

# Tissues, Pathology, and Diagnostic Microscopy

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### Histopathology as a biomarker of effect in ecotoxicological studies on natural populations of small mammals

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The search of suitable biomarkers is a crucial aspect in ecotoxicological studies, especially in natural populations inhabiting protected areas. Whereas histopathological evaluations are common tools in toxicological studies, there is scarce information of both structural and ultrastructural alterations induced by environmental pollution. Here, we compared different approaches of quantitative and qualitative pathological assessments on two species of small mammals exposed to metals and other common environmental pollutants: the greater white toothed shrew (*Crocidura russula*) and the wood mouse (*Apodemus sylvaticus*).

Specimens used in this study include 91 *C. russula* from polluted and reference sites of Doñana (National Park of Doñana, Spain), Garraf (Garraf Park, Spain), and Alentejo (Portugal), and 49 *A. sylvaticus* from Garraf. Both hepatic and renal tissues were analysed, focusing on a qualitative and semiquantitative assessment of pathologies based on the extension and severity of alterations (Alentejo [1], Garraf [2]) or quantitative analyses based on morphological differences of cells (Doñana [3]).

The results revealed liver as the main organ affected by the pollution in all disturbed sites. The damage observed that may be attributed to chronic exposure to pollutants includes cell cycle arrest (necrosis and apoptosis), inflammation, cytoplasmic vacuolation, microsteatosis and preneoplastic nodules. In Garraf, kidneys of the specimens exposed to environmental pollution showed a significant increase of medullar and cortical alterations, namely tubular dilatation and necrosis, and hyaline cylinders in the lumen of tubules from renal medulla, together with multifocal inflammation in cortical and medullar areas. In Garraf, the comparison of sympatric populations of mice and shrews showed that the latter species was more tolerant to pollution than *A. sylvaticus*. In Doñana, the specimens from the polluted site showed an increase of apoptosis and a tendency to increase cellular perimeter in the hepatic tissue. In Alentejo, increases of frequency and severity of necrosis, apoptosis, and vacuolation were observed in livers of animals from the polluted area. These results pointed out the necessity of pathological studies as a crucial management tool in protected sites.

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