

to confirm or reject a pathogenetic mechanism. The presence of a phospholipidosis can easily be proved by EM investigations on formalin-fixed tissue. Molecular epitopes are astonishingly stable on fixed tissue and can be demonstrated immunohistochemically even after many years in wet tissue and paraffin-embedded tissue. It is always worthwhile to test such material, as long as negative and positive controls are run simultaneously.

- Morphometry is important to prove or disprove numerical or volume changes in tissues and particularly to establish a no-effect level (13).

6.2. Tailor-made Mechanistic Studies

Additional studies may be envisaged, particularly for the following purposes:

- *To repeat any of the above investigations on properly sampled and prepared tissue*; for example after fixation with glutaraldehyde by EM (206) or more detailed cell kinetic studies (proliferation, single cell necrosis, etc.) (213).
- *Investigation of early findings and their development over time* (e.g., by sequential necropsies in a time-course study)
- *Reversibility studies*, with necropsies also after a treatment-free period
- *Mechanistic studies* to test hypotheses that cannot be tested with enough certainty on material already available
- *Special in vitro studies* with cell or tissue cultures, organ slices or the perfused target organ itself to investigate metabolism (214), effects on subcellular organelles (see also [Sec. 3.2](#)) or on gene expression (22,26–31), or dose–effect relationships at subcellular or molecular levels (215). Receptor studies (216,217) can be very helpful, while studies of intercellular communication (218) and initiation–promotion assays (219) may not contribute much.

7. INTERPRETATION OF PATHOLOGICAL FINDINGS

Interpretation of toxicological findings always has to start with a number of questions, such as:

- Is the study technically valid?
- Is the model valid?
- Is there indeed an adverse effect? Could the adverse effect be species-specific?
- Were there other relevant findings?