Ecotoxicological biomonitoring – principles, concepts and applications to vertebrate animals Peter Vladislavov Ostoich Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 1 Tsar Osvoboditel Blvd, Sofia 1606, Bulgaria PhD Thesis Sofia, 2019

The current work is a critical overview of the contemporary state of ecotoxicological research as applied to terrestrial vertebrates. The main objectives are to conduct a summary and analysis of the contemporary methods in this field of study, as well as to discuss three main types of pollutants (lead (Pb), cadmium (Cd), and radionuclides) and the model organisms used in the discipline (mainly European representative species of the families Cricetidae and Muridae). The first part introduces some basic concepts in the ecotoxicology of terrestrial ecosystems as well as discussing the background literature on the subject with its strengths and limitations. Examples are provided regarding the necessity of the current project and the gaps that exist in the literature.

With the focus shifting to methods, several classes of research techniques are discussed, including: 1) classical morpho-physiological and hematological indicators, 2) histopathological methods, 3) determination of the levels of toxicants in the body by spectrometric and other methods, 4) cytogenetic techniques, including metaphase analysis and sister chromatid exchanges (SCE), 5) studies of DNA damage, including the micronucleus test in its CBMN and *in vivo* variations, as well as most variations of the Comet test, 6) molecular biomarkers, including biomarkers of oxidative stress and determination of the concentration of detoxifying biomolecules, and 7) non-invasive sampling techniques. Several conclusions are drawn, including that every method, with the exception of the determination of toxic body burden, is insufficient by itself, and must be combined with other methods in order to produce informative results. The selection of methods always depends on the scope, objectives and underlying principles of the study conducted.

The chapter dealing with the major classes of pollutants discusses first several general principles responsible for the specific toxicity of chemical elements, including 1) dose and kinetics of retention and elimination, 2) chemical reactivity and known toxic compounds, 3) interactions with biological systems, 4) metabolic activation, and 5) age and sex-related effects. Continuing with lead (Pb), the chapter analyses the particular biokinetics and specific toxicity of this heavy metal, citing threshold doses for induction of deterministic effects (anemia, mental retardation and neurodegeneration, liver and systemic damage. The ecotoxicity of Pb is discussed, including its role in the "Coto Doňana" environmental disaster in Spain. Lead is confirmed as a genotoxic agent, and several eco-toxicological studies are discussed in detail such

as those, conducted by Ma et al. (1991), Metcheva et al. (2007) and Topashka-Ancheva et al. (2003). Shifting the discussion to cadmium (Cd), the analysis continues with the ecotoxicity and specific toxicity of that metal. Its genotoxicity is discussed in detail, including low-dose effects on DNA repair systems such as NHEJ and BER at very low (\leq 30µM) concentrations. The analysis continues with an overview of the environmental biomonitoring studies on cadmium, concluding that the metal poses a very high risk, even in very small doses, to individual organisms and ecosystems. The review of radionuclides starts with a discussion of natural radioactivity, then proceeds to anthropogenic enrichment and technogenic dispersal of radioactive isotopes. The ecological effects of three key anthopogenic radionuclides – ¹³⁷Cs, ⁹⁰Sr and ¹³¹I are reviewed in detail. The chapter provides an overview of the monitoring studies conducted in the 30-km Exclusion zone of the Chernobyl NPP and discusses contemporary concepts in radiobiology and radioecology. Conclusions are drawn regarding the suitability of small rodents, particularly *M. glareolus* and *M. oeconomus* as zoomonitors of radioactive contamination. The chapter ends with a discussion of the current challenges facing radioecology.

Following is a brief overview of the current concepts in the detoxication of heavy metals and radionuclides. Three stages of detoxication are discussed: 1) at the absorption level (sorbents, diet supplementation, etc.), 2) at the physiological level (metal chelation, specific antidotes), 3) at the elimination/excretion level (biological elimination systems, adaptive responses). The last chapter of the dissertation deals with suitable monitoring species, discussing in detail five European species of the family Cricetidae (*Microtus arvalis, Myodes glareolus, Microtus oeconomus, Chionomys nivalis*, and *Microtus guentheri*) as well as two representatives of Muridae (*Apodemus flavicollis* and *Mus spretus*). The comparative utility of the seven species for different types of ecotoxicological and radioecological monitoring is discussed and conclusions are drawn regarding the future role of these animals as zoomonitors of environmental contamination. The dissertation ends with a list of the main conclusions and achievements drawn from the conducted summary, systematization and critical analysis and an enumeration of the specific contributions of the work within the context of the scientific field.