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State, Application and Prospects of Radioecological Biomonitoring Using Small Mammals

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Abstract: The current review is focused on radiobiological and ecological effects on bioindicator small mammal species. Among the species commonly used for radioecological biomonitoring are those of the genus *Apodemus* and the family Cricetidae. Various ecotoxicological studies with laboratory mice have also been conducted as well as studies on large wildlife mammals. Biomarkers such as body burdens of selected radionuclides, haematological indicators for the high range of exposure doses, cytogenetic biomarkers for the mid-to-low exposure range and molecular biology biomarkers have been established. While radioecology has somewhat shrunk in scope during the last two decades, important studies are still being conducted. Some of the questions that require further attention are the long-term behaviour of radionuclides in terrestrial habitats as well as their impact on the genetic makeup and diversity of populations of organisms in contaminated areas.

Key words: radioecology, radionuclides, beta activity, terrestrial vertebrates

Introduction

Radioecology is considered one of the younger subdivisions of ecology (Tschurlovits 1995). Overall, the discipline can be defined as "the study of the effects of ionizing radiation to the living and non-living environment" (Hall & Giaccia 2006). The present article discusses the development of ecotoxicology over the past 50 years, with a focus on radioecological biomonitoring based on terrestrial small mammals and key issues such as studies on natural and anthropogenic radionuclide contamination.

Brief history of the discipline

The origins of radioecology predate the discovery of radioactivity itself. During the 19th century, miners at the town of Schneeberg in Germany were found to suffer from a lung condition of unclear origin known as the Bergkrankheit ("mountain disease"), which was proven to be lung cancer (Härtling & Hesse 1879). While the cause was unknown at the time, improved ventilation and wet drilling reduced mortality. Today, it is well known that the main causal agent was natural radioactivity in the tunnels, specifically radon and radon decay products emitted from uranium ore (Enderle & Friedrich 1999, Schultz 1999).

Since 1945, the effects of radiation on mankind and the environment have become a subject of widespread concern. Testing of nuclear weapons led to the development of detection techniques for anthropogenic radionuclides used by both military and civilian organizations. The period 1945-1986 was characterised, to a large extent, by secrecy. The Scandinavian monitoring station at the remote Faroe Islands has been particularly thorough in recording and releasing information (Aarkrog et al. 1981, 1982). Radioecological data on fallout and detailed analysis have been used to inform civil defence in Britain and elsewhere, although the public's fears about nuclear weapons and nuclear power were never allayed (Smith 2010, Ilyin 1995). The Partial Nuclear Test Ban Treaty (PTBT) of 1963 ended at-

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mospheric testing, which led to decreasing activities of radionuclides such as ^{90}Sr and ^{137}Cs in remote localities (Aarkrog et al. 1981, 1982, Nifontova, 1995) and decreasing global contamination.

The Chernobyl accident in 1986 was a turning point in the history of radioecology. It was the most significant release of radioactive contaminants in Europe and worldwide recorded to date with estimates varying in the 5.2-14 Exabecquerel (EBq) scale (Güntay et al. 1995), prompting a renewed global effort in detecting radionuclides in terrestrial ecosystems and studying their effects.

In the 1990s, there was a renewed interest in the natural radiation background. In Germany, where studies (reverse dose-estimation, epidemiology) initially were conducted on the uranium mines operated by the Wismut consortium (Enderle & Friedrich 1999), radon became a problem of general interest, culminating in several large projects aimed at creating maps for geogenic radon release, as well as studies on radon in homes in high-risk areas (Kemski et al. 2001). Radon has become of international concern, owing to its high contribution to the radioactive dose for people, as well as the tendency of α-particles to inflict high localised damage, and therefore, high micro-doses upon ingestion and inhalation (Darby et al. 2005, Hall & Giacchia 2006). Several other international projects have been put into place, e.g. the system of European high-mountain observatories designed to detect long-range (cross-border) contamination by natural and anthropogenic sources (Masson et al. 2016), which compare data from European locations as diverse as Moussala Peak (Bulgaria), Jungfraujoch (Switzerland), Pic du Midi (France) and Sonnblick (Austria). During this period, efforts have been put forward to integrate and model data for radiobiology and radioecology (for instance, the projects MELODI, Eurados, COMET and others). Radioecology continues to be an active area of research across the developed world.

Achievements and challenges to radioecology

Over the past 50 years, radioecology has provided a variety of tools and outcomes in measuring impact of radioactivity on the environment and important insight on the relationships between natural, anthropogenicallyenhanced and technogenic radionuclide contamination. The terrestrial ecosystems near the polar regions (e.g., tundra) are very vulnerable to anthropogenic radionuclide pollution. This is due to the slower turnover of matter and energy, as well as a dominance of lichens, which are particularly prone to accumulation of radiocesium ^{137}Cs and ^{134}Cs (Aarkrog et al. 1981, 1982, Nifontova 1995). Some of the consequences of the Chernobyl accident affected particularly Nordic countries (Sweden, Finland, Norway) (Chesser et al. 2001, Zibold et al. 1992).

Regardless of progress, radioecology faces several challenges today. After the Chernobyl accident (1986), the discipline experienced a renaissance (Tschurlovits 1995). However, in the decade 2000-2011, relatively little expenditure has been devoted to studies outside the 30-km Chernobyl Exclusion Zone (Okano et al. 2016). The Fukushima accident in 2011 renewed active research on the consequences of anthropogenic pollution. While the Chernobyl accident prompted inquiries into the effects of a reactor accident on terrestrial ecosystems, Fukushima posed uncertainties regarding the effects and bioaccumulation pathways of radionuclides into marine ecosystems of the Pacific Ocean. Important questions outside the context of Fukushima remain to be answered. Some of the most heavily contaminated sites for radioecological studies are facilities with restricted access [e.g., the Mayak Industrial Complex at Chelyabinsk-40 (Ozyorsk) and the plutonium production complex at Hanford in the Washington State (Burmistrov et al. 1998)].

Relatively little attention has been given to the leakage of radioactive and chemical pollutants from sites of former uranium mining and smelting operations (Kolev et al. 2014). The transfer of most radionuclides along terrestrial food chains and their bioaccumulation and biomagnification have been described (Chesser et al. 2000, 2006) and extensive efforts are made to measure and characterise radionuclide pathways within marine ecosystems of the Sea of Tohoku (Okano et al. 2016). One of the important issues to be addressed is trans-generational accumulation of genetic damage and genomic instability (Dubrova et al. 1996, Goncharova & Riabokon 1998, Bonisoli-Alquati et al. 2010, 2015). Some have observed that much lower environmental doses of radioactivity induce given genetic abnormalities as compared to doses required in laboratory studies (Christaldi et al. 1990b, Chesser et al. 2001, Ryabokon & Goncharova, 2006). Dubrova et al. (1996) have hypothesised that this is due to radiation-induced genomic instability. Nevertheless, these cogitations, as well as direct evidence of mutagenicity observed in barn swallows (*Hirundo rustica*) (Bonisoli-Alquati et al. 2010, 2015) are highly controversial (Furitsu 2010). Matson et al. (2000) has observed increased genetic variability in rodents of the Chernobyl Exclusion Zone. However, all these data have been re-interpreted as naturally occurring genetic variation (Chesser et al. 2000, 2001, 2006).

The question of radiation-induced genomic instability remains open to further research (Furitsu 2010, Okano et al. 2016).

Methods and indicators in the radioecological monitoring of terrestrial small mammals

Morpho-physiological indicators

Morpho-physiological indicators (Schwartz et al. 1968) are a conventionally used method in population studies on the effects of environmental factors on individual development. They enable to access physiological characteristics of animals based on a set of indirect indicators such as the relative mass of animal visceral organs. Animal body mass, index of fatness, liver, adrenal glands, testis index, etc., are used. The progeny of a monitor species can also be screened for the presence of anomalies (Gu et al. 1997). Growth performance and litter size, as well as the presence of anomalies in the progeny are important indicators of radiation effects, especially in areas where high doses are expected (Chesser et al. 2000, Ryabokon & Goncharova 2006). While comparing morpho-physiological indicators, animals of the same gender and approximately the same age are used,. In the context of radioecological monitoring, morpho-physiological indicators are not considered reliable and have niche applications at best.

Haematological indicators

Haematological methods are based on differential counting and estimation of several blood parameters in peripheral blood and estimation of the blood biochemical parameters (Fliedner et al. 2001). If radiation exposure is suspected, a fast depletion of the lymphocyte pool indicates serious radiation injury (Lamerton et al. 1960, Fliedner et al. 2001). While these indicators are most important in the high-dose range, they have historically been one of the first tools in studying the effects of ionizing radiation in rodents and mammalian species (Till & McCulloch 1961, Comas & Byrd 1967, Van Bekkum 1977).

Haematology remains a useful and important diagnostic tool in ecotoxicological studies but molecular methods such as the micronucleus test may provide higher resolution for genotoxic agents because of the appearance of effects at lower concentrations than for haematological studies (Fenech & Morley 1986, Mitkovska et al. 2012). Another consideration when applying haematological screening in the field of radioecology is that blood parameters can be changed by confounding factors in wild animals, such as age, gender, diet, exposure to non-radioactive contaminants, and the presence of infectious and parasitic diseases (Nieminen et al. 2015).

Cytogenetic techniques

Cytogenetic techniques are the gold standard in measuring medium-dose exposure to ionizing radiation in mammals (Natarajan et al. 1998). While different endpoints have been adapted for use in the context of radiation exposure, classically dicentric chromosomes are the most common aberration in human biodosimetry. Dicentrics have a low background frequency in mammals under normal conditions and their induction is an effect relatively specific for medium and high doses of ionizing radiation (Kulka et al. 2012).

While the scoring of chromosomal aberrations is a good indicator of general radiation exposure, it should always be correlated with other data such as the specific burden of radionuclides in each studied animal (Chesser et al. 2000, 2001, Beresford et al. 2008). One drawback of cytogenetic studies in wild animals is that the animal populations are not homogeneous with respect to age, sex , diet and other characteristics, meaning that large samples are needed and only general trends can be detected (Goncharova & Riabokon 1998, 2006).

Besides scoring large chromosomes, there are other cytogenetic methods to detect genotoxicity at lower levels. E.g. a promising method is the scoring of sister chromatid exchanges (SCEs) in rodents (Tapisso et al. 2009, Topashka-Ancheva & Gerasimova 2012). It is a sensitive biomarker for general genotoxicity but not specific for radiation injury (Topashka-Ancheva & Gerasimova 2012).

Determination of radionuclide body burden, total beta-activity and dose estimation of monitor rodent species

When measuring the radionuclide burden and estimating internal and external doses of monitor animal species, several considerations need to be taken into account. Body burden of radionuclides within most observed species of rodents varies intra-specifically even for rodents captured at the same location (Chesser et al. 2006). This is due to age and sex differences, as well as individual differences in feeding habits (Chesser et al. 2001, Beresford et al. 2008). In order to evaluate the effects of radionuclides on populations, each animal should be sampled individually. Another consideration is the combination of internal and external exposure. External exposure is usually expressed as a product of the dose rate in a given location and the estimated time of exposure:

Absorbed dose (Gy) = Dose rate (Gy/hr) x 8765.8 x Number of years of exposure (N)

It is important to note that different types of ionizing radiation differ in their potential to generate biological damage. This is based on their different ability to penetrate matter, different linear energy transfer (LET) and relative biological effectiveness (RBE) (Vasilev 2005). The Relative Biological Effectiveness (RBE) is measured (Hall & Giaccia 2006) by the following formula:

$$RBE = \frac{D_X}{D_R},$$

where Dx is the reference absorbed dose of a standard type of radiation (usually medium-energy γ-rays and X-rays, and Dr is the absorbed dose of radiation of type R, which causes the same amount of damage in biological tissues.

There are many ways of estimating radiation doses in monitor species of wild animals. Two methods are discussed further: total beta-activity and dose determination for particular radionuclides.

Total β-activity

The total β-activity is a well-established parameter when screening monitor species for radioactive contamination (Tschurlovits 1996). According to Thorn & Vennard (1976), the reference value for total β-activity should not exceed 4.8 Bq/kg, with higher values necessitating specific measurement of radionuclides in the animals. Several components of total β-activity should be taken into account. Most important are naturally occurring (^{40}K, ^{14}C, ^{238}U, ^{226}Ra) and anthropogenic radionuclides (^{90}Sr and ^{137}Cs; Vasilev 2005). Detecting total β-activity is a simple procedure, involving burning dried samples in a muffle furnace and using the ash to determine activities by a scintillation counter. While convenient and often used, the method still has its constraints. For an example of a study on the total β-activity see Table 1 representing results from a radioecological study of Rila Mts, SE Bulgaria (Iovtchev et al. 1995).

The results from the Rila Mts. indicate that *Myodes glareolus* slightly exceeds the reference

Table 1. Whole-body β-activity (Bq/g) in vertebrate monitor species from Rila Mts., SW Bulgaria (from Iovtchev et al. 1998)

Species	Number	Whole-body β activity (Bq/g)	Results
Apodemus flavicollis	41	2.6±0.1	Normal
Chionomys nivalis	22	3.8±0.1	Normal
Myodes glareolus	40	5.1±0.1	Normal
Cottus gobio gobio	7	2.7±0.1	Normal
Phoxinus phoxinus	10	3.1±0.1	Normal
Pitymys subterraneus	9	2.9±0.1	Normal
Rana temporaria	17	4.9±0.1	highest value – 25.2
Sorex araneus	9	3.3±0.1	Normal
Salmo trutta fario	46	4.6±0.1	Normal

Table 2. Results for ^{137}Cs and ^{90}Sr in monitor species of small mammals from the Chernobyl Exclusion Zone: areas with low, medium and high radioactive contamination (from Beresford et al. 2008).

Radionuclide and level of site contamination	Species	n	Whole-body activity concentration (kBq/kg FW)	
			Mean±SD	Range
Cesium-137				
Low	*Myodes glareolus*	3	3.8±0.8	3.1-4.7
	Apodemus flavicollis	18	3.1±2.0	1.3-9.8
Medium	*Myodes glareolus*	39	70.5±46.3	17.0-252
	Apodemus flavicollis	10	59.7±37.1	24.1-143
High	*Myodes glareolus*	2	2260±1290	1350-3180
	Microtus sp.	11	611±282	252-1140
	Apodemus flavicollis	2	145±53.3	108-183
Strontium-90				
Low	*Myodes glareolus*	3	7.7±4.1	3.1-10.3
	Apodemus flavicollis	18	7.4±5.2	1.4-21.1
Medium	*Myodes glareolus*	39	19.5±7.4	4.3-36.0
	Apodemus flavicollis	10	24.7±6.1	16.0-34.0
High	*Myodes glareolus*	2	81.3±22.1	65.6-96.6
	Microtus sp.	11	107±35.0	38.1-167
	Apodemus flavicollis	2	66.6±28.3	46.6-86.7

values for total β-activity set by Thorn & Vennard (1976). There is a high variation in the data for *Rana temporaria* and *M. glareolus*, indicating inter-individual differences in exposure. The amphibian species has presented the highest values, necessitating further study (Iovtchev et al. 1995). Even though the results confirm the need for further radioecological studies of the Rila Mts., the total β-activities are several orders of magnitude lower than those recorded in Chernobyl by Chesser et al. (2001).

Dose estimation from particular radionuclides

There are a range of methods available for measuring concentrations and dose rates from a given radionuclide. When measuring the activity of ^{137}Cs, germanium and NaI detectors are most often used (Chesser et al. 2000). For most radionuclides, radiochemical methods exist for determining their concentration in living and non-living matter (Kinova 1998, Mehra & Singh 2011, Ivanova et al. 2014). As an example, a study from the Chernobyl Exclusion Area can be given (Beresford et al. 2008; see Table 2). It demonstrates the utility of measuring radionuclides separately and confirms the observations that *M. glareolus*, due to its feeding habits, accumulates the highest amount of radionuclides compared to other studied rodents (Beresford et al. 2008; see also Chesser et al. 2000, 2001).

Molecular biology techniques applied to environmental biomonitoring

Micronucleus test

The micronucleus test is a well-established method for *in vivo* genotoxicity screening (Fenech & Morley 1986, Mitkovska et al. 2012). There are two main versions of the method – the cytokinesis-block micronucleus assay (CBMN) and the *in vivo* micronucleus assay, which uses different stains to detect DNA-containing fragments in rodent erythrocytes (Christaldi et al. 1990a, Rodgers & Baker 1999). While it is not quantitative enough to detect a dose-dependent biological response to radiation, it is still useful in both forms. It should be kept in mind that the observation of micronuclei is not the most sensitive method to detect genomic damage from ionizing radiation. Another drawback to the test is that it does not detect radiation damage specifically – micronucleus analyses are confounded by other factors such as age, the presence of non-radioactive pollution, as well as infectious and parasitic diseases (Mitkovska et al. 2012).

Comet Assay

The Comet Assay, or single-cell gel electrophoresis (SCGE) was discovered in 1984 in the context of radiation damage to cells (Ostling & Johanson 1984). While undoubtedly very useful in cell cultures, *in vivo* studies have shown that inter-individual variability strongly confounds the results. The results have been applied in environmental biomonitoring of barn swallows (*Hirundo rustica*) from Chernobyl and Fukushima (Bonisoli-Alquatti et al. 2010, 2015). These publications are controversial because of the methodology used.

Analysis of minisatellite and microsatellite instability

Dubrova et al. (1996) reported increased mutation rate in the minisatellite DNA of the children of parents who had been exposed to Chernobyl fallout, contradicting previous data from A-bomb survivors that found no significant genetic damage. While elevated minisatellite instability can point to an accumulation of trans-generational genomic instability in humans and animals, these findings have been contested. Many subsequent studies on minisatellite mutation frequency found no correlation between radiation dose and the effect of mutation frequency (Furitsu 2010).

Target organs and tissues for the toxicity of radionuclides in wild small mammals: haematopoietic toxicity, radiation toxicity to the reproductive system, mutagenesis

The biological effects of ionizing radiation in small mammals are well-studied (Bond et al. 1965, Hacker-Klom 1985, Hall & Giaccia 2006). Experimental studies in mice and rats have yielded many results that have been the subject of extrapolation to humans (Neel & Lewis 1991, Sankaranarayanan & Chakraborty 2000). Currently, scientists are less engaged with radiation exposure than two decades ago. The most important concepts in the radiobiology of radiation injury to mammals relevant to radioecotoxicology include hematopoietic injury and radiation damage to the reproductive system. Ecotoxicological studies on wild rodents typically deal with doses, which are much lower than those required for observable deterministic effects (changes in haematological parameters, teratogenesis).

Haematopoietic system

The lethality from acute total-body irradiation in mammals at the LD_{50} dose range is primarily due to damage to the haematopoietic system (Bond et al. 1965). It is the most radiosensitive key system in the body (Fliedner et al. 2001), being subjected to cytokine disturbances, proliferative arrest and destruction of haematopoietic stem cells. Due to radiation toxicity to the blood-forming organs, mammals are the most radiosensitive group of animals, with mean LD_{50}s ranging from 2.5-11 Gy (Bond et al. 1965). The course of acute radiation syndrome (ARS) is generally similar in humans and rodents (Fliedner et al. 2001). The hematopoietic phase of ARS can be described by the following three phases:

1. Phase 1 (days 1-3): acute lymphocytopenia; initial peak in blood levels of granulocytes, followed by a sharp decrease; beginning of thrombocyte depletion.

2. Phase 2 (days 3-7): measurable decreases in red blood cell (RBC) counts, haemoglobin and haematocrit, nadir in white blood cell (WBC) counts of all types of WBCs, beginning of the pathology of ARS – immune insufficiency, internal bleeding and inability to clot.

3. Phase 3 (days 7-30): autologous recovery of the hematopoietic system from stem cells; severest forms of generalised anaemia in rodents; bone marrow stem cell colonies appear in rodent spleens (Fliedner et al. 2001).

In the context of radionuclide pollution, acute radiation syndrome in wild mammals may not occur due to the dose-rate effect in radiobiology, which means that protracted exposure with a dose of several Gy is not biologically equivalent to the same radiation dose administered acutely (Hall & Giaccia 2006). Radiation damage is primarily due to two factors: 1) external γ- and, less significantly, β-radiation from the environment, and 2) internal α, β and γ radiation from incorporated radionuclides (Vasilev 2005). In the context of anthropogenic pollution, acute lethality of rodents was observed in the Chernobyl exclusion zone during the first 1-2 years after the accident (Chesser et al. 2001, 2006). Nevertheless, rodent populations recovered relatively quickly due to migration from surrounding areas. The locally residing rodent populations thrive around the Chernobyl in large numbers, even though doses from incorporated ^{137}Cs and ^{90}Sr are several orders of magnitude higher than in similar non-contaminated European regions (Chesser et al. 2001, Beresford et al. 2008). It is important to keep in mind that several key radionuclides are bone-seekers (i.e. preferentially accumulated in bone). This is the case with ^{90}Sr and ^{226}Ra (as well as all other radium isotopes), leading to increased bone marrow toxicity of these radioactive elements. Iodine radionuclides (most importantly ^{131}I), accumulate preferentially in the thyroid; ^{137}Cs as a sodium analogue accumulates mostly in soft tissues and nearly uniformly throughout the body with preferences for muscle and nervous tissue (Vasilev 2005). Seasonal variations in feeding habits lead to increased accumulation of ^{137}Cs during the fall season, when animals' feeding habits are intensified (Zibold et al. 1992).

Reproductive system

Sterility

Radiation effects on the reproductive systems of mammals are well documented at doses in excess of 2-4 Gy and can cause temporary sterility in both male and female animals (Hall & Giaccia 2006, Ryabokon & Goncharova 2006).

Mouse spermatogonia are remarkably radioresistant, being able to recover nearly completely the production of spermatozoa, even after irradiation with the strongest dose of 15 Gy, with the lowest dose of 0.5 Gy producing a measurable but small percentage decrease in haploid germ cells (Hacker-Klom 1985). Usually, the dose required to cause permanent sterility in wild animals are well in excess of the lethal doses (Hall & Giaccia 2006).

Radiation teratogenesis: in utero effects of radiation

A subject of concern is radiation-induced teratogenesis in wild animals. This effect occurs probabilistically at doses exceeding 1 Gy and in rats and mice is most severe during the organogenesis period (Fig. 1).

The above figure is an illustration of the damage to rodent embryos that occurs after an acute total-body irradiation with 2 Gy of photons (γ-rays or X-rays). Irradiation of pregnant mice, depending on the time post-conception, results in:

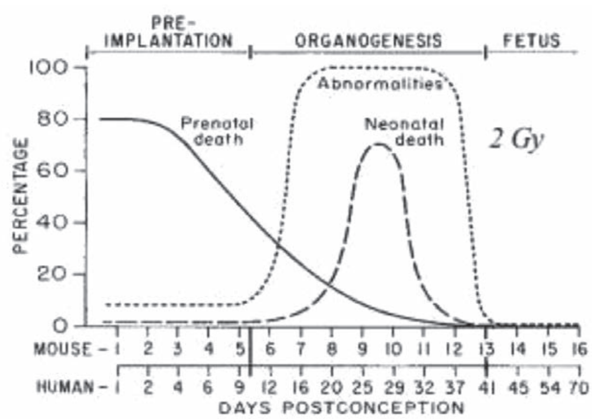

Fig. 1. Peaks of prenatal death (foetus resorption) (days 1-5), neonatal abnormalities (days 7-12) and neonatal death (days 9-10) in the progeny of mice, irradiated with 2 Gy of γ-rays (RBE=1); data for humans are also given based on epidemiological evidence from the Hiroshima and Nagasaki bombings (from Hall & Giaccia 2006).

1. Pre-natal death (resorption) of the foetuses with a probability of 80% (days 1-3 post-conception), decreasing to 0% at day 12.

2. Visible neonatal abnormalities (probability of nearly 100% in days 7-12 post-conception).

3. Neonatal death (with probabilities approaching 70% in days 9-10 post-conception).

During the stage of organogenesis *in utero* the most sensitive mammalian organ is the brain, followed closely by the eyes and gross skeletal and organic abnormalities (Hall & Giaccia 2006). One difficulty, which arises during studies of radiation teratogenesis in rodents is that small rodents frequently eat the abnormal newborns. Radiation effects *in utero* are very significant and severe in all mammalian species at doses exceeding 1 Gy. The ecological significance is diminished by the above-mentioned phenomenon of eating abnormal newborns, thus, radiation teratogenesis, while a severe adverse effect is rarely visible in wild-dwelling rodents and gross malformations are seldom propagated trans-generationally (Goncharova & Riabokon 1998).

Genetic effects: mutagenesis, genomic instability and population dynamics

The mutagenic effects of radiation have been a subject of widespread concern since the arrival of the atomic age (Tschurlovitz 1996). One of the main mammalian models for radiation mutagenesis have been laboratory mice. In the 1950s, Russell and Russell conducted a large experiment at several gene loci of 1,000,000 mice exposed of doses up to 10 Gy (protracted exposure). Russell et al. (1958) concluded that, for different genes mutation frequency varies by a factor of about 35. Their experimental data, combined with human epidemiological data from the A-bomb survivor study), have led scientists and radioprotection committees to estimate the doubling dose to be 1 Gy of chronic exposure to low-Linear Energy Transfer (LET) γ-rays or X-rays (Russell et al. 1958, Sankaranarayanan et al. 2000).

Data on the mutagenic effects of radionuclide pollution in populations of wild rodents are contradictory. Some authors have reported increased mutagenesis from Chernobyl at sites as distant from the site of the accident as Northern Sweden and Italy (Christaldi et al. 1990a, 1990b). These data have not been confirmed; studies from the Chernobyl exclusion zone have shown higher genetic variability of rodent populations from the contaminated zone than in control areas (Chesser et al. 2000, Matson et al. 2000). Using cytogenetic techniques, observations have been made from highly contaminated areas in Belarus, showing an increased number of chromosome aberrations, an euploidy and similar phenomena in mice from irradiated areas, as well as in their progeny (Goncharova & Riabokon 1998, see Table 3). The data presented both prove the utility of using cytogenetic biomarkers in animals from areas with high radiation exposure and give evidence for trans-generational accumulation of chromosomal damage in rodents from the most highly contaminated areas. Other data from studies with minisatellite DNA seem to confirm the hypothesis of radiation-induced genomic instability (Dubrova et al. 1996). What remains certain is that radiation is a powerful mutagen, inducing both somatic and heritable mutations. Further studies are needed to measure the mutagenic potential of radionuclides in terrestrial rodent species.

Table 3. Cytogenetic abnormalities in *Myodes glareolus* from reference areas (1), medium-radioactive (2) and highly radioactive (3, 4) areas in Belarus near the site of the Chernobyl accident (from Goncharova & Riabokon 1998).

Site	Year	Number of animals	Number of cells scored	Aberrant cells	Polyploid cells
1	1986	10	997	0.4	0.5
	1988	3	310	0.65	0
	1991	6	741	1	3.51
	1986-1991	19	2048	0.69	1.51
2	1991	20	2164	1.11	4.25
	1992	17	1995	1.22	1.65
	1991-1992	37	4159	1.17	3.01
3	1986	18	2011	1.71	1.19
	1988	21	2380	1.75	8.87
	1991	16	1824	2.54	9.27
	1986-1991	55	6215	1.96	6.5
4	1986	16	1743	1.27	0.23
	1987	36	3973	1.14	7.5
	1988	27	2883	1.77	5.86
	1991	30	4166	1.86	12.31
	1986-1991	109	12765	1.53	7.71

Small mammals as monitor species for environmental radioactivity

Utility of rodents (Cricetidae and Muridae) and shrews (Soricidae) as monitors of environmental radioactivity

The usefulness of rodents and other small mammals in the context of environmental monitoring has been discussed at length elsewhere (Talmage & Walton 1991, Talmage & Walton 1992, Andras et al. 2006, Topashka-Ancheva & Gerasimova 2012). There are several reasons for using rodents as biomonitors:

1. They have a fast life cycle (fast reproduction, relatively speedy maturation, fast turnover of

local populations). This means that rodents can be used to detect quickly changes in the environmental outlook, including seasonal variations in pollution levels (Andras et al. 2006);

2. Compared with larger animals, rodents have smaller home ranges, which means observing local populations is a good way to monitor pollution in given small areas (Talmage & Walton 1992);

3. Their small size, abundance, high reproductive rate, short gestation period and relative docility mean they are easy animals to work with, both in the field and in laboratory settings (Talmage & Walton 1991);

4. Many studies have been conducted on the comparative genetics of rodents and humans, ensuring that rodents are well-suited model animals for estimating effects in human populations (Neel & Lewis 1991);

5. Rodents are well characterised, meaning that a lot is known about their biology and behaviour (Talmage & Walton 1991, 1992).

Many of them, such as *Myodes glareolus, Apodemus flavicollis*, *A. sylvaticus* and *Peromyscus maniculatus* (Wagner, 1845) are the subject of many past and present ecotoxicological monitoring studies. This ensures that standard protocols exist and results are easy to compare (Talmage & Walton 1991, Chesser et al. 2001, 2006, Andras et al. 2006, Mitkovska et al. 2012). The insectivore species of the family Soricidae are located at the higher levels of the food chain and are often considered as especially useful in the monitoring of heavy metals and radionuclides (Talmage & Walton 1991, 1992, Chesser et al. 2000). Nevertheless, certain drawbacks exist when using them for monitoring studies:

1. They do not survive and breed in captivity due to their extremely fast metabolism.

2. They are less studied than rodents.

Comparative analysis of the utility of wild rodents in radioecological biomonitoring

Wild rodents have been established as monitor species for both heavy metals and radionuclides (Talmage & Walton 1991). So far, the most extensive studies have been conducted with European wild rodents in the vicinity of Chernobyl after the accident (Goncharova & Riabokon 1998, Chesser et al. 2000, 2001, Beresford et al. 2008). Rodents have different utility based on their life spans, food preference and tendency to accumulate toxicants. In series of studies, Chesser et al. have established that the bank vole is an excellent monitor species for determining the bioaccumulation of radionuclides, having body burdens and dose levels far exceeding those of *Apodemus* spp. (Chesser et al. 2000, 2001, 2006). This tendency is given by Beresford et al. (2008, see Table 2). The authors have confirmed that *M. glareolus* preferentially accumulates radiocesium isotopes due to its food preferences being mostly herbivorous (Beresford et al. 2008). This is in agreement with broader, more extensive studies (Chesser at al. 2000, see Table 4). The data demonstrate the utility of *M. glareolus* in monitoring environmental radioactivity but highlights two other tendencies: the higher concentration of radiostrontium in the bones of *M. oeconomus* and thevery high concentrations of ^{90}Sr in the skeletons of the insectivore *S. araneus*. This is explained, in part, by biomagnification due to the higher position of Soricidae in the food chain, which means that, for a given body weight, they accumulate substantially more radionuclides and heavy metals (Talmage & Walton 1992, Chesser et al. 2000).

Monitoring studies with other animal species

Studies have been conducted with other wildlife, mainly deer and rabbits (Zibold et al. 1992). Even though these animals are less suited to monitoring due to their larger home ranges and tendency to migrate (Talmage & Walton 1992), they can still be useful in assessing the effects of radionuclides in a given ecosystem, including seasonal variations in body burden. An example of such a study is the monitoring of roe deer (*Capreolus capreolus*) conducted in Oberschwaben, Germany, in 1987-1991 (Fig. 2). These data on the body body burden of radiocesium measured from the meat of roe deer varies significantly from season to season, with clear peaks in autumn, which are correlated with specific activities measured in mushrooms (Zibold et al. 1992).

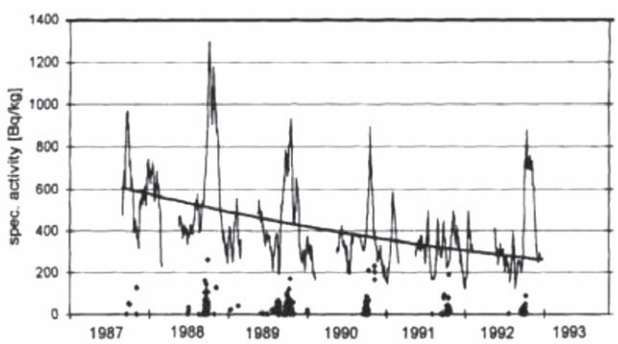

Fig. 2. Specific activities of ^{137}Cs in the meat of roe deer *Capreolus capreolus* (solid line graph) caught in Oberschwaben, Germany, in the period 1987-1991 (Zibold et al. 1992). The bar graphs represent specific activities of ^{137}Cs in mushrooms, which constitute one of the main sources of food for roe deer in the summer–autumn season.

In addition to cesium acquired by the consumption of mushrooms, another reason is the more intensive grazing of *C. capreolus* in the autumn season (Zibold et al. 1992). While the monitoring of other wildlife can be informative, it is primarily rodents (Muridae and Cricetidae), which constitute the most useful species for radioecological monitoring.

Current concepts in radioecological biomonitoring

Studies related to natural radioactivity; areas with naturally elevated radiation background; long-range transmission of radionuclides and its monitoring

There are areas with naturally elevated radiation background. While such regions have been the subject of some studies, most ecotoxicological research has focused on anthropogenic contamination with radionuclides. Still, areas with naturally elevated radiation background deserve a mention in the context of the radioecology of terrestrial vertebrates. Some of the most important locations with higher-than-average natural background radiation are Ramsar in Iran, Kerala in India, Guarapari in Brazil and Yangjiang in China. Most of this increased background is due to external γ-radiation and emission of radon and thoron and their daughter radionuclides (Mortazavi 2015). In Guarapari, cytogenetic studies have been conducted in humans inhabiting high-natural background radiation (HNBR) areas, although no conclusive results have been reached (Barcinski et al. 1975).

The effects of high natural radioactive background on humans and biota have been the subject of much discussion. A relatively recent comprehensive review on studies of humans (most of which have been inconclusive, with a few collaborative studies in China and India), indicates increased cancer risk among populations from HNBR areas (Hendry et al. 2009). Few studies have been conducted with mammals from HNBR areas; an example of one such study is the work of Testov (1996), who has studied rodents

Table 4. Levels of ^{90}Sr in bones of monitor species of small mammals (Bq/g) and dose range estimates (mGy/d) due to strontium in mammals from highly contaminated (Red Forest), medium-contaminated (Glyboke Lake) and reference areas within the Chernobyl Exclusion Zone (from Chesser et al. 2000).

Location/ perimeter in Chernobyl	Apodemus agrarius	Apodemus flavicollis	Apodemus sylvaticus	Microtus arvalis	Microtus oeconomus	Myodes glareolus	Sorex araneus	Total
Red Forest Grassland			n=12	n=21			n=1	n=34
Bq/g			389 (79.4-871)	314 (21.1-1374)			39	332 (21.1-1374)
Dose (mGy/day)			1.3 (0.3-3.0)	1.1 (0.1-4.8)			0.1	1.1 (0.1-4.8)
Red Forest Woodland		n=3	n=4	n=1				n=8
Bq/g		330 (36.5-582)	370 (204-815)	131				325 (36.5-815)
Dose (mGy/day)		1.1 (0.1-2.0)	1.3 (0.7-2.8)	0.5				1.1 (36.5-815)
Glyboke Lake	n=8	n=1	n=1	n=10	n=3	n=13	n=9	n=45
Bq/g	262 (109-765)	167	171	752 (368-1502)	1068 (370-1609)	271 (74-497)	630 (104-2275)	497 (74-2275)
Dose (mGy/day)	0.9 (0.4-2.6)	0.6	0.6	2.6 (1.3-5.2)	3.7 (1.3-5.6)	0.9 (0.3-1.7)	2.2 (0.4-7.9)	1.7 (0.3-7.9)
Chistogalivka reference zone	n=2			n=25	n=9	n=1	n=10	n=48
Bq/g	30 (30-30.5)			89 (12-677)	76 (32.3-239)	23	83 (56.1-168)	79 (1.2-677)
Dose (mGy/day)	0.1			0.3 (0.04-2.3)	0.3 (0.1-0.8)	0.1	0.3 (0.2-0.6)	0.3 (0.004-2.3)
Chernobyl Zone Summary:	n=10	n=4	n=17	n=57	n=12	n=14	n=20	n=135
Bq/g	216 (30.3-765)	289 (36.5-582)	372 (79.4-871)	289 (12-1502)	324 (32.3-1609)	253 (22.7-479)	327 (39-2275)	297 (1.2-2275)
Dose (mGy/day)	0.7 (0.1-2.6)	1 (0.1-2.0)	1.3 (0.3-3.0)	1 (0.04-5.2)	1.1 (0.1-5.6)	0.9 (0.1-1.7)	1.1 (0.1-7.9)	1 (0.004-7.9)

from several regions of Russia and Ukraine, including two areas with high natural background radiation (Ural and Uhta) and two with high anthropogenic contamination (East Urals Radioactive Trace (EURT) near Ozyorsk, Russia, and the Pripyat near Chernobyl. They analysed *Myodes rutilus, M. glareolus, Microtus oeconomus, M. arvalis, Apodemus agrarius, A. sylvaticus, A. flavicollis* and *Mus musculus*. The animals in HNBR areas show no statistical difference with respect to population genetics, morpho-physiological indicators and cytogenetic parameters in comparison with animals from control areas (Testov 1996).

Another issue related to both natural and man-induced radiation is the long-range transfer of radionuclides in terrestrial ecosystems. Long-range atmospheric transfer occurs through both natural events, such as volcanic eruptions, and due to anthropogenic sources. Technogenic radionuclides have been detected in remote areas such as the Faroes Islands and the Yamal Peninsula, indicating global contamination due to atmospheric nuclear testing in the period 1945-1965 (Aarkrog et al. 1981, 1982, Nifontova 1995). Tundra ecosystems are especially sensitive to global radioactive pollution (Nifontova 1995, Vasilev 2005).

For the purpose of monitoring long-range transfer of radionuclides, the European Economic Area (EEA) has established a Europe-wide network of high-mountain observatories, which have equipment to detect both natural and anthropogenic radionuclides in the atmosphere. These activities have been useful in determining fallout from Chernobyl, as well as, more recently, detecting contaminated air masses from the Fukushima accident (Masson et al. 2016). Although high-mountain observatories are useful in maintaining awareness of the global radiological outlook, the behaviour and transfer of radionuclides in high-mountain ecosystems is still an active area of research.

Anthropogenic enrichment of naturally occurring radionuclides; uranium mines and mine tailings

Anthropogenic enrichment of uranium and radium in certain areas represents an ongoing problem. While in Europe there are only two operational uranium mines, sites of former uranium extraction present an ongoing hazard to the environment (Tschurlovits 1996, Kolev et al. 2014). There have been few studies on the transfer of anthropogenically enriched uranium, radium and other radionuclides to small mammals, mostly from North America, such as vole species and *Peromyscys maniculatus* (see Talmage & Walton 1991, Thomas 2000). These data should be extrapolated with caution to European mammalian species. Radiological monitoring studies on the sites of former European uranium mining locations are regularly conducted and can be consulted when preparing and designing studies for biomonitoring of small mammals (e.g. Kolev et al. 2014).

Studies of surface waters and sediments are useful starting points for assessing the environmental impact of former mining activities. Nevertheless, they need to be supplemented by data for ecosystem transfer to plants and animals in order to be able to draw conclusions about the effects of contaminated sites on the environment. Regarding the waste products of the nuclear fuel cycle, little data are available on how uranium mine tailings interact with the terrestrial biota, although several European studies are in progress. Most available information has shown that uranium, radium and their radioisotopes in tailings are only one component of a complex pollution pattern in many areas, including toxic metals (lead, copper and vanadium) and, depending on the extraction method of uranium, various quantities of sulphuric and nitric acid and different salts thereof (Kolev et al. 2014).

Studies of contamination with anthropogenic radionuclides: migration in the soil, bioaccumulation and biomagnification in terrestrial food chains

Most radioecological studies on small mammals have been conducted in the context of pollution with anthropogenic radionuclides; the majority of studies address the radiological consequences of the Chernobyl accident (Chesser et al. 2001, Goncharova & Riabokon 1998, Beresford et al. 2008, Christaldi et al. 1990b). The migration of radionuclides in soil and along terrestrial food chains has been described elsewhere (Kinova 1998, Zibold et al. 1992, Nifontova 1995). It was determined that different species of small mammals have distinct profiles for the uptake, retention and bioaccumulation of radionuclides within the Chernobyl exclusion zone (Chesser et al. 2001, Beresford et al. 2008).

Future and prospective developments in radioecological biomonitoring with terrestrial small mammals

New studies on natural radioactivity and its effects on the biota

Although some efforts have been made to monitor the impact of natural radioactivity in free-living small mammals, currently little research is addressing the is-

Table 5. Contents of ^{134}Cs and ^{137}Cs in Apodemus speciosus (male adult animals) living in Fukushima and two control areas (Aomori and Toyama) (Bq/kg wet weight) (from Okano et al. 2016).

Bq/kg wet weight						
	2013			2014		
	134 Cs	137 Cs	n	134 Cs	137 Cs	n
Site 1 (Fukushima)	2326 (1771-4137)	4331 (3335-7806)	12	4770 (1140-12907)	12312 (2874-40131)	10
Site 2 (Fukushima)	1380 (882-1766)	2815 (1632-3761)	4	2359 (954-8480)	6426 (2456-21738)	8
Site 3 (Aomori)	0 (0-11)	4 (0-21)	10	0 (0-0)	5 (3-8)	5
Site 4 (Toyama)	0 (0-0)	0 (0-3)	10	0 (0-0)	0 (0-6)	8

sue of biomonitoring of the terrestrial biota in HNBR areas. Iovtchev et al. (1995) have measured total β-activities in vertebrate species inhabiting the Rila Mts., Bulgaria. While the Rila Mts. are not an HNBR area, there are slightly elevated levels of natural radioactivity in comparison with the national average (2.6 mSv/a) due to their granite structure and high elevation (Moussala Peak, 2925 m a.s.l.), leading to increased exposures to space radiation. For total β-activities of small mammals inhabiting the mountain, see Table 1.

Answering questions about anthropogenic radionuclides. Future concepts in the biomonitoring of anthropogenic pollution in the Chernobyl Exclusion Zone

A number of small mammal studies have been conducted in the area of the Chernobyl Exclusion Zone (Goncharova & Riabokon 1998, Chesser et al. 2000, 2001, 2006, Beresford et al. 2008). While the danger to humans and ecosystems from radionuclides is decreasing in the 30-km zone, many topics are still open to further research. Dubrova et al. (1996) and Ryabokon & Goncharova (2006) have presented evidence for transgenerational genomic instability induced by radioactivity from the Chernobyl accident. This means that, for ecosystems and humans exposed to ionizing radiation, a possibility exists that future generations will have an increased tendency to acquire transmissible germline mutations. Nevertheless, research in this area has been regarded as highly controversial. Some of the questions which remain to be answered are:

1. Is there a trans-generational accumulation of genomic instability within highly contaminated terrestrial ecosystems?

2. Is there a change in the genetic structure of populations of small mammals exposed to high levels of ionizing radiation?

While a significant amount of research has so far been devoted to rodent populations in the Chernobyl Exclusion Area, these questions do not have definitive answers yet.

Studies of other contaminated areas: Ozyorsk (Chelyabinsk-40), Fukushima and others

Over the last two decades significant amount of data have emerged on the radioactive contamination of another site in the former Soviet Union. The watershed of the Techa River is located in the Eastern Urals and flows from the grounds of the former nuclear complex "Mayak" and the closed city of Chelyabinsk-40. The river is well known for its radioactive contamination and has been the site of several radioactive accidents (Burmistrov & Linkov 1998, Burmistrov et al. 1999). In the period 1995-2005, the Techa River Basin has been the site of several US/EU/Russian collaborative studies to determine the radioecological status of the area and estimate radiation doses to residents. During the main period of the study, it was discovered that most of the remaining radioactive pollution in the vicinity of the Mayak Industrial Complex is due to ^{90}Sr, which contributed also to most of the anthropogenic bone marrow dose for residents in the area (Burmistrov et al. 1999).

No biomonitoring studies with small rodents have been conducted to study the impact of this site on terrestrial ecosystems. More recently, the industrial complex has been mentioned in the context of a release of Ruthenium-106 (^{106}Ru) in the atmosphere, which was detected in Europe in 2017; however, the secretive nature of the Mayak Enterprise has prevented the international community from verifying or disproving the occurrence of a new accident at the complex.

Another site of interest is the immediate vicinity of the Fukushima Dai-Ichi Power Plant in northern Honshu, Japan. Due to the timely shutdown of the reactors at Fukushima, the accident was much smaller in magnitude than Chernobyl (400-800 PBq, compared to 5,200 PBq; see Steinhauser et al. 2014). Bonisoli-Alquati et al. (2015) have focused on barn swallows from Fukushima. Okano et al. (2016) have measured radionuclide concentrations and genetic damage in Japanese field mice (*Apodemus speciosus*) in 2013 and 2014 (Table 5). While not much higher than concentrations in the reference areas, the measured values for radiocesium still provide evidence for the radioactive contamination of the environment close to the damaged power plant and for bioaccumulation of the element in *A. speciosus*. Although the authors report effects on mice spermatogenesis, there is still much research to be done before radionuclide contamination from the Fukushima accident can be said to significantly affect any measured biological endpoints, especially germline mutations.

The future of radioecological biomonitoring lies in the following directions:

1. Exploring radionuclide-contaminated sites which have received little attention: the basin of the Techa River, the Semipalatinsk test site, sites of former uranium mining and refining.

2. Introducing new methodologies to assess biological endpoints more accurately.

3. Exploring the two key issues of areas with high natural background radiation and the presence or absence of cross-generational genomic

4. Introducing and developing non-invasive methods for assessing radioactive contamination. In recent years, Bondarkov et al. (2011) have reported a system for external *in vivo* measurements of body burden of ^{90}Sr and ^{137}Cs near Chernobyl, which does not require sacrificing animals in order to obtain radionuclide data. Additionally, Beresford et al (2008) and Vetikko & Saxen (2010) have reported the use of predictive software models (the ERICA tool) to estimate radiation doses to terrestrial and freshwater biota from physical models of radionuclide deposition. While such systems and tools are undoubtedly useful, non-invasive sampling in the context of small-mammal radioecology is in its infancy and further studies are needed.

Conclusions

While small-mammal radioecology has achieved much, several challenges remain to be addressed in future research:

1. Gaining access and conducting measurements in the vicinity of closed facilities.

2. Development and implementation of non-invasive samplings.

3. Solving the question of genomic instability due to radionuclides contaminations. Even though radioecological research has achieved a lot in the recent past, the challenges and questions that remain to the discipline are practically boundless, ensuring that scientists will not run out of problems to solve. This area of study, in particular biomonitoring activities with small mammals, will likely continue to be active and productive in the immediate future.

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References

Aarkrog A., Dahlgaard H., Hallstadius L., Holm E. & Lippert J. 1981. Environmental radioactivity in the Faroes in 1980. Riso National Laboratory Archives.

Aarkrog A., Dahlgaard H., Hallstadius L., Holm E. & Lippert J. 1982. Environmental radioactivity in the Faroes in 1981. Riso National Laboratory Archives.

Andras P., Krizani I. & Stanko M. 2006. Free-living rodents as monitors of environmental contaminants at a polluted mining dump area. Carpathian Journal of Earth and Environmental Sciences 1(2): 51-62.

Barcinski M., Do Ceu Abreu M., De Almeida J., Naya J., Fonseca L. & Castro L. 1975. Cytogenetic investigation in a Brazilian population living in an area of high natural radioactivity. American Journal of Human Genetics 27(6): 802-806.

Beresford N., Gaschak S. & Barnett C. 2008. Estimating the exposure of small mammals at three sites within the Chernobyl exclusion zone – a test application of the ERICA Tool. Journal of Environmental Radioactivity 99: 1496-1502. DOI: 0.1016/J.JENVRAD.2008.03.002.

Bond V., Fliedner T. & Archambeau J. 1965. Mammalian radiation lethality: a disturbance in cellular kinetics. New York, NY: New York Academic Press.

Bondarkov M., Maksimenko A., Gaschak S., Zheltonozhsky V., Jannik G. & Farfan E. 2011. Method for simultaneous 90Sr and 137Cs in vivo measurements of small animals and other environmental media developed for the conditions of the Chernobyl Exclusion Zone. *Savannah River National Laboratory HPJ Special Issue*, pp. 1-27. DOI: 10.1097/HP.0b013e318224bb2b.

Bonisoli-Alquati A., Voris A., Mousseau T., Möller A., Saino N. & Wyatt M. 2010. DNA damage in barn swallows (*Hirundo rustica*) from the Chernobyl region detected by use of the comet assay. Comparative Biochemistry and Physiology 151(1): 271-277. DOI:10.1016/j.cbpc.2009.11.006.

Bonisoli-Alquati A., Koyama K., Tedeschi D., Kitamura W., Suzuki H, Ostermiller S., Arai E., Moller A. & Mousseau T. 2015. Abundance and genetic damage in barn swallows from Fukushima. Nature Reports 9432(5): 1-8. DOI: 10.1038/srep09432.

Burmistrov D. & Linkov I. 1998. Radioactive contamination of the Techa River. In: Linkov I. & Wilson R. (Eds.): Air Pollution in the Ural Mountains. Amsterdam: Kluwer Academic Publishers, pp. 423-428. (ISBN 978-94-011-5208-2).

Burmistrov D., Kossenko M. & Wilson R. 1999. Radioactive contamination of the Techa River and its effects. In: Harvard Academic Review (TTS 9906-543), 747-755.

Chesser R., Rodgers B., Wickliffe J., Gaschak S., Phillips C. & Baker R. 2001. Accumulation of 137-cesium and 90-strontium from abiotic and biotic sources in rodents at

Chornobyl. Environmental Toxicology and Chemistry 20: 1927–1934. doi.org/10.1002/etc.5620200910.

Chesser R., Sugg D., Lomakin M., Van den Bussche R., DeWoody A., Jagoe C., Dallas C., Whicker F., Smith M., Gaschak S., Chizhevsky I., Lyabik V., Buntova E., Holloman K. & Baker R. 2000. Concentrations and dose rate estimates of 134,137-cesium and 90-Strontium in small mammals at Chornobyl, Ukraine. Environmental Toxicology and Chemistry 19(2): 305-312. doi.org/10.1002/etc.5620190209.

Chesser R. & Baker R. 2006. Growing up with Chernobyl. American Scientist 94(1): 542-549.

Christaldi M., D'Arcangelo E., Ieradi L., Mascanzoni D., Mattei T. & Van Axel Castelli I. 1990a. ^{137}Cs determination and mutagenicity tests in wild *Mus musculus domesticus* before and after the Chernobyl accident. Environmental Pollution 64(1): 1-9. doi.org/10.1016/0269-7491(90)90091-P.

Christaldi M., Ieradi L., Mascanzoni D. & Mattei T. 1990b. Environmental impact of the Chernobyl accident: mutagenesis in bank voles from Sweden. International Journal of Radiation Biology 59(1): 31-40. doi.org/10.1080/09553009114550031.

Comas F. & Byrd B. 1967. Hemopoietic spleen colonies in the rat. Radiation Research 32(2): 355-365. DOI: 10.2307/3572229.

Darby S., Hill D., Auvinen J., Barros Dios H., Baysson F., Bochiccio H., Deo R., Falk F., Forastiere M., Hakama I., Heid L., Kreienbrock M., Kreuzer L., Lagarde I., Makeleinen C., Muirhead W., Oberaigner G., Pershagen A. & Doll R. 2005. Radon in homes and risk of lung cancer: collaborative analysis of individual data from 13 European case-control studies. Revista Portuguesa de Pneumologia 11(2): 190-191. DOI: 10.1136/bmj.38308.477650.63.

Dubrova Y., Nesterov V., Krouchinsky N., Ostapenko V., Neumann R., Neil D. & Jeffreys A. 1996. Human minisatellite mutation rate after the Chernobyl accident. Nature 380: 683-686. DOI: 10.1038/380683a0.

Enderle G. & Friedrich K. 1999. Uranium mining in East Germany ("Wismut"): health consequences, occupational medical care and workers' compensation. International Archives of Occupational and Environmental Health 72, Supplement 3: M42-M49. https://doi.org/10.1007/PL00014215.

Fenech M. & Morley A. 1986. Cytokinesis-block micronucleus method in human lymphocytes: effect of in vivo ageing and low dose X-irradiation. Mutation Research 161(1): 193-198. doi.org/10.1016/0027-5107(86)90010-2.

Fliedner T., Feinendegen L. & Hopewell J. (Eds) 2002. Chronic irradiation: tolerance and failure in complex biological systems. British Journal of Radiology 2002a, Supplement 26: 57-71.

Fliedner T., Friesecke I. & Beyrer K. 2001. Medical management of radiation accidents. London: British Institute of Radiology. pp. 162–172. DOI: 10.1093/occmed/kql011.

Furitsu K. 2010. Trans-generational effects of radiation exposure from the Chernobyl Nuclear Plant Accident: A review of studies using mutation markers of repeat DNA sequences. RRI Bulletins 139(1): 165-178.

Goncharova R. & Riabokon G. 1998. Biological effects in natural populations of small rodents in radiation-polluted territories. Dynamics of chromosome aberration frequency in a number of generations of European bank vole (*Clethrionomys glareolus*, Schreber). Radiatsionnaya Biologiya i Radioekologiya 38(5): 763-768. PMID:9876498.

Gu Y., Kai M. & Kusama T. 1997. The embryonic and fetal effects in ICR mice irradiated in the various stages of the preimplantation period. Radiation Research 147(6): 735-740.

Güntay S., Powers D. & Devell L. 1995. The Chernobyl reactor accident source term: development of a consensus view. IAEA: INIS 41(8): 183-193.

Hacker-Klom U. 1985. Long-term effects of ionizing radiation on mouse spermatogenesis. Acta radiologica: Oncology 24(4): 363-367. doi.org/10.3109/02841868509136066.

Hall E. & Giaccia A. 2006. Radiobiology for the radiologist. New York, London: Lippincott Williams and Wilkins. ISBN: 978-1608311934.

Härtling F. & Hesse W. 1879. Der Lungenkrebs, die Bergkrankheit in den Schneeberger Gruben. Vierteljahresschrift für Gerichtliche Medizin 30(1): 3 parts.

Hendry J., Simon S., Wojcik A., Sohrabi M., Burkard W., Cardis E., Laurier D., Tirmarche M. & Hayata I. 2009. Human exposure to high natural background radiation: what can it teach us about radiation risks? Journal of Radiological Protection 29(0), A29-A42. DOI: 10.1088/0952-4746/29/2A/S03.

Ilyin L. 1995. Chernobyl: myth and reality. Moscow: Megapolis. 397 p. ISBN:590078701X, 9785900787015.

Iovtchev M., Metcheva R. & Peev D. 1998. Radiation monitoring 1993-1996: total beta activity in samples from Rila Mountain, Bulgaria. Observatoire de montagne de Moussala OM2, Vol. 7, pp. 174-181. ISBN: 954-9820-80-4.

Ivanova K., Badulin V., Kunovska B. & Yovcheva M. 2014. Natural uranium concentrations in surface water and sediments from former uranium mining locations for 2012-2013. Radiation Protection Journal 2: 47-53. ISSN 1314-9199.

Kemski J., Siehl A., Stegemann R. & Valdivia-Manchego M. 2001. Mapping the geogenic radon potential in Germany. Science of the Total Environment 272 (1-3): 217-230. doi.org/10.1016/S0048-9697(01)00696-9.

Kinova L. 1998. Radionuclide status in certain areas of Rila Mountain. Observatoire de montagne de Moussala OM2, vol. 7, pp. 240-248. ISBN: 954-9820-80-4.

Kolev S., Van Meir N. & Ivanova K. 2014. Assessment of the current state of radionuclides and metals content in the surface and groundwater at the region of Eleshnitsa former uranium mine. Radiation Protection Journal 2(1): 35-42. ISSN 1314-9199.

Kulka U., Ainsbury L., Atkinson M., Barquinero J.F., Barrios L., Beinke C., Bognar G., Cucu A., Darroudi F., Fattibene P. & Gil O. 2012. Realising the European network of biodosimetry (RENEB). Radiation Protection Dosimetry 151(4): 621-625. DOI: 10.1093/rpd/ncu266.

Lamerton L., Pontifex A., Blackett N. & Adams K. 1960. Effects of protracted irradiation on the blood-forming organs of the rat: I. Continuous exposure. British Journal of Radiology 33(1): 287–301. DOI: 10.1259/0007-1285-33-389-287.

Masson O., Bieringer J., Brattich E., Dalheimer A., Estier S., Penev I., Ringer W., Schlosser C., Steinkopff T., Steinmann P., Tositti L., Van Beek P. & Vismes-Ott A. 2016. Variation in airborne ^{134}Cs, ^{137}Cs, particulate ^{131}I and ^{7}Be maximum activities at high-altitude European locations after the arrival of Fukushima-labeled air masses. Journal of Environmental Radioactivity 162-163: 14-22. doi.org/10.1016/j.jenvrad.2016.05.004.

Matson C., Rodgers B., Chesser R. & Baker R. 2000. Genetic diversity of *Clethrionomys glareolus* populations from zones of highly contaminated sites in the Chornobyl Region, Ukraine. Environmental Toxicology and Chemistry 19: 2130–2135. doi.org/10.1002/etc.5620190824.

Mehra R. & Singh M. 2011. Measurement of Radioactivity of ^{238}U, ^{226}Ra, ^{232}Th and ^{40}K in Soil of Different Geological Origins in Northern India. Journal of Environmental Protection 2(1): 960-966. DOI: 10.4236/jep.2011.27110.

Mitkovska V., Chassovnikarova T., Atanasov N. & Dimitrov H. 2012. Environmental genotoxicity evaluation using a micronucleus test and frequency of chromosome aberrations in free-living small rodents. Journal of Biosciences and Biotechnology 1(1): 67-71. ISSN: 1314-6246.

Mortazavi S. 2015. High radiation background areas in Ramsar, Iran. RIRBM Protocols 12(1): 151-157. PMID: 11769138.

Natarajan A., Santos S. & Darroudi F. 1998. ^{137}Cesium-induced chromosome aberrations analyzed by fluorescence in situ hybridization: eight years follow up of the Goiania radiation accident victims. Mutation Research 400(1): 299-312. https://doi.org/10.1016/S0027-5107(98)00021-9.

Neel J. & Lewis S. 1991. The comparative radiation genetics of humans and mice. In: Neel J. & Schull W. (Ed.): The children of atomic bomb survivors: a genetic study. Washington, DC: National Academic Press, pp. 451-487. ISBN-10: 0-309-04537-1.

Nieminen P., Huitu O., Henttonen H., Finnila M., Voutilainen L., Itamies J., Karja V., Saarela S., Halonen T., Aho J. & Mustonen A. 2015. Physiological condition of bank voles (*Myodes glareolus*) during the increase and decline phases of the population cycle. Comparative Biochemistry and Physiology. A. Molecular and Integrative Physiology 187(1): 141-149. https://doi.org/10.1016/j.cbpa.2015.05.007.

Nifontova M. 1995. Radionuclides in the moss lichen cover of tundra communities in the Yamal Peninsula. Science of the Total Environment 160(1): 749-752. https://doi.org/10.1016/0048-9697(95)04408-S.

Okano T., Ishiniwa H., Onuma M., Shindo J., Yokohata Y. & Tamaoki M. 2016. Effects of environmental radiation on the testes and spermatogenesis in wild large Japanese field mice (*Apodemus speciosus*) from Fukushima. Science Reports 6(23601): 1-8. DOI: 10.1038/srep23601.

Ostling O. & Johanson K. 1984. Microelectrophoretic study of radiation-induced DNA damages in individual mammalian cells. Biochemical and Biophysical Research Communication 123(1): 291–298. https://doi.org/10.1016/0006-291X(84)90411-X.

Russell W., Russell L. & Kelly E. 1958. Radiation dose and mutation frequency. Science 128 (3338), 1546-1550. DOI: 10.1126/science.128.3338.1546.

Ryabokon N. & Goncharova R. 2006. Transgenerational accumulation of radiation damage in small mammals chronically exposed to Chernobyl fallout. Radiation and Environmental Biophysics 45(1): 167-177. DOI: 10.1007/s00411-006-0054-3.

Sankaranarayanan K. & Chakraborty R. 2000. Ionizing radiation and genetic risks: XI. The doubling dose estimates from the mid-1950s to the present and the conceptual change to the use of human data on spontaneous mutation rates and mouse data on induced mutation rates for doubling dose calculations. Mutation Research 453(2): 107-127. https://doi.org/10.1016/S0027-5107(00)00108-1.

Schultz H. 1999. Die Zentrale Betreuungsstelle Wismut im Rückblick: 1991-1999. Die BG (July 1999), pp 404-408.

Schwartz S., Smirnov V. & Dobrinskii L. 1968. The method of morpho-physiological indicators in the ecology of terrestrial vertebrates. Sverdlovsk: RAN publishing. (In Russian.)

Smith M. 2010. Architects of Armageddon: The Home Office Scientific Advisers' branch and civil defence in Britain, 1945-68. British Journal for the History of Science 43(2): 149-180. doi.org/10.1017/S0007087409990392.

Steinhauser G., Brandl A. & Johnson T. 2014. Comparison of the Chernobyl and Fukushima nuclear accidents: a review of the environmental impacts. Science of the Total Environment 470(1): 800-817. DOI: 10.1016/j.scitotenv.2013.10.029.

Talmage S. & Walton B. 1991. Small mammals as monitors of environmental contaminants. Reviews of Environmental Contamination and Toxicology 119(1): 47-145. https://doi.org/10.1007/978-1-4612-3078-6_2.

Talmage S. & Walton B. 1992. Small mammals as environmental monitors. In: ReView: The Laboratory. Oak Ridge, TN: Oak Ridge National Laboratory, pp. 53-57.

Tapisso J., Marques C., da Luz Mathias M. & da Graça Ramalhinho M. 2009. Induction of micronuclei and sister chromatid exchange in bone-marrow cells and abnormalities in sperm of Algerian mice (Mus spretus) exposed to cadmium, lead and zinc. Mutation Research/Genetic Toxicology and Environmental Mutagenesis 678(1): 59-64. DOI: 10.1016/j.mrgentox.2009.07.001.

Testov B. 1996. Physiological adaptation of small mammals to radioactive pollution. In: Luyks F. & Frissel M. (Eds): Radioecology and the restoration of radioactive-contaminated sites. Dordrecht: Kluwer Academic Publishers, pp. 245-253. DOI: 10.1134/S1607672917050143.

Thomas P. 2000. Radionuclides in the terrestrial ecosystem near a Canadian uranium mill-Part II: Small mammal food chains and bioavailability. Health Physics 78(6): 625-632.

Thorn M. & Vennard J. 1976. The toxicity of Sr-90, Ra-226 and Pu-239. Nature 263(1), 555–558. PMID:10832921.

Till J. & McCulloch E. 1961. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. Radiation Research 14(1): 213-222. DOI: 10.1667/RRAV01.1.

Topashka-Ancheva M. & Gerasimova T. 2012. Genomic sensitivity in small mammals – a suitable system in the genetic monitoring. In: Begum G. (Ed.): Ecotoxicology. Vienna, Austria: InTech Open, pp. 125-146. DOI: 10.5772/1185.

Topashka-Ancheva M., Metcheva R. & Teodorova S. 2003. A comparative analysis of the heavy metals loading of small mammals in different Bulgarian regions. II. Chromosomal aberrations and blood pathology. Ecotoxicology and Environmental Safety 54(2): 188-193. https://doi.org/10.1016/S0147-6513(02)00052-0.

Tschurlovits M. 1996. Recent developments in radioecology. IAEA: *INIS* 37(2): 427-430.

Van Bekkum D. 1977. The appearance of the multipotential hemopoietic stem cell. In: Baum S.D. & Ledney J. (Eds): Experimental Hematology Today. New York, Berlin: Springer. ISBN 978-3-662-25807-1.

Vasilev G. 2005. Radioecology: radiation, ecology, man. Sofia, Bulgaria: Tita Consult. (In Bulgarian.)

Vetikko V. & Saxen R. 2010. Application of the ERICA assessment tool to freshwater biota in Finland. Journal of Environmental Radioactivity 101(1): 82-87. DOI: 10.1016/j.jenvrad.2009.09.001

Zibold G., Drissner J., Erb W., Herrmann T., Hund M., Lindner G., Wilhelm C., Wolf J. & Zech W. 1992. Mechanisms of cesium transfer in forest ecosystems obtained from continuous monitoring of roe deer contamination. IRPA 1(9): 1709-1712. ISBN 1-55048-657-8.

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