Examination of Organ Toxicity

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Abstract

As a result of their anatomy and physiology, the organs and tissues of animals and man may show different morphological responses and sensitivity to xenobiotics. Toxic responses can manifest systemically (e.g., the immune system) or may produce specific toxic effects in a single organ system (skin) or single organ (liver). Organ toxicity may therefore result from a direct and primary effect on a target organ or as a result of secondary effects in organs and tissues that have a physiological dependence on the primary target (Haschek 2009). The

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assessment of organ toxicity must therefore take into account the complex physiological interdependence of tissues and organs within the body.

Keywords

Toxicologic Pathology · Target Organ · Histopathology · Clinical Chemistry · Organ weight · Necropsy

Introduction

While there have been advances made in the field of in vitro toxicology testing, the safety assessment of novel drugs and agrochemicals relies on in vivo studies utilizing appropriate laboratory animal species. These laboratory animal species are administered the drug or chemical by an appropriate route of administration, and the potential for target organ toxicity is assessed using a variety of endpoints. These endpoints include clinical observations in life, clinical chemistry of blood and urine, necropsy with organ weight measurement, and the collection and histopathology examination of tissues to assess the potential for structural changes associated with toxicity. The robust assessment of target organ toxicity relies on an integration of all these endpoints to provide a weight of evidence approach to assess the potential for toxicity within tissues and organ systems.

Basic Principles for the Examination of Organ Toxicity

Toxicological alterations of the morphology and structure of organs and tissues are detected by anatomical pathology examination following single or multiple doses of a drug or chemical administered to a laboratory species. During the in-life phase and at the end of an animal study, clinical pathology parameters (e.g., hematology and urinalysis) are assessed and provide important biomarkers for functional metabolic disturbances and maybe the first indicators for potential organ toxicity (e.g., liver or kidney). Both anatomical and clinical pathology disciplines offer a broad diagnostic repertoire to analyze organ toxicity in a well-considered, stepwise, so-called tiered approach concept.

Gross Pathology

After the in-life phase of an animal experiment, all study animals are necropsied according to a standardized, systematic procedure. As a rule, the study design is based on the most recent effective guidelines (e.g., OECD guideline for the testing of chemicals or US EPA Health Effects Test Guidelines OPPTS); however, the study design should always be adapted if the mode of action of a chemical is already known. All observations and macroscopic lesions identified at necropsy are recorded in a validated electronic data system for each single study animal. A standard study

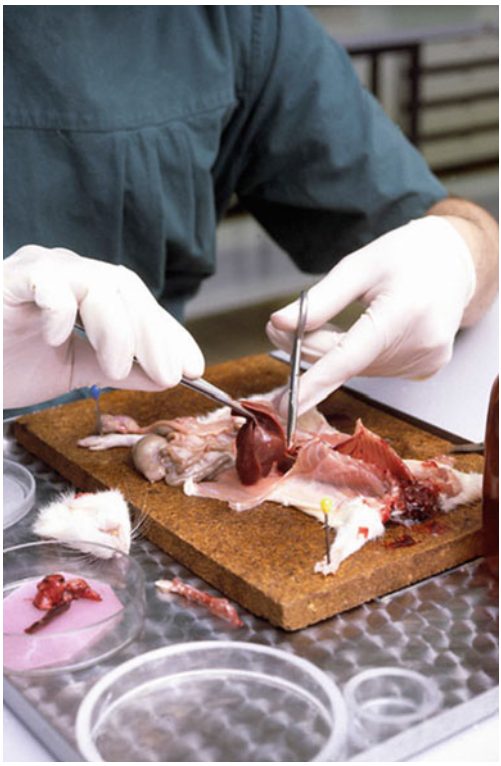


Fig. 1 Necropsy of laboratory animals with organ and tissue collection. The figure shows the removal of the liver from the abdominal cavity of a Wistar rat

protocol lists a wide range of tissues and organs that have to be removed for further histopathology examination. Some selected organs (e.g., liver, kidneys, adrenal glands) are weighed. Organ weights are often sensitive indicators of a treatment-related effect and may provide initial information on potential target organs during or shortly after necropsy (e.g., *increased liver or thyroid gland weights* are often recorded for compounds that act as enzyme inducers). Macroscopic observations during necropsy also have the potential to alert the toxicologist to possible target organs (e.g., *yellow-brown or clay-like discoloration of the liver* is indicative of a degenerative change, and the finding of a *mass* could turn out to be a chronic inflammatory process, an abscess, or a malignant tumor). The careful consideration of organ weights and macroscopic findings are an essential part of the detection of organ toxicity in experimental animal studies (Fig. 1).

Histopathology

The histopathological examination (by light microscopy) of a diverse range of organs and tissues by a well-trained toxicological pathologist is one of the most important and time-consuming elements in the assessment of organ toxicity

(Greaves 2012). With the advances in digital technology, slide scanners can now produce digital whole slide images from histopathology slides that can be reviewed by a pathologist in a similar way to a microscope. The minimum scope for histopathology examination is dictated by the various guidelines already mentioned above. In principle, organs and tissues are selected that are considered highly relevant determinants of basic metabolic and detoxification processes (e.g., the liver), organs that act as portals of entry for drugs/chemicals and may have been in direct contact with the test compound (gastrointestinal tract, lungs, skin), and organs that are crucial for excretion (e.g., kidneys, urinary bladder, and biliary system in the liver). Furthermore, representative samples are also examined from the immune and hematopoietic systems (two lymph nodes, one close to the site of exposure, thymus, spleen, bone marrow), the skeletal system (bone, joints, skeletal muscle), and the nervous system (various coronal sections from the brain including the cerebrum and cerebellum, two to three cross and longitudinal sections from the spinal cord, and one peripheral nerve). The cardiovascular system (arteries, veins) is examined as a constituent of many of the routine organ samples but specifically in one or more targeted sections from the heart and one section of the aorta. The reproductive system (including the testes, epididymides, prostate, accessory sexual glands and ovaries, oviducts, uterus, vagina, and mammary gland) is also included in the organs and tissues examined (Fig. 2).

A thorough histopathological examination is essential as toxicological changes can manifest microscopically in the absence of alterations to organ weights and clinical pathology parameters.

Fig. 2 Histopathology. Scope of examination for one single test animal in a carcinogenicity study (*left* paraffin blocks, *right* histological slides)

Diagnostic Approach, Procedures, and Considerations

As a minimum, all organ and tissue alterations from the high-dose and concurrent control group are recorded systematically in a validated data entry system to produce a pathology finding incidence table. The pathologist chooses an appropriate morphological diagnosis for the lesion observed and may describe the findings in more detail in the narrative pathology report. The pathologist must also grade the severity of findings where appropriate in order to help establish the presence of a dose–response. The grading system used by the pathologist will take into account the type of study (duration of exposure) and the nature of findings observed. The pathologist relies on his knowledge of the spontaneous pathology of the test species used to help differentiate spontaneous from treatment-related findings. A thorough understanding of the potential impact of any histopathology findings on the test species used also helps the pathologist to ascribe adversity to any treatment-related lesions present (Kerlin et al. 2015; Palazzi et al. 2016). The provision of accurate and up-to-date historical control data for spontaneous lesions in test species concerned may also be required to help support the pathologist's conclusions.

There has been an ongoing debate as to whether the study pathologist should have knowledge of which animals are treated and which animals are controls to guarantee a more *objective examination*. However, the approach of *blind reading* is not recommended by toxicological pathologists or their societies for the initial histopathology examination. The risk of introducing "bias" or the potential to overlook or to misinterpret minor treatment-related variations in the morphology of organs is high, and blind reading should therefore not be performed. However, blind reading of histological slides is often used at a later stage during slide evaluation, e.g., to find a no-observed-effect level (NOEL) for a specific organ toxicity and to allow the study pathologist to consistently identify a subtle or borderline lesion. "Blinding" slides with codes can also be performed if there are different opinions on the interpretation of a lesion between pathologists and the lesion is being considered by a pathology working group (PWG). A PWG is a formal and well-documented process to resolve different opinions on the diagnosis and relationship to treatment of pathology findings from a toxicology study by an independent panel of expert pathologists, which also includes the study and peer-reviewing pathologist. These experts undertake a "blind reading" so as not to be biased (Fig. 3).

Clinical Pathology Parameters

In addition to the analysis of anatomical pathology data after completion of an animal study, the analysis of the clinical pathology data will add significant value for the detection of organ toxicity. For clinical pathology, blood and urine samples are taken during the in-life phase of a study at scheduled time points and, as a minimum, at least once before the final sacrifice of the test animals. Diverse hematology parameters are measured and calculated (e.g., number of red and

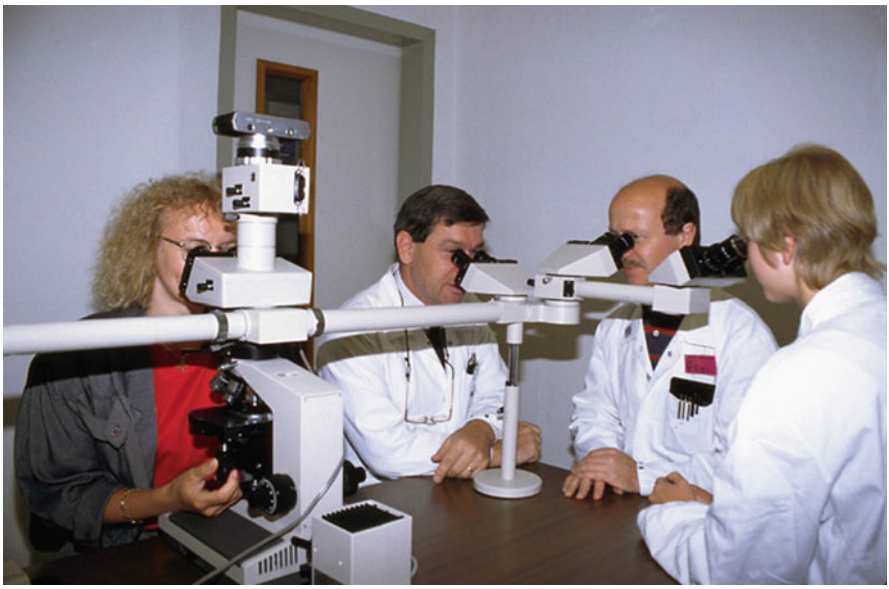


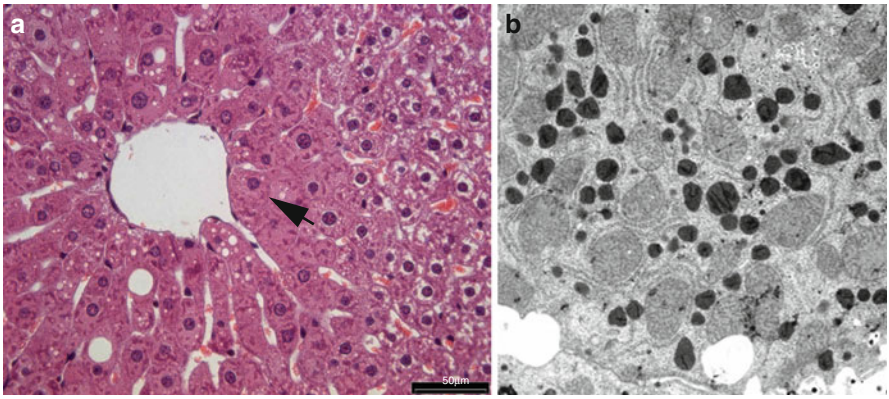
Fig. 3 Evaluation of histological slides by light microscopy. Pathologists during an internal review of histopathological findings at a multiheaded microscope

white blood cells, hemoglobin concentration, mean corpuscular volume, coagulation tests, and differential blood count). As these tests are highly automated, the results can provide an initial assessment of such things as anemia and inflammation prior to the histopathology results. Blood sample analysis also includes clinical chemistry parameters (e.g., enzymes, biochemical analytes in plasma, like transaminases, urea, creatinine, electrolytes, serum protein levels) that may indicate organ toxicity in the liver or the kidneys. As a rule, the selection of the standard minimum panel of clinical pathology parameters in animal experiments follows guideline recommendation and aims to detect major metabolic impacts of a potential toxic compound. If the mode of action of a test item is known, clinical pathology tests may be specifically designed and additional parameters analyzed (e.g., hormones). The clinical pathology data are another important component and, together with the gross pathology, organ weights, and histopathology data analysis, allow the detection of specific organ toxicity with a high degree of certainty.

Results of a Well-Concerted Combination of Anatomical and Clinical Pathology Data Analyses

For the majority of cases, standard approaches like the analysis of hematoxylin and eosin-stained histological slides by light microscopy are sufficient to detect organ toxicity. However, there are also exceptions where more sophisticated methods are required. A liver cell swelling (*centrilobular hypertrophy of hepatocytes*) noted by

light microscope may indicate a degenerative or an adaptive response of the liver parenchyma (Fig. 4a). A degenerative process that leads to liver cell death is much more critical than an adaptive process, where the morphological change is based on a physiological and fully reversible response of the liver tissue. There are a number of different chemicals, so-called enzyme inducers, that produce an adaptive liver cell swelling by a proliferation of the smooth endoplasmic reticulum (SER). A



c

Table
Quantitative measurements of the peroxisomal enzyme activity in B6C3F1 mice

Dose groups	Males	Females
Control	4.03±0.91[a]	5.28±0.41
500 ppm	6.05±1.42**	5.91±0.82
1500 ppm	9.31±2.00***	8.88±0.74***
4000 ppm	23.14±5.33***	22.05±1.60***
8000 ppm	43.37±2.18***	43.90±1.52***

The data show the activity of the cyanide-insensitive palmitoyl-CoA –oxidation. Results are presented as milliunits (mU) per milligram protein as group mean ± standard deviation

*p<0.05
**p<0.02
***p<0.002

Fig. 4 Example of a successful contemporary approach using three different methods to analyze organ-specific lesions and to correlate structure and function: (**a**) Centrilobular liver cell hypertrophy (*arrowhead*) is detected in a histological slide by light microscopy examination. (**b**) The liver cell hypertrophy is characterized by electron microscopy examination as proliferation of specific cell organelles in the cytoplasm, the peroxisomes (here stained as black rounded bodies). (**c**) The clinical pathology examination of the cyanide-insensitive palmitoyl-CoA oxidation in liver homogenates resulted in a statistically significant functional increase and shows a clear dose–response relationship

proliferation of the SER is the morphological correlate for an induction of the cytochrome P450 enzyme superfamily (CYP) and major enzymes in the metabolism of xenobiotics (toxic chemicals and drugs). Morphologically, a definitive diagnosis can be made by using electron microscopy of the liver, and clinical biochemistry allows a diagnosis by the analysis of specific enzymes (e.g., ethoxyresorufin-*O*-deethylase and pentoxyresorufin *O*-dependentylase).

Both methods are also appropriate approaches to identify another group of substances that also induce a centrilobular hypertrophy of hepatocytes, e.g., peroxisome proliferators. The latter result in an accumulation of specific intracytoplasmic cell organelles, the peroxisomes, which play a significant physiological role in lipid metabolism. Peroxisomes can be selectively stained by cytochemical or immunohistochemical methods and can be morphologically quantified. Results from the latter techniques correlate well with a significant increase of the cyanide-insensitive palmitoyl-CoA oxidation that can be analyzed from liver homogenates taken during necropsy (Fig. 4b, c (table)).

These examples show the complementary use of clinical pathology and anatomical pathology approaches to assure the accurate diagnosis and interpretation of certain types of organ toxicity and to contribute to the understanding of the mode of action of xenobiotics.

International Activities on Harmonization in the Use of Diagnostic Terms

For many years, the major scientific societies of toxicological pathology in Europe (European Society of Toxicologic Pathology [ESTP] and British Society of Toxicological Pathology [BSTP]) and the United States (Society of Toxicologic Pathology [STP]) have worked on harmonizing the nomenclature and diagnostic criteria used in toxicology studies. These activities were guided by the need for pathologists globally to use the same pathomorphological diagnostic criteria in the description of findings from toxicity studies using drugs and chemicals. For example, a *hyperplasia of the mammary gland* should be always differentiated from an *adenoma of the mammary gland* using the same diagnostic criteria regardless of geographical location. As pharmaceutical and chemical companies become more globalized, preclinical safety and toxicology studies for a single compound may be performed in research facilities in different geographical regions. As a result of these trends, it was considered extremely important to come to a common understanding in the use of diagnostic criteria. Initially, the primary focus was given to proliferative lesions in rodents as these findings were easier to harmonize among the international community of toxicological pathologists. As a result working groups of toxicological pathologists from Europe and America published a series of *International Classification of Rodent Tumours* for rats and mice between 1992 and 2001. The subsequent use of these published criteria significantly helped to harmonize the diagnosis of tumors in rodent oncogenicity studies. However, there will always be borderline lesions and growth patterns of tumors where harmonized criteria do not fit

the lesion and the pathologist has to make their own informed judgment based on experience.

On the basis of an initiative of the European Society of Toxicologic Pathology (ESTP) and the US Society of Toxicologic Pathology (STP), a further important step forward was started in 2005. In conjunction with the German-based Registry of Industrial Toxicology Animal-data (RITA), a collaborative process of review, update, and harmonization of existing diagnostic nomenclature, documents, and databases of rodents was initiated. The British Society of Toxicological Pathology (BSTP) and the Japanese Society of Toxicologic Pathology (JSTP) joined this process in 2006. This project, known as INHAND (International Harmonization of Nomenclature and Diagnostic Criteria for Lesions in Rats and Mice) project, includes efforts to harmonize nomenclature not only for proliferative lesions but also for non-proliferative lesions at a level that gains international acceptance (Mann et al. 2012). Up to 2020, INHAND nomenclature has been published for the cardiovascular (Berridge et al. 2016), integument (Mecklenburg et al. 2013), soft tissues/skeletal (Fossey et al. 2016), respiratory (Renne et al. 2009), hepatobiliary (Thoolen et al. 2010), urinary (Frazier et al. 2012), nervous (Kaufmann et al. 2012), endocrine (Brändli-Baiocco et al. 2018), hematolymphoid (Willard-Mack 2019), special senses (Ramos et al. 2018), reproductive systems (Creasy et al. 2012), gastrointestinal tract (Nolte et al. 2016), and the mammary gland (Rudmann et al. 2012). Guides for non-rodent species are in preparation. The INHAND nomenclature is also available electronically at the goRENI webpage and is presented at the international annual meetings of the societies of toxicological pathology to discuss the practical use of these harmonized diagnostic criteria.

Summary and Future View

Organ toxicity is the result of physiological dysfunction and structural alteration. Clinical pathology and histopathological examination are complementary approaches that underpin the detection and characterization of organ toxicity. Despite the many advances in molecular biology (genomics, metabolomics), the use of routine clinical pathology measurements and histopathological examination of hematoxylin and eosin-stained tissue sections are unlikely to be replaced in the near term as a first-tier approach for detecting organ toxicity in animal toxicology studies. The latter techniques can be complemented by more sophisticated examination using electron microscopy, immunohistochemistry, and molecular pathology approaches, once the target organs have been identified, and may help to identify the mode of action of the toxicity in question.

Steady advances have been made in the validation and use of in vitro systems for predicting organ toxicity. These have mainly involved the evolution of primary cell cultures into more sophisticated microphysiological systems (MPS) that contain more than one cell type (Truskey 2018). With advances in stem cell biology, these MPS can be humanized, to allow for a direct comparison of results between laboratory animals and man.

Cross-References

- ▶ Biomolecules Versus Smaller Chemicals in Toxicology: ICH, EU, and US Recommendations
- ▶ Examination of Acute and Repeated-Dose Toxicity
- ▶ International Regulation of Toxicological Test Procedures
- ▶ Specific Toxicity Tests for Neurotoxicity, Immunotoxicity, Allergy, Irritation, Reprotoxicity, and Carcinogenicity

References

Berridge BR, Mowat V, Nagai H, Nyska A, Okazaki Y, Clements PJ, … Wells MY (2016) Non-proliferative and proliferative lesions of the cardiovascular system of the rat and mouse. J Toxicol Pathol 29(3_Suppl). https://doi.org/10.1293/tox.29.3s-1

Brändli-Baiocco A, Balme E, Bruder M, Chandra S, Hellmann J, Hoenerhoff MJ, … Rosol TJ (2018) Nonproliferative and proliferative lesions of the rat and mouse endocrine system. J Toxicol Pathol 31(3_Suppl). https://doi.org/10.1293/tox.31.1s

Creasy D, Bube A, De Rijk E, Kandori H, Kuwahara M, Masson R, Nolte T, Reams R, Regan K, Rehm S, Rogerson P, Withney K (2012) Proliferative and nonproliferative lesions of the rat and mouse male reproductive system. Toxicol Pathol 40:40S–121S

Fossey S, Vahle J, Long P, Schelling S, Ernst H, Boyce RW, … Leininger J (2016) Nonproliferative and proliferative lesions of the rat and mouse skeletal tissues (bones, joints, and teeth). J Toxicol Pathol 29(3_Suppl). https://doi.org/10.1293/tox.29.3s-2

Frazier KS, Seely JC, Hard GC, Betton G, Burnett R, Nakatsuji S, Nishikawa A, Durchfeld-Meyer B, Bube A (2012) Proliferative and nonproliferative lesions of the rat and mouse urinary system. Toxicol Pathol 40(4 Suppl):14S–86S

Greaves P (2012) Histopathology of preclinical toxicity studies: interpretation and relevance in drug safety evaluation, 4th edn. Academic/Elsevier, Amsterdam

Greaves P, Chouinard L, Ernst H, Mecklenburg L, Pruimboom-Brees IM, Rinke M, … Yoshida T (2013) Proliferative and non-proliferative lesions of the rat and mouse soft tissue, skeletal muscle and mesothelium. J Toxicol Pathol 26(3_Suppl). https://doi.org/10.1293/tox.26.1s

Haschek WM, Rousseaux CG, Wallig MA (2009) Fundamentals of toxicologic pathology, 2nd edn. Elsevier, Amsterdam

Kaufmann W, Bolon B, Bradley A, Butt M, Czasch S, Garman RH, George C, Gröters S, Krinke G, Little P, McKay J, Narama I, Rao D, Shibutani M, Sills R (2012) Proliferative and non-proliferative lesions of the rat and mouse central and peripheral nervous systems. Toxicol Pathol 40(4 Suppl):87S–157S

Kerlin R, Bolon B, Burkhardt J, Francke S, Greaves P, Meador V, Popp J (2015) Scientific and regulatory policy committee: recommended ("best") practices for determining, communicating, and using adverse effect data from nonclinical studies. Toxicol Pathol 44(2):147–162

Mann P, Vahle J, Keenan CM, Baker JF, Bradley AE, Goodman DG, Harada T, Herbert R, Kaufmann W, Kellner R, Nolte T, Rittinghausen S, Tanaka T (2012) International harmonization of toxicologic pathology nomenclature: an overview and review of basic principles. Toxicol Pathol 40:7S–13S

Mecklenburg L, Kusewitt D, Kolly C, Treumann S, Adams ET, Diegel K, … Bradley A (2013) Proliferative and non-proliferative lesions of the rat and mouse integument. J Toxicol Pathol 26 (3_Suppl). https://doi.org/10.1293/tox.26.27s

Nolte T, Brander-Weber P, Dangler C, Deschl U, Elwell MR, Greaves P, … Ward JM (2016) Nonproliferative and proliferative lesions of the gastrointestinal tract, pancreas and salivary glands of the rat and mouse. J Toxicol Pathol 29(1_Suppl). https://doi.org/10.1293/tox.29.1s

Palazzi X, Burkhardt J, Caplain H, Dellarco V, Fant P, Foster J, Francke S, Germann P, Gröters S, Harada T, Harleman J, Inui K, Kaufmann W, Lenz B, Nagai H, Pohlmeyer-Esch G, Schulte A, Skydsgaard M, Tomlinson L, Wood C, Yoshida M (2016) Characterizing "adversity" of pathology findings in nonclinical toxicity studies. Toxicol Pathol 44(6):810–824

Ramos MF, Baker J, Atzpodien E-A, Bach U, Brassard J, Cartwright J, … Zhang H (2018) Nonproliferative and proliferative lesions of the rat and mouse special sense organs(ocular [eye and glands], olfactory and otic). J Toxicol Pathol 31(3_Suppl). https://doi.org/10.1293/tox.31.97s

Renne R, Brix A, Harkema J, Herbert R, Kittel B, Lewis D, March T, Nagano K, Pino M, Rittinghausen S, Rosenbruch M, Tellier P, Wöhrmann T (2009) Proliferative and non-proliferative lesions of the rat and mouse respiratory tract. Toxicol Pathol 37(7 Suppl):5S–73S

Rudmann D, Cardiff R, Chouinard L, Goodman D, Küttler K, Marxfeld H, Molinolo A, Treumann S, Yoshizawa K (2012) Proliferative and nonproliferative lesions of the rat and mouse mammary, zymbal's, preputial, and clitoral glands. Toxicol Pathol 40(6 Suppl):7S–39S

Thoolen B, Maronpot RR, Harada T, Nyska A, Rousseaux C, Nolte T, Malarkey DE, Kaufmann W, Küttler K, Deschl U, Nakae D, Gregson R, Vinlove MP, Brix AE, Singh B, Belpoggi F, Ward M (2010) Proliferative and nonproliferative lesions of the rat and mouse hepatobiliary system. Toxicol Pathol 38(7 Suppl):5S–81S

Truskey G (2018) Human microphysiological systems and organoids as in vitro models for toxicological studies. Front Public Health 6. https://doi.org/10.3389/fpubh.2018.00185

Willard-Mack CL, Elmore SA, Hall WC, Harleman J, Kuper CF, Losco P, … Keenan CM (2019) Nonproliferative and proliferative lesions of the rat and mouse hematolymphoid system. Toxicol Pathol 47(6):665–783. https://doi.org/10.1177/0192623319867053

Resources

European Society of Toxicologic Pathology (ESTP). http://www.eurotoxpath.org/. Accessed 08 Mar 2013

goRENI – The standard reference for nomenclature and diagnostic criteria in toxicologic pathology. http://www.goreni.org/. Accessed 08 Mar 2013

International Harmonization of Nomenclature and Diagnostic Criteria (INHAND). www.toxpath.org/inhand.asp

OECD Guidelines for the Testing of Chemicals. http://www.oecd.org/env/chemicalsafetyandbiosafety/testingofchemicals/. Accessed 08 Mar 2013

Registry of Industrial Toxicology Animal-data (RITA). http://reni.item.fraunhofer.de/reni/public/rita/index.php. Accessed 08 Mar 2013

Society for Toxicologic Pathology (STP). http://www.toxpath.org/

US EPA Health Effects Test Guideline OPPTS. www.epa.gov/oppts/pubs/frs/home/guidelin.htm. Accessed 08 Mar 2013