Chapter 4

Pathology of the Liver and Gallbladder

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Abstract

The liver is a major metabolic organ, and the first site of contact of xenobiotics follows oral ingestion or administration. The primary functional unit of the liver is the hepatic lobule. Gradients for metabolizing enzyme and oxygen tension in the hepatic lobule determine toxification and detoxification of ingested xenobiotics with immediately toxic agents causing cell damage in the periphery of the hepatic lobule, while enzymatic generation of toxic metabolites typically affect centrilobular hepatocytes. A wide spectrum of spontaneous degenerative changes occurs in the liver, some changes are age-, species-, and strain-dependent, and the job of the pathologist is to assess the significance of a potential treatment-induced liver changes against the background changes in a particular study. Because the liver has a high regenerative capacity following insult, proliferative changes, including benign and malignant neoplasms, are common, especially in the rodent liver. These proliferative changes also occur with an age-, species-, and strain-related background, and the pathologist must weight any proliferative response against this background incidence. Unique to the liver is a class of proliferative lesions designated as foci of cellular alteration which are localized presumptively preneoplastic responses. Several cell types in addition to hepatocytes are present in the liver, and each needs to be addressed by the pathologist in safety assessment of liver changes. Key to understanding the judgment involved in documenting and categorizing the broad spectrum of liver lesions is the pathology narrative where criteria for assessing hepatopathology is spelled out by the pathologist.

Key words Hepatic lobule, Cell death, Apoptosis, Fatty change, Degeneration, Hypertrophy, Hyperplasia, Foci of cellular alteration, Hepatocellular adenoma, Hepatocellular carcinoma

1 Introduction

The purpose of this chapter is to present and discuss some practical considerations in understanding and interpreting common hepatic responses in rat and mouse toxicity and safety assessment studies as diagnosed and described by the study pathologist. We have deliberately avoided elaborating on morphological features and subtle diagnostic criteria as these are well documented in manuscripts and other textbooks.

The liver is a multifunctional and biochemically diverse organ capable of rapid responses to insult and stimuli to maintain optimal function. Because of its high metabolic activity and exposure to

Thomas J. Steinbach et al. (eds.), *Toxicologic Pathology for Non-Pathologists*,
https://doi.org/10.1007/978-1-4939-9777-0_4,

exogenous xenobiotics via the portal blood supply, the liver is a frequent site of toxicity. As the first major organ to be exposed to ingested material, the liver provides protection to other organ systems by "first pass" removal of potentially toxic materials but may also be at increased risk for hepatic injury from xenobiotics and/or their metabolites.

2 Structure and Function

The liver is a major body organ located in the cranial aspect of the abdomen and represents 1–4% of adult body weight in most species. It is comprised of individual lobes in and contains a gallbladder in all common laboratory species except rats. There is a dual blood supply to the liver with approximately 75% of blood coming from the gastrointestinal tract via the portal vein and 25% from the hepatic artery. The functional microscopic units of the liver are multiple hepatic lobules where the dual blood supplies get mixed and enter at the portal area of each lobule. While a three-dimensional structure of the liver has been described (Teutsch et al. 1999), most pathologists typically describe their microscopic findings based on a two-dimensional description of the hepatic lobule.

The hepatic lobule unit can be viewed from different perspectives. The classic hepatic lobule considered in two dimensions as viewed in typical histological sections is used by most pathologists to localize lesions. This classic lobule is a polygonal structure with peripherally located portal triads and a central hepatic vein (Fig. 1). The portal triad consists of a portal vein, a hepatic artery, and a bile duct. Extraneous small bile ducts, hepatic arteries, and lymphatic venules also comprise the portal tracts. Mixed portal vein and hepatic arterial blood flows from the portal region to the sinusoids and then the central vein and hepatic vein and ultimately enters the posterior vena cava moving on to the heart with ultimate systemic distribution throughout the body. An alternative depiction of the microscopic functional unit of the liver is the hepatic acinus (Fig. 1), where blood flow is defined as flowing from the portal triads at each pole of the unit to the central vein. This unit is based upon the gradients of metabolic functions and oxygen tension. A mixture of oxygenated and nutrient-rich blood enters zone 1 which includes the periportal hepatocytes, zone 2, the middle zone of hepatocytes, and zone 3, which includes those cells closest to the central vein and possessing the lowest oxygen tension. A third type of view of the hepatic unit is the portal lobule, which is based upon bile flow (Fig. 1). Bile flow is opposite the flow of blood in the acinus and classic lobule. These alternative depictions are useful in understanding the dynamics of hepatic physiology and responses.

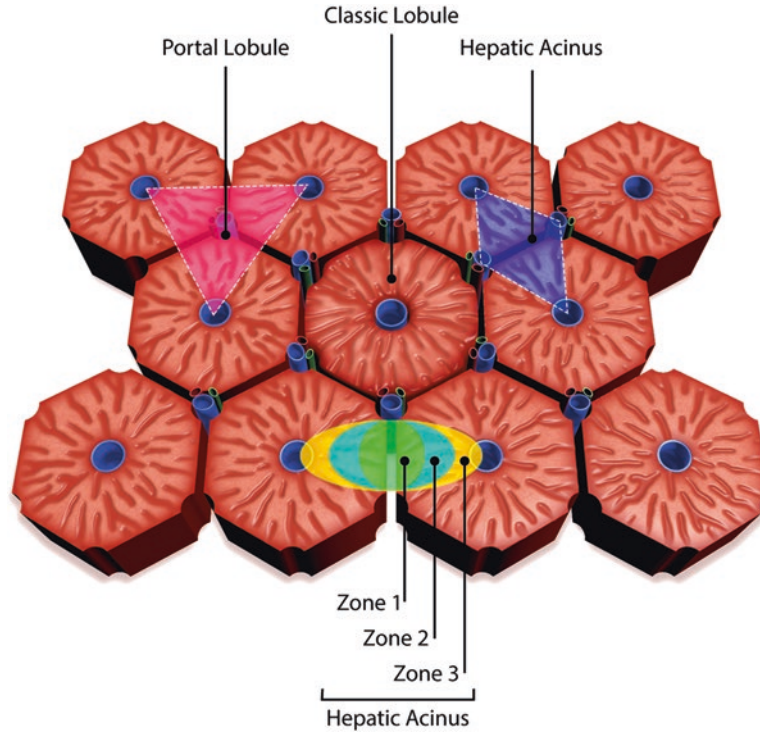

Fig. 1 Idealized drawing of liver lobules identifying the structure of functionally different hepatic lobules. The classic lobule is defined as peripherally located portal triads each consisting of hepatic artery, portal vein, and bile duct and a central hepatic vein. The hepatic acinus (blue shading) is a functional depiction based on blood flow from the portal areas to the central vein. This hepatic acinus may also be depicted based on oxygen tension gradients (multicolored shading) with the highest oxygen gradient in the portal zone (zone 1). The portal lobule (pink shaded triangle) is based on bile flow from the central vein to the portal bile duct

3 Structure and Cellular Components of the Hepatic Lobule

Hepatocytes, the primary cellular component of the liver, occupy positions throughout each hepatic lobule. They are arranged in linear plates extending from the portal triad to the central vein and are separated by vascular sinusoids. There are functional gradients (Fig. 2) in hepatocyte enzymatic content depending upon their lobular position and with respect to oxygenation and blood supply (Teutsch et al. 1999). In general, centrilobular hepatocytes are slightly larger than portal hepatocytes, due to their abundant intracellular endoplasmic reticulum, and have a high proportion of drug metabolizing enzymes. Functional gradients also apply to various non-parenchymal cell types in the liver.

Other cells of the liver include biliary cells that form bile ductules and a spectrum of sinusoidal-lining cells including endothelial cells, Kupffer cells, and stellate cells. The sinusoidal endothelial

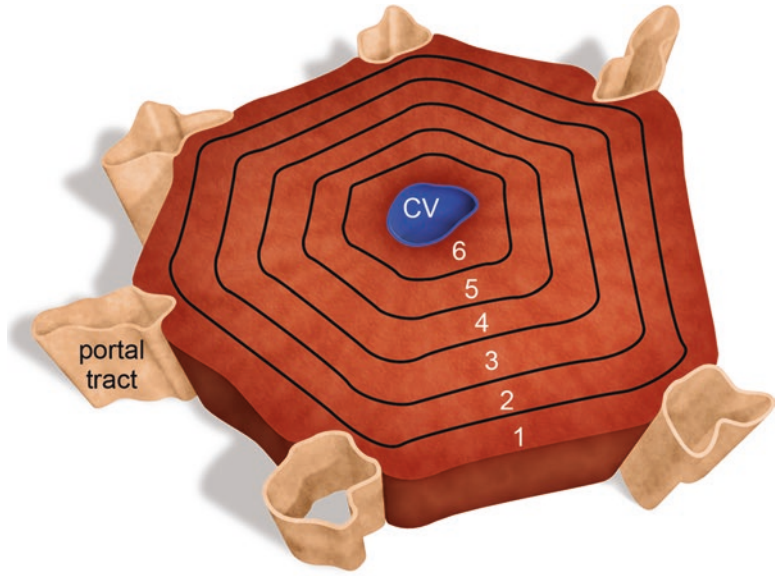

Fig. 2 Depiction of an hepatic lobule showing gradient zones of enzymatic content with the highest level of several endogenous and inducible drug metabolizing enzymes in zone 6. Oxygen tension is greatest in zone 1 with a diminishing gradient moving toward the central vein (CV). (Figure adapted from Teutsch et al. 1999)

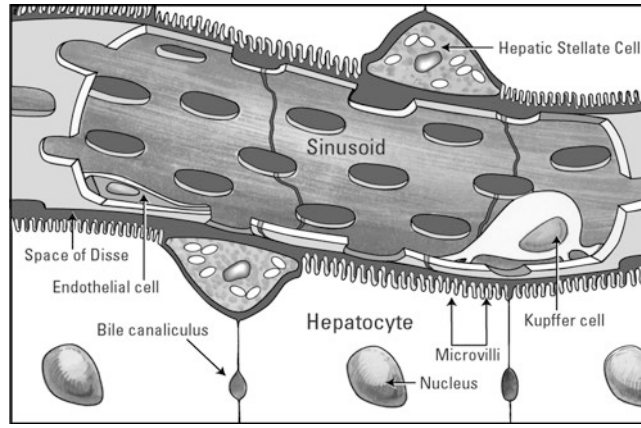

Fig. 3 Diagrammatic representation of the cellular and structural components of an endothelial lined fenestrated hepatic sinusoid showing positions of hepatocytes and other cellular components of the liver

cells are fenestrated and can use pinocytosis to aid in the transfer of cell-free components from blood to the perisinusoidal Space of Disse bringing molecules in direct contact with the hepatocytes (Fig. 3). Kupffer cells are from the mononuclear phagocytic cell system, are situated on the sinusoidal endothelial lining, function as macrophages which engulf and digest cellular debris and foreign substances, and are the source of cytokines during hepatic injury.

Pit cells, a type of natural killer large granular lymphocyte, as well as dendritic cells, that are regulators of liver immunity, line the perisinusoidal space and are among the first lines of defense. A small population of stellate (Ito) cells located along the sinusoidal surface stores lipids and vitamin A and, if activated, initiates the production of collagen seen in hepatic fibrosis.

The liver is the major filter of blood from the gastrointestinal tract prior to its systemic circulation and functions to metabolize and detoxify chemicals that are then secreted into the bile. Endogenous and inducible metabolizing and detoxifying enzymes are concentrated in a gradient within each hepatic lobule (Fig. 2). The liver also carries out metabolism of fats, proteins, and carbohydrates; stores glycogen, minerals, and vitamins; and synthesizes plasma proteins and clotting factors. A variety of hepatic lesions identified by the pathologist during microscopic examination, including necrosis, inflammation, cholestasis, steatosis, vascular changes, and neoplasia, can cause perturbations in any one or more of the diverse functions of the liver.

4 How Pathologists Diagnose and Document Liver Lesions and the Importance of the Pathology Narrative

In most toxicity and safety assessment studies, the pathology findings are tabulated as individual diagnoses with specific modifiers by treatment groups. Studies differ in what strains and species are used, in the range of doses and duration of exposures, and with differing study objectives. Thus, there may be significant differences among studies in how the study pathologist diagnoses, grades, documents, and interprets tissue changes. Consistent with a best practice approach to conducting a pathology evaluation, a pathology narrative provides the basis for the judgments made by the study pathologist for a given study and is an effective means of communicating the interpretative aspects of a given pathology evaluation (Morton et al. 2006). Descriptions in the pathology narrative can convey to the reader the degree to which a spontaneous or treatment-related lesion conforms to classical published morphological features and how severity grading was applied for well-defined as well as for subtle lesions.

Pathologists may differ in the threshold they use for detecting and recording lesions during the evaluation of a safety assessment study. There are age-related common background spontaneous lesions seen in control animals of all types of studies. Some pathologists, therefore, establish a threshold for the occurrence of such lesions and document the presence of commonly seen lesions only when they exceed the background level in incidence and/or severity, or if the lesions occur at an earlier age than is typically seen. What constitutes an acceptable background level of a common lesion is

based to some degree on historical control data and the age of the animals but mostly depends on the experience and judgment of the study pathologist. To help ensure consistency within and across studies, laboratories with multiple pathologists may establish a policy or common practice for defining spontaneous age-associated background lesions. Since background lesions may be exacerbated by treatment, understanding the diagnostic threshold used by the study pathologist is important and ideally should be defined in the narrative portion of the pathology report for certain lesions that are considered treatment-related.

With respect to the documenting lesions in the liver, pathologists may be "lumpers or splitters" in how they categorize lesions. In addition to indicating the severity of a liver lesion, the pathologist will typically identify the anatomical or lobular distribution of a given lesion and also indicate if the lesion is acute, subacute, or chronic. Thus, what is basically the same type of lesion may have several different modifiers. In the tabulation of a given lesion with multiple different modifiers, there is an opportunity to combine what are basically similar lesions for assessing a potential treatment effect. Subcategorizing a lesion excessively with too many modifiers along with different severity grades may mask what is a true effect. For example, in a given dose group, one diagnosis may be "liver, inflammation, acute, neutrophilic, moderate," while another diagnosis is "liver, inflammation, subacute, mixed inflammatory cell, minimal." Since these subcategories probably actually reflect inter-animal variability of the same process, it would be reasonable to combine the two categories for purposes of assessing a significant treatment-related response. In this instance, a combined diagnosis of "liver, inflammation" with a description of the spectrum of features associated with that diagnosis could be presented in the pathology narrative. In addition to considerations regarding combining and dividing lesions based on documenting subtle variations in lesion characteristics, it is important to maintain consistency in categorizing lesion variations and lesion severity in a chronic study to determine if lesions progress in extent or severity or if there is evidence of lesion recovery following cessation of treatment. Evidence for the progression or regression of treatment-related lesions can be explained in the pathology narrative.

5 Discussion of Hepatic Responses in Rats and Mice

Detailed morphological descriptions of rat and mouse hepatic responses with associated photographic examples are documented in many publications (Thoolen et al. 2010; Harada et al. 1989, 1999; Maronpot et al. 1987; Eustis et al. 1990; NNLA 2019; Bannasch and Zerban 1990; Cattley et al. 2013; Deschl et al. 2001; Foster 2018; Frith et al. 1994; Greaves 2012; Haschek et al. 2010)

as well as in other earlier publications. The following discussion of commonly occurring rat and mouse liver responses will focus on how the study pathologist documents and interprets tissue changes rather than detailed morphologic features and diagnostic criteria.

6 Liver Enlargement (Hepatomegaly and Hepatocyte Hypertrophy)

Non-neoplastic liver enlargement can be a relatively common occurrence in rodent toxicity studies, and liver weight is a sensitive metric to identify a treatment-related liver response, whether that response is due to overt toxicity or to microsomal enzyme induction. The pathologist may look at toxicokinetics or pharmacokinetics (TK or PK) and/or absorption, distribution, metabolism, and excretion (ADME) data to provide an interpretative perspective of a particular liver response. Liver weights are typically expressed as absolute weights and as weight expressed relative to body weight. The latter is usually more useful, particularly if there are systemic effects that affect body weight. Relative liver weight increases of 15–20% may not present with easily recognized morphological changes during histopathological evaluation but may still be a significant response to treatment. Relative liver weight increases of greater than 20% usually have an altered morphological feature such as hepatocellular hypertrophy or hepatic inflammatory cellular infiltrates to define the likely cause of the increased weight. However, depending upon the type of microscopic change, for example, marked centrilobular hypertrophy, even very low levels of relative liver weight increases may be supported by microscopic effects. Generally, liver weight increases at 90 days along with concurrent hepatocellular hypertrophy, and degenerative changes such as cytoplasmic vacuolization and/or hepatocyte necrosis are multiple contributing factors in the risk of development of hepatocellular tumors in the rat and mouse. Liver weight increases serve as a sensitive quantitative response to liver toxicity or hepatic enzymatic induction and represent an important component in the assessment of liver responses to xenobiotic exposure (Hall et al. 2012; Allen et al. 2004; Maronpot et al. 2010).

7 Clinical Chemistry

Just as liver weight measurements should be considered in evaluating effects on the liver, several routine serum measurements used in conjunction with microscopic examination of stained liver sections can provide an indication of the degree of liver toxicity (Wiedmeyer 2018). Alanine aminotransferase (ALT) and sorbitol dehydrogenase (SDH) have their highest tissue levels in the liver, and elevated serum levels indicate hepatocellular leakage or injury. Aspartate

aminotransferase (AST) and alkaline phosphatase (ALP) occur in several tissues and are thus less specific for the liver, but serum increases in these analytes in conjunction with elevated serum ALT and/or SDH help to confirm liver damage. Additional clinical chemistry analytes that may be useful in assessing liver integrity and function include serum bilirubin, total bile acid, glutamate dehydrogenase (GDH), and gamma-glutamyl transferase (GGT).

During the course of conducting toxicity and safety assessment studies in rodents, routine clinical chemistry analyses are carried out. Generally, serum enzymes levels greater than twofold above normal will have a correlated morphological microscopic change provided the liver histology sample is taken close to the time serum was collected for analysis. Sometimes twofold elevations of a liver-associated analyte show statistical significance that suggests liver toxicity, even if this degree of elevation falls within laboratory historical control ranges. In these instances, the study pathologist is charged with determining if there is a morphological change that correlates with the statistically flagged analyte. In the absence of identifying a morphological change that could explain the statistically flagged value, some other explanation may be needed to account for the clinical chemistry finding such as diet, serum hemolysis, or normal variability.

8 Frequently Occurring Non-neoplastic Liver Lesions in Rodent Studies

8.1 Hepato-diaphragmatic Nodule

This is a rounded, herniated nodular protrusion of normal hepatic parenchyma that was occupying a defect in the diaphragm. It is considered a developmental anomaly occasionally seen in rats, especially F344 rats, and may be seen in fetuses and should not be considered a treatment-related lesion (Eustis et al. 1990).

8.2 Cytoplasmic Alterations

Cytoplasmic alterations that occur in the rodent liver include glycogen accumulation or depletion, fatty change, phospholipidosis, and hydropic and cystic degeneration. While the nonspecific diagnostic term of "cytoplasmic vacuolization" may be used for hepatocyte glycogen accumulation, fatty change, and phospholipidosis, definitive proof for a specific diagnosis requires special staining (Thoolen et al. 2010). However, an experienced pathologist can recognize morphologic features consistent with fatty change or glycogen accumulation on a hematoxylin and eosin (H&E)-stained section of the liver. Hydropic and cystic degeneration are generally diagnosed based on H&E-staining.

8.2.1 Glycogen Accumulation and Depletion

Abnormal glycogen retention or depletion is unusual but suggests a functional failure to normally metabolize glycogen and should be documented when present. Glycogen accumulation is characterized by clear or rarefied cytoplasm surrounding a centrally located

nucleus and can be demonstrated by special staining (periodic acid Schiff (PAS) with and without diastase). Most pathologists expect some degree of cytoplasmic vacuolation in hepatocytes consistent with glycogen and do not diagnose it unless there is an apparent difference among treatment groups. Being nocturnal feeders, glycogen is stored in rodent hepatocytes and is gradually mobilized as a source of energy throughout the day (Greaves 2012; Thoolen et al. 2010; Eustis et al. 1990; Harada et al. 1999; Malarkey et al. 2005). Thus, glycogen accumulation in hepatocytes is more readily apparent in non-fasted animals and may be seen to some degree in animals that are fasted overnight. Animals with decreased food intake, such as moribund animals or those with restricted diet, are prone to have depleted their glycogen stores. The dynamics of glycogen accumulation and its subsequent mobilization (depletion) are important conditions to be aware of, and a reason for randomizing the sacrifice timing of treated and control animals in a toxicity study (Malarkey et al. 2005).

8.2.2 *Fatty Change*

Lipidosis and steatosis are synonyms for fatty change which reflect perturbation of lipid metabolism and can be a treatment-related change (Thoolen et al. 2010; Cattley and Popp 2002; Evans and Lake 1998; Greaves 2012; Haschek et al. 2010). Macrovesicular and microvesicular fatty change are two forms of fatty change that are diagnosed separately but can occur together. Both represent abnormal storage of fat within hepatocytes, both are potentially reversible, and both may occur along with the presence of cytoplasmic glycogen and hepatocellular lesions such as necrosis. When accompanied by significant inflammatory foci, it is referred to as steatohepatitis. Macrovesicular fatty change is an imbalance between uptake of blood lipids and hepatocyte secretion of lipoproteins, while microvesicular fatty change is usually considered a more serious hepatic dysfunction and may reflect a mitochondrial perturbation. An experienced pathologist can recognize characteristic morphological features in hematoxylin and eosin-stained sections to provisionally diagnose macrovesicular and microvesicular fatty change, but confirmation with an oil-red-O or Sudan black stain is needed for definitive diagnosis. Since fatty change is potentially reversible, consistency in diagnosis and severity grading is important in studies that include a reversibility cohort.

8.2.3 *Phospholipidosis*

Phospholipidosis is a drug-induced storage disease involving the lysosomes. Phospholipidosis is a response seen following treatment with cationic amphophilic compounds and represents a lipid disorder where xenobiotic and phospholipid complexes are deposited in hepatocyte lysosomes. It can resemble microvesicular fatty change in an H&E-stained section. Definitive diagnosis of phospholipidosis requires either electron microscopy (for the presence of lamellated and crystalloid inclusions) or immunohistochemical

staining for lysosomal-associated membrane protein (LAMP) and acidophilin (Chatman et al. 2009; Halliwell 1997).

8.2.4 Hydropic Degeneration

Sometimes referred to as cloudy swelling, hydropic degeneration refers to a cytoplasmic swelling attributed to perturbation of cell membrane integrity (Thoolen et al. 2010). It is typically associated with exposure to toxic xenobiotics, may have a centrilobular or periportal localization, and is often a precursor to cell death.

8.2.5 Cystic Degeneration

A traditional synonym for this change is spongiosis hepatis. It is usually localized and somewhat focal and is formed by cystic enlargement of perisinusoidal stellate (Ito) cells. This change is more common in rats than in mice and is more often seen in older animals (Eustis et al. 1990; Harada et al. 1999; Thoolen et al. 2010). Although this is a spontaneous age-associated change, there is some evidence for xenobiotic induction of cystic degeneration, and, thus, it is recommended to document this change whenever present.

8.3 Amyloidosis

Extracellular deposits of amyloid, a form of misfolded insoluble protein, is rare in rats but seen in some strains of mice as a spontaneous aging lesion (Harada et al. 1999) and may be influenced by husbandry factors (Lipman et al. 1993). It is usually a systemic disease manifested in several organs. In the liver, it appears in perisinusoidal and periportal locations as well as in blood vessels. It can be identified microscopically with Congo red staining and polarizing birefringence. The occurrence of amyloid is either primary or secondary to chronic illness, and it may be exacerbated by treatment or stress. It is usually diagnosed as a systemic disease rather than specifically in the liver.

8.4 Mineralization

Hepatic mineralization is rare and may reflect a dietary-calcium disturbance (NNLA 2019). Dystrophic mineralization often accompanies necrosis, inflammation, or neoplasia. It can be identified with Alizarin Red or von Kossa special stains for calcium. When secondary to a pronounced necrotic or inflammatory lesion, it may not be separately diagnosed but could be mentioned in the pathology narrative.

8.5 Pigment

Hepatic pigment includes lipofuscin, iron, porphyrin, bile, or possibly an unusual xenobiotic metabolite (Thoolen et al. 2010). It typically reflects an ongoing process. Lipofuscin is commonly referred to as “wear and tear” pigment, is age-associated, can occur as a background change, and may be exacerbated by treatment. Iron pigment is normally very low to undetectable in the adult liver. When present it is most often related to hemosiderin deposition from the breakdown of erythrocytes, and thus a treatment-

related cause should be considered. Since definitive diagnosis of a specific pigment requires special staining, a generic diagnosis of pigment and an indication of its location and severity are typically sufficient in initial histopathological evaluation. Should there be evidence of a dose-related or treatment-related response, definitive special staining is warranted.

8.6 Hepatocellular Hypertrophy

As a distinct form of cytoplasmic change, hepatocellular hypertrophy is a common adaptive metabolic response following administration of some xenobiotics. It is identified by hepatocytes that are larger than normal and have a finely granular, pale staining cytoplasm. There is usually a lobular localization with centrilobular hepatocellular hypertrophy most commonly seen. Since there is often a dose-related response, severity grading based on the degree of hepatocyte enlargement and lobular extent of the hypertrophy is usually helpful. Hepatocellular hypertrophy is typically reflected by increased relative liver weight. Common etiologic agents include enzyme inducers (e.g., phenobarbitone), peroxisome proliferators (e.g., fibrates), and AhR receptor agonists (e.g., dioxins). While this change is reversible following adaptation to a higher level of hepatic homeostasis or following cessation of exposure to the causative agent, severe and prolonged hypertrophy can result in occlusion of sinusoidal space, oxygen deprivation, and hepatocyte death with the latter most evident around central veins. Since hepatocellular hypertrophy is initially an adaptive physiological response, it is usually not associated with any elevation of circulating hepatic enzymes reflective of liver damage. However, when severe and prolonged and associated with centrilobular cell death, some elevation of serum ALT and AST can be present, and there is increased risk of development of hepatocellular tumor development in mouse (Allen et al. 2004; Hall et al. 2012).

8.7 Increased Mitosis

Mitogenesis:
Increase in the presence of hepatocyte mitotic figures may be documented in safety assessment studies and, when present above a very low threshold, is usually given a severity grade and indication of its lobular localization. There are different situations where increased mitoses will be present in the liver, and it is important to try to determine the underlying cause. Some xenobiotics are inherently mitogenic, and their administration results in increased numbers of mitotic figures in routinely stained liver sections. Upon withdrawal of treatment with a mitogenic agent, there is typically an increase in hepatocellular apoptosis as the liver cellular mass adjusts back to a normal physiological number of hepatocytes. An increase in mitotic figures occurs as a recovery response after prior loss of hepatocytes from necrosis. Hence, it is important to determine if the response is secondary to earlier necrosis. During

pregnancy there is an increase in hepatocellular mitosis presumably to provide maternal energy support during gestation.

8.8 Cell Death

There has been considerable dialogue in recent years centered around recommended nomenclature for cell death (Elmore et al. 2016; Elmore 2007). Depending upon the lexicon used by pathologists and organizations, there are preferences for use of "single cell necrosis" by some and "apoptosis" by others for the same change seen in H&E-stained sections. Special caspase stains are used to more definitively identify the programmed cell death pathway referred to as apoptosis. In general, the term necrosis is appropriate when small to large groups of contiguous dead cells often accompanied by secondary inflammation are present.

8.8.1 Apoptosis

A diagnosis of hepatocyte apoptosis implies there is an active cell death of individual hepatocytes in the absence of accompanying inflammation. The morphologic features in H&E-stained sections are often sufficiently characteristic to make the diagnosis of apoptosis without the need for special stains. The diagnosis typically has some modifiers indicating location within hepatic lobules and severity of the response. A very low level of hepatocyte apoptosis may occur spontaneously, and some pathologists do not diagnose apoptosis unless it exceeds a threshold. If believed to be an important factor in a study, the pathology narrative can be used to define the threshold for diagnosis and to describe the location and extent of the observed apoptosis.

8.8.2 Necrosis

Documentation of necrosis in safety assessment studies generally includes important modifiers that localize the cell death and provide an indication of its extent and severity (Cattley et al. 2013; Thoolen et al. 2010). Necrosis can be focal, multifocal, random, or generalized. When generalized, there may be a zonal distribution (i.e., centrilobular, midzonal, or periportal) suggesting a mechanistic toxicological injury (see below). Differential distribution among hepatic lobes reflecting the lobar pattern of portal blood flow from the gastrointestinal tract may be discerned. In typical studies, at least two and sometimes three different liver lobes are sampled for histopathology, and an occasional single focus of microscopic hepatocellular necrosis may occur in one or two of the sampled lobes as a background lesion. Usually, these are very small focal necrotic lesions, may be found in controls as well as in treated animals, are documented by the study pathologist with indication of a minimal severity, have a random lobular localization, and may be mentioned in the pathology narrative as background changes. It is recommended to document focal necrosis even if considered a background lesion since there may be an exacerbation associated with treatment. Many pathologists using a diagnosis of focal necrosis then use a

severity score to reflect multifocality. As would be expected, larger areas of necrosis are more serious indication of hepatotoxicity, and their lobular location may reflect their pathogenesis.

The most common location of hepatic necrosis in safety assessment studies is centrilobular and can often be attributed to toxicity from a metabolite of the test agent generated by the high centrilobular content of endogenous or inducible metabolizing enzymes (Fig. 4). A good example of this is acetaminophen toxicosis. Alternatively, centrilobular necrosis can also be a response to tissue anoxia since centrilobular hepatocytes reside in a low oxygen gradient area. Periportal necrosis (Fig. 5) typically reflects more direct damage from hepatic toxins carried there in the portal blood. Midzonal necrosis (Fig. 6) is not commonly seen but may be a response to specific toxins. Regardless of its lobular localization, necrosis may be accompanied by congestion, hemorrhage, inflammation, and biliary stasis and can extend between lobules as a bridging necrosis. Since necrosis may reflect a dose-related response, severity grading is important.

Fig. 4 Centrilobular necrosis. Blue granular areas surrounding the central vein represent necrotic liver parenchyma. This is a common site of necrosis because of a low gradient of oxygen (relative anoxia) and the presence of CYP metabolizing enzymes in this area of the lobule. In severe cases, the areas of necrosis may extend into the mid-lobular areas and also may form a bridging necrosis by extending to centrilobular areas of adjacent lobules

Fig. 5 Periportal necrosis. Blue granular areas representing necrosis surround the portal triads and sometimes may extend between adjacent portal areas to form a bridging necrosis. This pattern of necrosis may be seen with exposure to highly reactive agents such as acrolein and elemental phosphorus

8.9 Inflammation

Hepatic inflammation is generally documented as a focal, multifocal, or generalized infiltration of specific inflammatory cells and categorized under a diagnosis of "infiltration, neutrophilic" or "infiltration, mixed leukocyte" rather than being diagnosed as "hepatitis", which is a nonspecific term for inflammation of the liver. Small foci of mononuclear cell infiltrates, often without any obvious associated cell death, are common background lesions in rodent livers, and most pathologists only document these focal mononuclear cell infiltrates when they occur above a specific threshold. Since their occurrence and frequency can be exacerbated by treatment, it may be necessary to adjust the threshold for diagnosis appropriately to better identify any potential treatment-related effect.

Inflammatory cell infiltration:
The specific cell type of infiltrating inflammatory cell is usually defined during histopathology evaluation (Cattley et al. 2013; Thoolen et al. 2010; NNLA 2019). Neutrophilic infiltrates indicate an acute response to liver injury and necrosis, but occasional lymphocytes and monocytes may also be present in what is a primary neutrophilic infiltrate. Mononuclear infiltrates are typically

Fig. 6 Midlobular necrosis. Blue granular areas representing necrosis and localized mid-way between portal areas and central veins are uncommon but have been seen after administration of ferrous sulfate and may be associated with exposure to some phytotoxins

associated with a low level of obvious cell death and usually represent a more chronic response. A mixed neutrophilic and mononuclear response with relatively equal components of each cell type would be expected in a chronic, active inflammatory process. Since the dose of a given hepatotoxic agent and the timing after initial insult might influence the morphological features of the inflammatory infiltrate, consideration should also be given to combining related inflammatory responses in assessing dose-related responses and determining no adverse effect levels. Hepatic fibrosis can occur as a response to prolonged repeated hepatotoxicity.

8.10 Vascular Changes

Hemorrhage and congestion can accompany hepatic necrosis and inflammatory responses and is often present as a relatively minor secondary response. In those situations, hemorrhage and congestion may not be separately diagnosed but rather described in the pathology narrative.

Angiectasis:
Angiectasis is a cystic or cavernous localized widening of hepatic sinuses that is occasionally observed in chronic studies (Thoolen et al. 2010). Because it is often macroscopically visible on natural and cut surfaces of the liver, the technician may diagnose it as a

gross lesion at the time of necropsy or even tissue trimming. It may be present secondary to hemodynamic changes in blood flow associated with hepatic neoplasia. It has been induced by treatment.

8.11 Hyperplasias

While any one of the different cell types that reside in the liver may undergo a diffuse or localized increase in number, the most frequent type of hyperplasia involves hepatocytes. Bile duct epithelium and oval cell hyperplasias are most commonly diagnosed in chronic studies, occur spontaneously, and may be exacerbated by treatment. Rarely there can be Kupffer cell hyperplasia in rats and mice and stellate (Ito) cell hyperplasia in mice.

8.11.1 Hepatocellular Hyperplasia

There is a wide spectrum of morphological features of hepatocellular hyperplasia (Thoolen et al. 2010). Most hyperplasias are focal or multifocal rather than diffuse. Because of their focality and uncommon occurrence, they are sometimes misdiagnosed as foci of cellular alteration. Foci of cellular alteration are classified based on their phenotype in H&E-stained sections (see below) and are much more common than hyperplasias. In contrast to foci of cellular alteration, more extensive regions of hepatocellular hyperplasia have been reported as regenerative and non-regenerative hyperplasia. Non-regenerative hyperplastic responses span multiple hepatic lobules and are seen primarily in chronic studies. Non-regenerative hyperplastic nodules are rare, while a diagnosis of regenerative hepatocellular hyperplasia is associated with previous or concurrent hepatocellular damage.

8.11.2 Foci of Cellular Alteration

Hepatic foci of cellular alteration are typically classified based on the cytoplasmic tinctorial H&E features (Harada et al. 1989; Eustis et al. 1990; Thoolen et al. 2010). They occur as an age-associated and strain-related change with some foci of cellular alteration occurring spontaneously, while other phenotypes are more likely a response to treatment (Tables 1 and 2). Commonly occurring spontaneous foci of cellular alteration can be exacerbated by treatment and thus appear earlier in treated animals during the course of a study (Table 3).

Foci may be induced but are a common background change, especially in rats. They are rarely seen in any rat strains in short-term (≤90 day) toxicity studies, but in Fischer rats, there is an almost 100% incidence in both males and females by 2 years of age. In contrast, 28% and 38% incidences in male and female Sprague Dawley rats, respectively, have been documented at 24–26 months of age (Newsholme and Fish 1994). Basophilic foci are most common in F344 and female Sprague Dawley rats.

The importance of foci of cellular alteration relates to their presumptive preneoplastic potential based on their early appearance following treatment with known rodent liver carcinogens, their

Table 1
Foci of cellular alteration in rats

Rarely seen in control animals at 90 days of age
Occurs in 75–100% of animals by 2 years of age Numbers per liver ~ 500/cm^3 Males – Basophilic and clear cell phenotypes most common Females – Basophilic phenotype most common
Incidence varies by sex and strain – F344 > SD rats

Table 2
Foci of cellular alteration in mice

Rarely seen in control animals at 90 days of age
Occurs in 15–30% of animals by 2 years of age Numbers per liver ~100's Eosinophilic and clear cell phenotypes most common
Incidence varies by sex and strain More common in males

appearance before development of hepatic adenomas and carcinomas (Fig. 7), and the morphological continuum between large foci of cellular alteration and hepatocellular neoplasia.

The most common, spontaneously occurring foci of cellular alteration (in decreasing order) are basophilic foci, eosinophilic foci, clear cell foci, and mixed cell foci. The occurrence is species and strain dependent in rats and mice and rarely recognized in humans (Thoolen et al. 2010) (Table 4). They are reported to be in about 30% of control mice with the eosinophilic and clear cell phenotypes most common. They increase as the animals age and can be more numerous and appear earlier following exposure to known hepatocarcinogenic agents. Because foci of cellular alteration are a common early response to known rodent carcinogens and because of their morphological similarity to hepatocellular adenomas, they are presumptively preneoplastic. Consequently, in routine safety assessment studies, they are always diagnosed and may be given a severity grade based on size and number of foci on routine H&E sections. Additionally, stereological methods may be employed to assess for preneoplastic growth. They may be present in liver that also contain hepatocellular adenomas and carcinomas. In some short-term rodent models, quantitative stereology has been used to evaluate immunohistochemically stained foci of cellular alteration as an experimental means to identify potential hepatocarcinogens (Tsuda et al. 2003) (Fig. 7).

Table 3
Predisposing factors influencing the incidence of liver tumors in various species[a]

	Mouse	Rat	Dog	Man
Infectious hepatitis	+	–	–	+
Hepatitis viruses	–	–	–	+++
Helicobacter hepaticus	+	–	–	–
Cirrhosis	+	++	+++	++++
Alcohol	–	–	–	+
Toxins	+	+	–	–
Immune-mediated	–	–	–	+
Hepatocarcinogens	+++	+++	?	++++
Aflatoxin[b]	+	+	?	+

[a]The "+" indicates that the factor has been shown to play a role in liver cancer development with "+" through "++++" indicating the frequency and/or magnitude this factor plays in hepatocarcinogenesis in various species. "–" indicates not a known or major factor
[b]Aflatoxin is the only unequivocal human hepatocarcinogen, while there are dozens of known rodent hepatocarcinogens

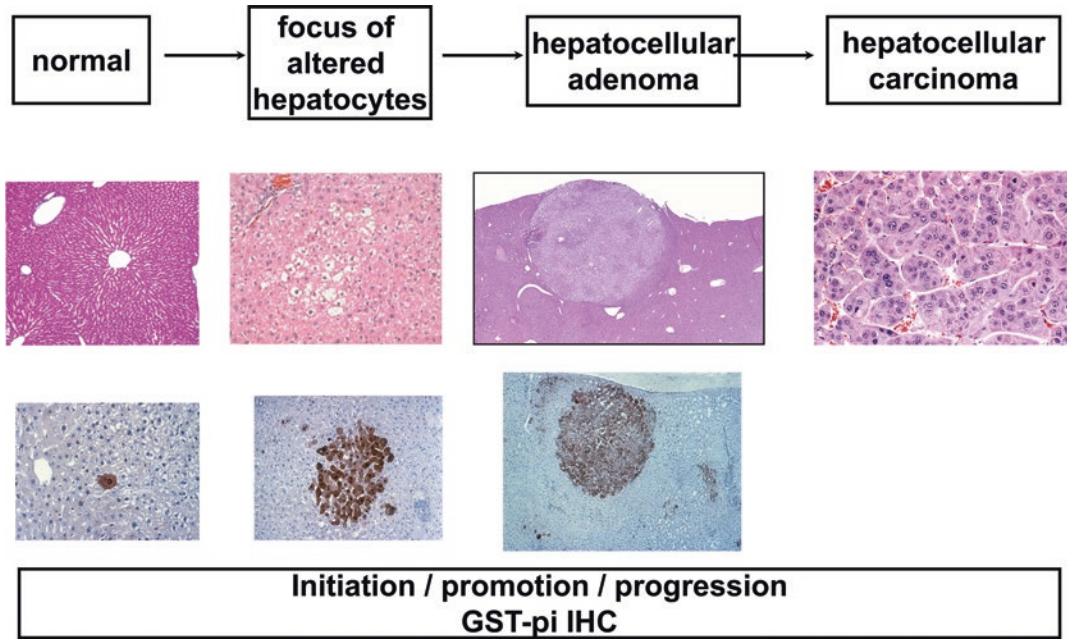

Fig. 7 This figure depicts the proposed process of rodent hepatocarcinogenesis whereby early, initiated hepatocytes may be detected using immunohistochemistry for glutathione-S-transferase-pi (GST-pi), an immunohistochemical (IHC) marker of pre-neoplastic and neoplastic hepatocytes. GST-pi expression can be demonstrated in individual cells, foci of altered hepatocytes (FAH), and adenomas, which can progress and become hepatocellular carcinomas (HCCs). The occurrence of progress is also evidenced by the development of adenomas arising from FAH and HCCs arising from adenomas. Also note that there are hundreds to thousands of FAH in adult rodents, conveying that the vast majority of FAH do not progress

Table 4
Comparative incidences of hepatocytic proliferative lesions[a]

	Mouse	Rat	Dog	Man
Hyperplasia	+	+	+++	+
Preneoplastic lesions	+++	++++	–	+
Foci of altered hepatocytes	++	++	–	–
Large/small cell lesions[a]	–	–	–	+
Hepatocellular adenoma	++++	+	+	+
Hepatocellular carcinoma	++++	++	+	+
Hepatoblastoma[b]	+++	–	–	+

[a]This table is an overview of the frequency and the comparative occurrences of various hepatocytic proliferative lesions in various species. The "–" (not or rarely encountered) and "+" through "++++" indicating the frequency and/or magnitude of hepatocarcinogenesis in each species
[b]Hepatoblastoma differs in man and mouse, where in man it is a disease of childhood and in mouse it is a late-onset neoplasm of adults

8.11.3 Bile Duct Hyperplasia

Bile duct hyperplasia is an age-associated change commonly seen in chronic rat and mouse studies (Thoolen et al. 2010), but it can also be observed in other common laboratory species. Some pathologists only record bile duct hyperplasia when it exceeds their threshold for diagnosis. When present it may be associated with a minimal peribiliary mononuclear cell infiltrate that may also be below a threshold for diagnosis for some pathologists. As for any relatively common background lesion, any threshold for diagnosis can be described in the pathology narrative. When bile duct hyperplasia is diagnosed, it may be given a severity grade since it has some morphological features suggestive of progression to cholangiofibrosis and cholangiocellular neoplasia.

8.11.4 Oval Cell Hyperplasia

Hyperplastic oval cells appear to arise from terminal ductal cells of bile ducts and, thus, are related to biliary hyperplasia. Oval cell hyperplasia is relatively rare, is seen following severe hepatotoxicity, and is also seen as an initial and high incidence response to some known hepatocarcinogens (Deschl et al. 2001; Foster 2018). Even when part of a constellation of other hepatic changes, it is recommended to be diagnosed and given a severity grade.

8.12 Other Non-neoplastic Changes

Several other non-neoplastic hepatic changes are occasionally seen and are described in multiple publications and texts (Greaves 2012; Cattley et al. 2013; Foster 2018; Frith et al. 1994; Harada et al.

1999; Haschek et al. 2010). These are not covered here but include nuclear and cytoplasmic inclusions, intracellular and extracellular crystals, karyocytomegaly, chronic passive congestion, thrombosis, infarction, endothelial cell hypertrophy, endothelial cell hyperplasia, and biliary cysts.

9 Frequently Occurring Neoplastic Liver Lesions in Rodent Studies

Morphological features of rat and mouse liver tumors are well documented in the literature (Harada et al. 1989, 1999; Eustis et al. 1990; Thoolen et al. 2010; Maronpot et al. 1989). Assessment of liver tumor responses in chronic rodent studies utilizes incidence, latency or time to appearance, presence of multiple tumors in a given liver, and concurrent and historical control data in determining the strength of evidence in categorizing the test agent's carcinogenicity. Hepatocellular tumor responses are the most common tumor target sites in long-term rat and mouse studies with important strain and species differences in background incidences of adenomas and carcinomas. Rats are generally more resistant to spontaneous hepatocellular tumor development than mice, whereas up to 49–90% of some strains of mice are susceptible. There is some experimental evidence indicating regression of hepatocellular neoplasms in rodents that are chemical dependent (Malarkey et al. 1995). A statistically significant increased incidence and/or earlier than normal appearance of adenomas and carcinomas is regarded as a positive response in a 2-year chronic study. In addition to hepatocellular neoplasms, biliary cells may give rise to cholangial tumors. Mixed hepatocellular-cholangial tumors also occur in rodents, and benign and malignant endothelial cell tumors can originate in the liver.

9.1 Hepatocellular Adenoma

Diagnostic features of hepatocellular adenomas are well documented in the literature (Harada et al. 1989, 1999; Eustis et al. 1990; Thoolen et al. 2010; Maronpot et al. 1989). The older terminology of "neoplastic nodule" has essentially been replaced by hepatocellular adenoma. Since hepatocellular adenomas have a well-established background incidence, especially in some strains of rats and mice, assessment of a treatment-driven response will be based on factors including reduced latency, increased incidence, and increased multiplicity. Since the diagnostic borderline between very large foci of cellular alteration and hepatic nodular hyperplasia and hepatocellular adenomas is often not distinct, it is helpful to evaluate any hepatocellular adenoma response in light of the severity grade of foci of cellular alteration based on the incidence and multiplicity of these

putative preneoplastic lesions. Tinctorial and morphological features of hepatocellular adenomas can be described in the pathology narrative as these features vary depending upon the test agent.

9.2 Hepatocellular Carcinoma

There are a few clear examples that show the development of hepatocellular carcinoma arising within hepatocellular adenomas. This finding supports the belief that there is a morphological continuum between adenomas and carcinomas. Indeed, for a given neoplasm, arriving at a confident diagnosis of carcinoma may be challenging, especially if based on a relatively small area in what is otherwise a large adenoma. Furthermore, in studies using known hepatocarcinogens, some individual animals have both adenomas and carcinomas in their livers, supporting progression of neoplastic growth but not necessarily excluding the possibility of independently arising carcinomas. Molecular biological evidence also supports independently arising liver tumors (Hoenerhoff et al. 2009, 2011). Consequently, many pathologists recommend combining the incidence and multiplicity of adenomas and carcinomas for assessing a carcinogenic response. When dealing with a situation where there are multiple adenomas or carcinomas or both adenomas and carcinomas present in the same liver, it is recommended that information be captured and discussed in the pathology narrative.

Hepatocellular carcinomas may present in several different morphological types including solid, glandular, trabecular (the most common type), and anaplastic, with frequent pulmonary metastases (25–50%). Different morphological types of carcinomas are generally combined when tabulating tumor frequency with descriptions of their morphological features provided in the pathology narrative.

9.3 Hepatoblastoma

Hepatoblastomas are poorly differentiated hepatic neoplasm seen primarily in male mice and consisting of primitive-appearing basophilic cells that often arise within or adjacent to hepatocellular adenomas. Hepatoblastomas are typically not combined with either adenomas or carcinomas in carcinogenicity studies. A statement of how diagnoses are recorded when a hepatoblastomas arise within hepatocellular adenomas can be addressed in the pathology narrative. There has been molecular evidence that hepatoblastoma have distinctive cancer gene mutations not present in the associated hepatocellular adenoma (Kim et al. 2005). As is the case with hepatocellular adenomas and carcinomas, hepatoblastomas can occur spontaneously in untreated male and female mice but have not been reported in rat (Turusov et al. 1973). Metastasis in males may be as high as 36% (Turusov et al. 2002).

9.4 Cholangioma and Cholangiocarcinoma

Cholangiomas and cholangiocarcinomas are rare (<0.1%) in treated and control rodents but have been observed as a response to specific hepatotoxic agents (Deschl et al. 2001; Foster 2018). Since they both arise from biliary cells, reporting them separately as well as collectively may be appropriate. Cholangial neoplasms may be present in studies that also have increased incidences of hepatocellular neoplasms.

9.5 Hepatocholangioma and Hepatocholangiocarcinoma

These mixed cell tumors are rare in treated and control rodents but have been seen following treatment with specific xenobiotics. These neoplasms contain neoplastic elements of both hepatocytes and bile duct epithelium, and benign and malignant forms may be reported separately as well as combined (Deschl et al. 2001; Foster 2018). They may occur in studies with cholangial and hepatocellular tumor responses. Whether these mixed tumors are diagnosed as benign or malignant is based on the most malignant cellular component.

9.6 Hemangiomas and Hemangiosarcomas

Hepatic hemangiomas and hemangiosarcomas arise from the endothelial lining of vascular spaces (sinusoids), may present as single or multiple lesions, and are well described in the literature (Deschl et al. 2001; Foster 2018). They are not commonly seen in untreated rats but are relatively common in mouse (<2%). They can occur in livers that have other types of neoplasms.

9.7 Other Liver Neoplasms

Less commonly seen liver neoplasms include stellate cell tumors (Ito cell tumors) in mice, histiocytic sarcomas that can arise in the liver or represent metastasis from other organs, and lymphohematopoietic neoplasms that are typically systemic neoplasms (i.e., malignant lymphoma) (Deschl et al. 2001; Foster 2018; Harada et al. 1999). A relatively common large granular lymphocytic leukemia (previously called mononuclear cell leukemia) occurs spontaneously and in high incidence in F344 rats, and its reduced latency and increased incidence have been associated with a wide range of xenobiotics (Maronpot et al. 2016).

10 Gallbladder

Non-neoplastic and neoplastic gallbladder lesions occur sporadically in control mice and generally are not a common response to xenobiotic exposure. Calculi, cholecystitis, mucosal papillary hyperplasia, and adenomas are described in the literature (Deschl et al. 2001; Harada et al. 1999; Thoolen et al. 2010).

Representative photomicrographs and associated literature references can be found in the INHAND publication (Thoolen et al. 2010), National Toxicology Program (Nonneoplastic Lesion Atlas 2019), and pathology textbooks (Haschek et al. 2010; Harada et al. 1999; Bannasch and Zerban 1990; Foster 2018).

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