: RPM_Division-GPR_Division@hc-sc.gc.ca

For biologic products:
Health Canada
Biologics and Genetic Therapies Directorate
Centre for Policy and Regulatory Affairs Division
Regulatory Affairs Division
200 Tunney's Pasture Driveway
Ottawa, Ontario K1A 0K9
Address Locator: 0701A
Telephone: (613) 957-1722
Fax: (613) 941-1708
Email: bgtd_ora@hc-sc.gc.ca

For natural health products:
Health Canada
Natural Health Products Directorate
Qualicum Tower A
2936 Baseline Rd
Ottawa, Ontario K1A 0K9 (courier: K2H 1B3)
Telephone: 1-888-774-5555
Fax: (613) 948-6810
Email: NHPD_DPSN@hc-sc.gc.ca

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4.1 Other Regulatory Guidances

European Medicines Agency, Committee for Medicinal Products for Human Use, Draft Guideline on Detection of Early Signals of Drug-induced Hepatotoxicity in Non-clinical Studies, Doc. Ref. EMEA/CHMP/SWP/150115/2006, June 2006.

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Food and Drug Administration, Guidance for Industry, Drug-Induced Liver Injury: Premarketing Clinical Evaluation, July 2009.

TABLE 1 - Parameters routinely examined during assessments for hepatotoxicity

Parameter	Components	Collection Times	Advantages
Clinical Pathology	Clinical chemistry* - Hepatocellular leakage enzymes: ALT, AST - Cholestasis indicators: bilirubin, ALP, GGT - Function indicators: albumin, urea nitrogen - Metabolism indicators: electrolytes, acid/base balance, glucose, triglyceride, cholesterol - Additional standard chemistries: hemogram, leukogram, coagulogram, urinalysis	Intervals throughout all phases of study, but more frequently early in the study	- Certain hepatic changes can only be detected with clinical pathology parameters, especially clinical chemistry parameter data sets specific for hepatic enzymes. - Complementary to histopathology
Morphologic Pathology	Histopathology Gross pathology Hepatic weight	Study termination (There may also be an interim necropsy)	- Critical for identification of certain hepatic changes - Critical for determination of pathogenesis/mechanism of change - Complementary to clinical pathology
Live phase	Clinical observations Body weight Food consumption	Throughout live phase	- In itself does not identify selected hepatic change, but does provide complementary data and clinical consequence to hepatic changes, includes accumulation of parent substrate and metabolite(s)
Expression	metabolism and transport: inhibition/induction	All	- Provides complementary data for morphologic pathology findings - Critical for determining certain potential interactions in man

*The clinical chemistry parameters listed are relevant examples and are not inclusive of all parameters that may be measured. This table was abstracted and adapted from the United States Food and Drug Administration (FDA) (2000a).

APPENDIX A - Clinical Enzymes and Chemistry

Alanine aminotransferase (ALT)

The highest concentration of ALT is present in hepatocytes, and in smaller amounts in skeletal muscle and intestinal epithelium. ALT is considered more sensitive and specific than AST for liver inflammation and hepatocyte necrosis. It rises very rapidly in plasma of patients with acute damage to the hepatocytes. While the absolute numerical value of ALT increase is not directly proportional to the degree of liver damage, the arbitrary value of 3xULN can always be considered to be abnormal if the value persists. The trend of the value, rather than the actual numerical value (unless it is extremely high, e.g. 10xULN) usually correlates well with the development of disease. If the hepatic injury is caused by biliary obstruction, then the increase in ALT is slower and is usually accompanied by increased ALP and GGT.

Aspartate aminotransferase (AST)

AST is found in many tissues (liver, skeletal muscle, heart, kidney, brain, erythrocytes, lung and pancreas) and may increase even if there is no hepatic injury. The increase in AST is usually less than the increase in ALT. AST higher than ALT may suggest, but not prove, alcohol-induced injury.

Alkaline Phosphatase (ALP)

ALP is a nonspecific screening test and may be increased by causes unrelated to liver (e.g. bone, kidney, breast, etc.). High ALP usually means that either the liver has bile duct damage or blockage or a condition causing increased bone cell activity is present. If other liver tests such as bilirubin, AST, or ALT are also high, usually the ALP is coming from the liver. If GGT or 5'-nucleotidase is also increased, then the high ALP is likely due to liver disease. If either of these two tests is normal, then the high ALP is likely due to a bone condition.

γ-Glutamyltransferase (GGT)

Although present in many different organs, GGT is found in particularly high concentrations in the epithelial cells lining biliary ductules. It is a very sensitive indicator of hepatobiliary disease, but is not specific. Levels are elevated in other conditions including renal failure, myocardial infarction, pancreatic disease, alcohol use, and diabetes mellitus. Its major clinical use is to exclude a skeletal source of an elevated serum alkaline phosphatase level.

Bilirubin

Bilirubin is the most common screen for hepatic function. It has been noted that measures of conjugated bilirubin (CB) are seldom obtained and the direct-reacting bilirubin fraction is an overestimate (Navarro and Senior 2006). Increased total or unconjugated bilirubin may be a result of hemolytic, sickle cell or pernicious anemias or a transfusion reaction. If conjugated bilirubin is elevated, there may be some kind of blockage of the liver or bile ducts, hepatitis, trauma to the liver, cirrhosis, a drug reaction, or long-term alcohol abuse. Drug-induced

hyperbilirubinemia may occur as a side effect due to inhibition of bilirubin UDP-glucuronyltransferase 1A1 (UGT1A1) activity by certain drugs. This is predominantly unconjugated bilirubin and is not associated with liver injury or indicators of hepatobiliary damage. If bilirubin is elevated, it should be determined if the increase is due to CB in order to differentiate cholestasis from hepatocellular injury. ALP should be determined for the same reason. An increase in INR may precede an increase in serum TB level. TB measurements are useful, but if the increase is due to liver toxicity it is normally accompanied by a much more rapid increase in ALT. If the cause of increased bilirubin is biliary obstruction, then the increase in ALT is slower (days to weeks/months) and is accompanied by increased ALP and GGT, and there is much less risk of severe acute liver failure. If the cause of increased bilirubin is haemolysis, it has to be diagnosed by other means.

Prothrombin time and International Normalized Ratio (INR)
Most coagulation factors are synthesized by the liver, including factors I (fibrinogen), II (prothrombin), V, VII, IX, and X and have much shorter half-lives than that of albumin. The prothrombin time is useful in assessing severity and prognosis of acute live disease. Deficiency of one or more of the liver-produced factors results in a prolonged prothrombin time. Prolongation of the prothrombin time in cholestatic liver disease may result from vitamin K deficiency. Other explanations for a prolonged prothrombin time apart from hepatocellular disease or vitamin K deficiency include consumptive coagulopathies, inherited deficiencies of a coagulation factor, medications that antagonize the prothrombin complex. Vitamin K deficiency diagnosis can be excluded if an administration of vitamin K 10 mg corrects or improves the prothrombin time within 24 hours. This implies that hepatic synthetic function is intact. Significant prolongation of the prothrombin time that is unresponsive to vitamin K infusions suggests a poor prognosis in patients with fulminant liver disease.

Bile acids
Bile acids are synthesized from cholesterol in the liver, conjugated to glycine or taurine, and excreted in the bile. Bile acids facilitate fat digestion and absorption within the small intestine. They recycle through the enterohepatic circulation; secondary bile acids form by the action of intestinal bacteria. Elevated level of serum bile acids is an indication of hepatobiliary dysfunction. Normal bile acid levels in the presence of hyper-bilirubinemia suggests haemolysis or Gilbert’s syndrome.

Serum total bile acid has been used as an indicator for hepatic function with various types of chemical-induced hepatotoxicity. This test provides diagnosis of hepatocellular dysfunction (more than ALT), but will not provide a definitive diagnosis of the nature of the hepatotoxicity. The role of this test is progressing rapidly and its assessment may be significant in the future. Additional experimental work may be considered to examine other features of liver failure such as encephalopathy, decreased albumin, decreased clotting factors, etc.