Available online at www.sciencedirect.com

ScienceDirect

Environment International 34 (2008) 880–897

ENVIRONMENT INTERNATIONAL

www.elsevier.com/locate/envint

Review article

Effects of non-human species irradiation after the Chernobyl NPP accident

S.A. Geras'kin [a,*], S.V. Fesenko [a,b], R.M. Alexakhin [a]

[a] *Russian Institute of Agricultural Radiology and Agroecology, Obninsk, Russia*
[b] *International Atomic Energy Agency, Agency's Laboratories, Seibersdorf A-2444, Austria*

Received 23 July 2007; accepted 14 December 2007
Available online 30 January 2008

Abstract

The area affected by the Chernobyl Nuclear Power Plant accident in 1986 has become a unique test site where long-term ecological and biological consequences of a drastic change in a range of environmental factors as well as trends and intensity of selection are studied in natural settings. The consequences of the Chernobyl accident for biota varied from an enhanced rate of mutagenesis to damage at the ecosystem level. The review comprehensively brings together key data of the long-term studies of biological effects in plants and animals inhabiting over 20 years the Chernobyl NPP zone. The severity of radiation effects was strongly dependent on the dose received in the early period after the accident. The most exposed phytocenoses and soil animals' communities exhibited dose dependent alterations in the species composition and reduction in biological diversity. On the other hand, no decrease in numbers or taxonomic diversity of small mammals even in the most radioactive habitat was shown. In a majority of the studies, in both plant and animal populations from the Chernobyl zone, in the first years after the accident high increases in mutation rates were documented. In most cases the dose–effect relationships were nonlinear and the mutation rates per unit dose were higher at low doses and dose rates. In subsequent years a decline in the radiation background rate occurred faster than reduction in the mutation rate. Plant and animal populations have shown signs of adaptation to chronic exposure. In adaptation to the enhanced level of exposure an essential role of epigenetic mechanisms of gene expression regulation was shown. Based on the Chernobyl NPP accident studies, in the present review attempts were made to assess minimum doses at which ecological and biological effects were observed.

Keywords: Chernobyl NPP accident; Radioactive contamination; Doses; Ecological and biological effects

Contents

1. Introduction . . . 881
2. Effects of ionizing radiation on plants . . . 881
    2.1. Forest trees . . . 881
    2.2. Herbaceous plants . . . 883
    2.3. Genetic effects . . . 884
3. Effects of ionizing radiation on animals . . . 885
    3.1. Soil- and entomofauna . . . 885
    3.2. Amphibians . . . 886
    3.3. Hydrobionts . . . 886
    3.4. Mammals . . . 887
        3.4.1. Mouse-like rodents . . . 887
        3.4.2. Agricultural and domestic animals . . . 889
        3.4.3. Genetic effects . . . 889

---

* Corresponding author.
E-mail address: stgeraskin@gmail.com (S.A. Geras'kin).

0160-4120/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.envint.2007.12.012

4. Lessons learned 891
Acknowledgements 895
References 895

1. Introduction

The Chernobyl accident has no analogs in both area of radioactive contamination and absorbed doses to biota species. The total release of fission products (ignoring inert radioactive gases) was estimated to be 1.85×10^{18} Bq (IAEA, 2006). The resulting large-scale and nonuniform radioactive contamination of the area affected as well as the variety of biota responses at different levels of biological organization – from molecular and cellular to ecosystem – have made investigations of ecological and biological effects of the Chernobyl accident a source of actually unique information.

Most affected by radiation were natural and agricultural ecosystems in the 30-km Chernobyl NPP zone. The Chernobyl accident occurred in late April, the period of accelerated growth and formation of the reproductive organs, when plant communities and many of the representatives of soil mesofauna were most radiosensitive. The maximum biota exposure fell within the first 10–20 days after the accident when the major contributors to the absorbed dose were short-lived radionuclides. Immediately after the accident investigations of ecological and biological consequences of the Chernobyl accident were launched in the affected regions. These studies had revealed numerous facts of radiation damage to plants and animals.

In this review, only information from investigations, which provide detailed data on doses to biota species and contamination of the environment, has been collated. Altogether 250 references were reviewed, of which 80 articles were finally used for the review. The remainder papers were rejected because they did not contain adequate dosimetric information for sites where biological effects were studied.

There have been several reviews on ecological and biological effects observed following the ChNPP accident published at the international level (Sokolov et al., 1993; UNSCEAR, 1996; IAEA, 2006; Moller and Mousseau, 2006; Hinton et al., 2007). However, this paper for the first time brings together detailed data on biological effects in plants and animals inhabiting the areas affected by the accident at the Chernobyl NPP over twenty years. Furthermore, in the present review, based on the experience of Chernobyl NPP accident studies, attempts were made to critically evaluate minimal doses to biota species at which ecological and biological effects were observed.

The objectives of the present paper were: (i) to summarize the results from many long-term investigations, mainly published in the Russian language literature and (ii) to assess minimal doses to non-human species resulting in ecological and biological effects on different levels of biological organization for the conditions specific for the Chernobyl area.

2. Effects of ionizing radiation on plants

2.1. Forest trees

By the time of the accident, pine trees (*Pinus sylvestris* L.) aged 30–40 years dominated the forest stands of the 10-km Chernobyl NPP zone (Kozubov and Taskaev, 2002). High retention capacities of stand canopy with respect to radioactive fallout resulted in the fact that 60–90% of radionuclides falling on the forest were initially intercepted by the tree crowns (Tichomirov and Shcheglov, 1994). This led to high doses absorbed by the apical and leaf meristems, β-radiation being the main contributor to the absorbed dose. The rate of crown self clearing determines the value and duration of radiation exposure of forest trees. Within two months after the accident not less than 95% of the total radionuclide amount migrated from the canopy into the forest litter and accumulated during the following 7 year within the upper 3–5 cm soil layer (Tichomirov and Shcheglov, 1994). Even three years after the accident radioactive contamination in the forests of the 10-km Chernobyl NPP zone amounted to 1.45×10^5–4.1×10^5 kBq/m^2 (Tichomirov et al., 1993). Four zones were identified (Kozubov and Taskaev, 2002) varying in the extent of radiation damage to the forest:

1) zone of lethal effects, with an area of 600 ha, absorbed dose as of June 1, 1986 was 60–100 Gy. By the end of 1987, in addition to mass mortality of pine trees, the radiation damage to the crown of birch (*Betula pendula* Roth.) and black alder (*Alnus glutinosa* L.) was observed;
2) zone of sublethal effects (3800 ha), where 40–75% of trees dried, absorbed dose was 30–40 Gy. Necrosis of meristems and young shoots in 90–95% of pines was observed, together with the death of tree tops and suppression of growth;
3) zone of medium damage (11,900 ha), absorbed doses were 5–6 Gy. For this zone suppression of growth, partial abscission of needles on the shoot tops and damaged reproductive buds were typical;
4) zone of slight damage that covered the rest of the forest in the 30-km zone, absorbed dose was 0.5–1.0 Gy. Suppression of pine trees growth in some sites was observed, along with increasing by 10–12% of the number of hollow seeds in cones.

The first signs of the radiation injury in pine trees, yellowing and needle death, appeared within 2–3 weeks in close proximity to the Chernobyl NPP in an area of about 100 ha, where the absorbed dose to the needles and apical meristem exceeded 500 Gy. During the summer of 1986, the area of radiation damage expanded in the north-west direction up to 5 km, serious damage was observed at a distance of 7 km. Radiation burns and partial damage to pine bark were reported (Smirnov and Suvorova,

1996) at an external dose rate of 27 mGy/day. By the end of the vegetation period of 1986, in pines irradiated at absorbed doses of 10–60 Gy all shoots of the current growth, generative organs (male and female strobiles), most of dormant buds died; partial necrosis was observed in the needles of previous growth (Arkhipov et al., 1994). The mass yellowing of needles of pine aged 35–40 years was observed (Kozubov and Taskaev, 2007) at absorbed doses of 8–12 Gy, while in spruce of the same age this effect was observed at absorbed doses of 3.5–5 Gy.

In zone of sublethal effects, pine trees did not bear seeds for 5–7 years (Fedotov et al., 2006). Acute irradiation of the pine forest in 1986 at absorbed doses to the meristem of 10–12 Gy and more led to mass death of young cones and anthers, while the cones of the previous year of life continued their development and attained normal size at a dose of up to 25 Gy (Sokolov et al., 1994). The absorbed doses of 1–5 Gy significantly influenced the reproductive capacity of pine, which appeared as a reduced number of seeds per cone and increased fraction of hollow seeds (Fedotov et al., 2006). The period of acute irradiation at the early stage of the accident coincided with the micro- and macro-sporification, gametogenesis and early embryogenesis of pine. It had to be manifested in disorder of seed-buds of two generations (pine cones are developing for two years). Indeed, at a dose of 3.8–5.2 Gy partial female sterility was observed appeared as reduced gametophyte survival of seed-buds pollinated in 1986 and reduced embryonic survival of seed-buds pollinated in 1985 (Khromova et al., 1990). At doses of 7–9 Gy radiation effects appeared as growth inhibition of auxiblasts and needles.

The radiosensivity of Norway spruce (*Picea abies* L.) was observed to be greater than that of pines. Irradiation of spruce aged 25 years at doses of 8–10 Gy resulted in the death of young sprouts in 1986 and within 2–3 years in the death of most of the affected trees. In 40-year old spruce trees after an acute irradiation in 1986 at dose of 2.5–3.0 Gy the sprout mass was reduced by 40% and mass of 100 needles by 50% (Kozubov and Taskaev, 2007).

The deciduous forests in the Chernobyl NPP 30-km zone mainly consist of birch, aspen (*Populus tremula* L.), black alder and oak (*Quercus robur* L.). The deciduous species have proved to be by far the most tolerant to radiation exposure compared with conifers (Sarapult'zev and Geras'kin, 1993). Therefore, the radiation damage to the deciduous species crown was observed only in the immediate vicinity of the destroyed reactor at levels of radioactive contamination several times higher than for similar damage to conifers (Sokolov et al., 1994). Damage to birch and black locust (*Robinia pseudoacacia* L.) leaves was registered on plots where dose rate of γ-radiation was above 105 mGy/day (Smirnov and Suvorova, 1996). In birch, where the dose reached 500 Gy, young apical shoots partially died off, while by mid-August 1986, the foliage was mostly yellow and fell off (Sokolov et al., 1993). By the autumn, necrosis of some individual branches was recorded.

During the vegetation period of 1986, doses to plants and animals within forest ecosystems changed drastically. It was related with both decay of short-lived radionuclides and migration from the canopy into the forest litter and accumulation within the upper 1–3 cm soil layer of long-lived radionuclides. Up to 80% of the dose absorbed by forest trees was accumulated during the first month after the accident, the process of radiation injury of crowns of coniferous trees persisting till the autumn of 1986. Root systems of trees were not affected in the early period. Noticeable dying-off of thin roots began in the second half of 1987.

By the spring of 1987, the recovery of the affected trees (those which retained only a small fraction of their needles) began due to the growth of more resistant dormant cells partially protected from β-radiation by external tissues. Doses at which the recovery processes were observed amounted up to 50–60 Gy for Scots pine and 10–12 Gy for Norway spruce (Kozubov and Taskaev, 2002). Severely affected trees (15–20 Gy), because of the death of 1986 shoots, drastically reduced the mass of newly developed needles (Abaturov et al., 1991). The formation of 1.5–2.3 times larger needles than in the control and increase in their lifetime compensated for the losses. Decrease in the increment of shoots, compared to the control in 1987, was observed in the majority of trees starting from a dose of 0.43 Gy, a dose of 3.45 Gy caused full cessation of growth (Sidorov, 1994). The maximum drop in the growth increment was reported in the pine stands aged 40–50 and 50–60 years (Kozubov and Taskaev, 2007). In 1988–1989, it fell up to 40–45% of normal on exposure at doses of 20–25 and 40–60 Gy, respectively. Inhibition of tree growth and development processes was accompanied by a reduced photosynthesis and transpiration rate (Grodzinsky and Gudkov, 2006).

In the spring of 1987, shoots' development in irradiated trees was on average delayed by 10–12 days. The beginning of the recovery processes was accompanied by mass appearance (Abaturov et al., 1991; Arkhipov et al., 1994; Sokolov et al., 1994; Kozubov and Taskaev, 2007) of serious morphological disorders — changes in the shape and size of needles, development of "witch's brooms", shoot bundles, degradation of seed quality. The morphological deviations were most pronounced in the apical part of 10–12-year-old pines at absorbed doses of 32–80 Gy to the needles and 8–12 Gy in the apical meristem. Morphologically abnormal needles were distinct from the control in the content of total and individual proteins, organization of genome, peculiar features of its expression, karyotype and frequency of cytogenetic disturbances (Sorochinsky, 2003; Zelena et al., 2005). Specifically, twelve genes were significantly up- or down-regulated between normal and dwarf needles. Anomalous morphogenesis was accompanied by significant metabolic changes in cells (Sorochinsky, 2003).

Similar effects in the 5-km zone near the reactor have been reported in deciduous trees (leaf gigantism, changes in the leaf shape) (Arkhipov et al., 1994; Sokolov et al., 1994). In the spring of 1987, some male and female catkins branched, and were twisted in shape, and some of the anthers were necrotized. By mid-summer, the majority of birches acquired a peculiar coloration, the central part of the leaf lamina remaining green, while the peripheral portion became bright yellow. In the upper part of the crown, some very large dark-green leaves developed. During 1988, the birches regained their normal foliage.

Even 10 years after the accident radioactive contamination still directly affected the developmental processes of trees. In 1996, fluctuating asymmetry and the frequency of phenodeviants in black locust and rowan (*Sorbus aucuparia* L.) were positively related to the level of radioactive contamination (Moller, 1998). Since 1993, nearly 50–60% of all young coniferous trees aged 2–9 years from the highly contaminated sites of the Exclusion Zone have been demonstrating an increased level of abnormal morphogenesis (Zelena et al., 2005).

2.2. Herbaceous plants

Communities of herbaceous plants in the vicinity of the Chernobyl NPP are characterized by a wide diversity of species from different genera and families as well as by a wide variation of species-specific radiosensitivity. The deposition of radionuclides and associated doses to plants within the most contaminated sites in 30-km Chernobyl NPP zone were sufficient (Alexakhin et al., 2004) to cause mortality, sterility, and reduction in productivity of some species. The main contributor to the dose absorbed by plants in 1986 was β-radiation. Based on the instrumental measurements of γ-radiation exposure dose and absorbed dose of β- and γ-radiation, the contribution of external γ-radiation in agricultural and natural phytocenosis was found to be 5–10% of the overall dose (Alexakhin et al., 2004). Two thirds of those doses were received over the first month. The deposition of β-emitting nuclides onto critical plant organs resulted in significantly larger dose than animals living in the same environment.

When analyzing radiation effects on phytocenoses, the impact of ionizing radiation on reproductive systems needs to be estimated first. Winter wheat at an external dose rate of 1.6 mGy/day on day 15 after the accident exhibited a reduced number of seeds per ear, plant sterility reached 25%, yield capacity was 0.1 kg/m^2 (Smirnov and Suvorova, 1996). Reduction by half in the winter rye yield and partial sterility of grain at dose of 15 Gy over the first month was revealed (Suvorova et al., 1993).

Reduced number of racemes per bean plant (*Vicia faba* L.), reduced number of flowers in a raceme, flowers with changed color, flowers fallen on day 70, reduced number of seeds in beans and reduced productivity were found at absorbed doses of 2.5 Gy of external γ-radiation over 110 days of the growing season (Gudkov et al., 1999). Analysis of results from radiobiological experiments with this crop under controlled conditions indicates that the observed effect corresponds to doses of chronic γ-irradiation, at least 10 times higher of those registered in areas affected by the Chernobyl accident (Gudkov et al., 1999). These findings are in agreement with the information that currently in the Chernobyl zone internal exposure of plants and animals is responsible for 70–95% of total dose (Grodzinsky and Gudkov, 2006). That could be a reason of many misinterpretations in radiobiological studies where observed biological effects contradicted to dose levels at which such effects could potentially be registered. A significant contributor to the above disagreement is the fact that most of radionuclides taken up by plants are mainly concentrated in growth zones, where active cell division occurs. Therefore, real dose burdens to the most sensitive parts of plants, meristems, can be by one order of magnitude higher (Mikheev, 1999) than doses calculated based on the assumption of uniform radionuclide distribution within the plant tissues.

In wild plants partial or total sterility of seeds was observed (Suvorova et al., 1993) at doses for the first month following depositions ranging from 10 Gy (dandelion (*Taraxacum officinale* Wigg.) and arabidopsis (*Arabidopsis thaliana* L. Heynh)) to 40 Gy (vetch (*Vicia sativa* L.)). Clover (*Trifolium repens* L.), fireweed (*Chamaenerium angustifolium* L.) and silene (*Melandrium album* Mill.) from the 30-km ChNPP zone exhibited (Grodzinsky and Gudkov, 2006) some 30% increase in the fraction of non-vital pollen. Taskaev et al. (1992) failed to detect any significant differences in the germination and mass of 1000 seeds of 15 herbaceous species collected over the period of 1986–1989 in 12 sites within the 30-km ChNPP zone (γ dose rate in these sites in the autumn of 1986 varied within 0.05–192 mGy/day). In contrast to this study, Shershunova and Zainullin (1995) reported that quality of cocksfoot (*Dactylis glomerata* L.) seeds collected in 1986 on plots with contrasting contamination density (absorbed dose of external irradiation ranged from 0.01 to 20 Gy) showed a significant trend towards decrease in germination and mass of 1000 grains. The survey of wild vetch (*Vicia cracca* L.) populations in a plot with a dose rate of 0.4 mGy/day revealed prevalence of pods with 1–2 peas, the fraction of sterile peas reached 7% and fraction of embryonic lethals — 13% while in the control plot these values amounted to 4.5% and 3%, respectively (Smirnov and Suvorova, 1996). These findings are indicative of possible alterations in the species ratio in affected by radioactive contamination phytocenoses caused by damage to the reproductive sphere of the species most sensitive to irradiation.

Indeed, geobotanic examination in 1987 of natural herbaceous cenoses near a settlement of Yanov in the 30-km zone has shown that total numbers of plants reduced from 740 to 310 specimens per m^2 with increase in the dose rate of γ-radiation as of May 10, 1986 from 15 to 730 mGy/day (Suvorova et al., 1993). The numbers of some species declined along with the dose rate rise, whereas numbers of other species grew because of weakening of interspecies competition after disappearance of radiosensitive ones. A drastic cut in the number of herbaceous species in the second year following the accident (from 90 to 39 species) was observed at the dose rate of γ-radiation of 17 mGy/day on May 10, 1986. Species diversity, compared to the pre-accident year, was not restored by 1990; species abundance per 100 m^2 dropped approximately four times (Suvorova et al., 1993).

The phytocenoses status in the contaminated areas was influenced by loss in immune resistance in plants along with increase of virulence in some pathogens. The decrease in agricultural plants (wheat, rye, maize) disease resistance was shown (Dmitriev et al., 2007) in the course of field observations within the 10-km Chernobyl zone. Analysis of biochemical mechanisms underlying the decrease in disease resistance revealed a reduction in activity of proteinase inhibitors in plants. At the same time, the population structure of *Puccinia graminis* Pers., a casual agent of stem rust, had been changed in the 10-km Chernobyl zone by appearance of a new strain with high frequency of more virulent clones (Dmitriev et al., 2007). Brown rust and true mildew infection of winter wheat grew along with increase in the density of radioactive contamination. Low resistance to phytopathogen fungi remained in plants grown on uncontaminated soil.

An increased mutation rate in phytocenoses became apparent in 1987 as various morphological abnormalities. Morphoses were registered at reaching an external dose rate of γ-radiation of 4.2–6.3 mGy/day on May 10, 1986 (Suvorova et al., 1993). More frequently occurring abnormalities were morphoses such as fasciation and branching of stems, doubling, changes in racemes, color and size of leaves and flowers. Enhancement of vegetative mass of heather (*Calluna vulgaris* L.) and gigantism of some plant species were observed at external dose rates more than 24 mGy/day and 36 mGy/day, respectively (Smirnov and Suvorova, 1996). In ribwort plantain (*Plantago lanceolata* L.), both the rate of morphoses increased and seed productivity decreased along with the contamination density increase (Popova et al., 1992). Its seed reproduction in uncontaminated soil had revealed higher occurrence of morphoses, mainly in the raceme structure.

2.3. Genetic effects

A study of genetic processes in plant populations began in May 1986. Its main directions were as follows (Sokolov et al., 1993):

- study of the mutation process rate in plant populations as a function of dose rate;
- analysis of dose–effect relationship for different types of genetic disturbances (point mutations, chromosome aberrations in mitosis and meiosis);
- study of the mutation process dynamics in chronically exposed plant populations in several successive generations;
- analysis of microevolutionary and adaptation processes in exposed plant populations.

To estimate genetic effects in Scots pine populations from the accidental zone, mutation rate of enzyme loci in seed endosperm and chromosome aberration rate in seedlings and needles were used as criteria. In 1986, in most contaminated plots (absorbed dose of external γ-irradiation 10–20 Gy) frequency of induced enzyme loci mutations was 4–17 times and frequency of aberrant cells 1.5–7.2 times higher than in the control (Fedotov et al., 2006). Seedlings of Scots pine seeds of the 1987–1989 yield showed no differences in the frequency of chlorophyll mutations, though some morphologic mutations revealed noticeable abnormalities (Shevchenko et al., 1996). Genomic DNA of exposed pine trees was considerably hypermethylated (Kovalchuk et al., 2003). Moreover, hypermethylation appeared to be dependent upon the radiation dose absorbed by the trees. These findings suggest an essential role of epigenetic mechanisms in the formation of plant response to radiation exposure.

The links between both cytogenetic disturbances frequency and the mutation rate of enzyme loci and contamination densities were found to be supralinear (Fedotov et al., 2006), with the mutation rate per unit absorbed dose at low contamination levels of 5.3×10^{-3} (185–370 kBq/m^2 for ^{137}Cs) being 16 times higher than at high ones, 3.2×10^{-4} (14,800–20,350 kBq/m^2). In Scots pine populations exposed to doses above 5 Gy, gametic selection against some alleles was found, resulting in disorder in heterozygous trees and alteration of the genetic structure of next generations (Fedotov et al., 2006).

In 1987–1990, an increased yield of cytogenetic disturbances in needles was observed, which a declined more slowly than the contamination in the area (Sidorov, 1994). Similar results were obtained in study of biological effects on farm crops (Geras'kin et al., 2003). Extra exposure to γ-radiation of Scots pine seeds from the control and chronically treated populations revealed a radioadaptation effect based on the frequency of aberrant cells (Fedotov et al., 2006).

An aberrant cells frequency and number of aberrations per aberrant cell in seedlings root meristem of winter rye and wheat were increased along with the absorbed dose (Geras'kin et al., 2003; Suvorova et al., 1993). A significant excess of aberrations was registered from an absorbed dose of 3.1 Gy, inhibition of mitotic activity from 1.3 Gy, germination from 12 Gy, i.e. radiation damage to farm crops in 1986, according to the main tests, reminded the effect induced by acute γ-radiation at comparable doses.

In the autumn of 1989, aberrant cell frequencies in intercalary meristem of winter rye and wheat of the second and third generations grown at four plots with different levels of ^{137}Cs contamination (11.7–454 MBq/m^2) within the 10-km ChNPP zone significantly exceeded these parameters for the first generations (Geras'kin et al., 2003). The differences between cytogenetic indices obtained for the second and third generations were small and not statistically significant, so, the observed effect is not expressed below a threshold. It is important that cytogenetic disturbances were analyzed within the intercalary meristem of plants. It means that the overwhelming majority of radiation-induced alterations accumulated during the previous vegetation season were realized into mutations long before the samples were fixed for the cytogenetic analysis. Plants of all three generations were growing in identical conditions and were exposed to the same doses, so the most probable explanation of the registered phenomenon relates to a genome destabilization in plants grown from radiation-affected seeds. From these viewpoints, the results observed in this study and indicating a threshold character of the genetic instability induction may be a sign of an adaptation processes beginning, that is, the chronic low-dose irradiation appears to be an ecological factor creating preconditions for possible changes in the genetic structure of a population.

An essential role of genomic instability in the long-term consequences of radiation exposure is supported by the results on winter wheat in the 10-km ChNPP zone (Grodzinsky and Gudkov, 2006). In 1986, external doses absorbed by plants ranged from 9 to 20 Gy, resulting in a 60–80% plant fraction with morphological alterations; in 1987, the plant fraction with morphological alterations amounted to 60%. In plants grown from the seeds of the same generation on a control plot, morphological alterations (changes in linear sizes and shape, organs number, color, bushiness level, wax coating on leaves) were also frequent. The elevated level of mutagenesis persisted through many generations in both the 30-km zone and control plot.

During four years after the accident the frequency of aberrant cells in narrowleaf hawksbeard (*Crepis tectorum* L.)

significantly exceeded the control level and the relationship between cytogenetic damage and the radioactive contamination density was found to be nonlinear (Abramov et al., 2006). In 1987, on the plot in the 30-km zone with a dose rate of external irradiation of 4.8 mGy/day on May 1987, 6.2% of germs with an altered karyotype were observed at a chromosome aberration rate of 1.4%. In the period of 3–4 years after the accident a positive correlation was found between the aberration rate and frequency of seedlings with abnormal karyotype. The altered karyotypes were dominated by pericentral inversions and reciprocal translocations; several karyotypes with an extra (9th) chromosome were found. A nonlinear relationship between cytogenetic effect and dose rate was reported on the same plant within the East Urals Radioactive Trail, however, no altered karyotypes were observed (Shevchenko et al., 1998). These failed to be detected 7 years after the Chernobyl accident in the contaminated plots of the Bryansk region of Russia, whereas frequencies of aberrant cells on some plots were even higher than in the 30-km Chernobyl NPP zone (Shevchenko and Grinikh, 1995).

From 1986 until 1992 the frequency of embryonic lethal and chlorophyll mutations have been studied in populations of arabidopsis from the 30-km ChNPP zone (Abramov et al., 2006). In 1987, the dose of external irradiation on the study plots ranged 0.02–185 Gy (Abramov et al., 1992). Over the first 2–3 years after the accident, in spite of dose decline, the rate of embryonic lethal and chlorophyll mutations in populations was increased. In later years the frequency of lethal mutations declined, nevertheless the mutation rate in 1992 was by factors 4–8 higher than the spontaneous level. Throughout these 6 years study high doses induced fewer mutations per unit dose than low doses; that is, the effectiveness of irradiation decreases with increasing dose. Studies of the genetic structure of the arabidopsis populations have shown that high levels of radioactive contamination result in the impoverishment of genetic diversity of populations with time (Abramov et al., 1992). From this point of view radioactive contamination may be regarded as an ecological factor that potentiates the action of natural selection by eliminating radiosensitive genotypes. Seeds from these populations resisted higher concentrations of chemical mutagens and additional γ-ray exposures (Kovalchuk et al., 2004). Interestingly, the progeny of plants that were collected from the same experimental plots in years 1991 and 1992 were more resistant to mutagens than the progeny of plants collected in 1989 and 1990. Plants from experimental plots had (Kovalchuk et al., 2004) more than 10-fold lower frequency of extrachromosomal homologous recombination, significantly increased level of expression of radical scavenging and DNA-repair genes upon exposure to mutagens, and a higher level of genome methylation. These data suggest that adaptation to chronic radiation exposure is a complex long-term process. Although the mechanisms governing radioadaptation are not well understood, the data given by Kovalchuk et al. (2004) suggest that epigenetic regulation resulting in genome stability plays an important role in adaptation of plant population to chronic radiation exposure.

Thus, the above data allow the conclusion that the effects of plant communities observed in the Chernobyl affected areas were various: from enhanced rate of mutagenesis to damage at the ecosystem level. The severity of radiation effects was dependent on the dose received in the early period after the accident. Significantly exposed phytocenoses showed dose dependent alterations in the species compositions and loss of biodiversity. In the first years after the accident in a majority of populations from the Chernobyl zone an elevated level of mutagenesis was observed. In most cases the dose–effect relationship was supralinear and the yield of mutations per unit dose was higher at low doses and dose rates. In subsequent years the decline in the radiation background rate occurred faster than the reduction in the mutation rate. Populations of both woody and herbaceous plants showed signs of adaptation to chronic radiation exposure. From this point of view radioactive contamination may be regarded as an ecological factor that potentiates the action of natural selection by eliminating radiosensitive genotypes. In adaptation to the enhanced level of radiation exposure the epigenetic mechanisms of gene expression regulation play an essential role.

3. Effects of ionizing radiation on animals

Data on radiation effects in free-living animals after the Chernobyl accident are fragmentary and not as accurate as those for plants. It is connected with a mobile mode of life in most of animals, which noticeably complicates experimental works and simultaneously affects the accuracy of dose assessment. An additional source of complexity in the assessment of existing data on biological effects obtained in the field conditions arises particularly for many insects, because at different stages of the life cycle they occupy different environmental niches.

3.1. Soil- and entomofauna

The invertebrates comprise approximately 95% of all known species (Wilson, 1999), have unique physiological characteristics, and often are crucial components of ecosystems. The Chernobyl accident coincided with the most radiosensitive phase in the development of soil inhabitants: period of reproduction and molting of invertebrates after winter somnolency and spring warming up of the soil. Soil invertebrates are the least capable of migration. In the first years after the accident the majority of radionuclides are concentrated in the upper soil layer and forest litter. As a result, dwellers of forest litter were severely affected at a distance of 3–7 km from the Chernobyl NPP. Estimated from TLDs placed in the soil doses of about 29 Gy induced catastrophic (by a factor of 30) and doses of about 9 Gy noticeable (by a factor of 20) changes in the population density of forest litter community (Krivolutsky and Pokarzhevsky, 1992). In arable soils, even at absorbed dose on the surface of 86 Gy, soil animals were damaged relatively less (2–3 times, with no reporting in any of the animal groups of a catastrophic drop in the population size), probably because they were well protected against β-radiation by the soil layer (dose of β-radiation in the upper soil layer was 3–10 fold lower than in the litter), the main contributor to the total dose. Species composition was not affected as greatly as population size. Near the Chernobyl NPP, only 15 species of Oribatid mites, the most abundant soil-inhabiting group, were found compared to 25 species on the edge of the 30-km zone and 33 species on reference plot (Krivolutsky and Pokarzhevsky, 1992).

Due to enhanced radiosensitivity of the early stage of invertebrate development, radioactive contamination disturbed the process of reproduction of soil inhabitants. Among the inhabitants of pine forest litter, first instar larvae and nymphs failed to be detected. Adult earthworms are among the most radioresistant multicellular animals, but juvenile stages have the same LD_{50} as mice (Krivolutsky, 1983). As a result, near the Chernobyl NPP young earthworms (*Nicodrilus caliginosus* S., *Dendrobaena octaedra* S.) did not survive or hatch from cocoons in the autumn of 1986, although earthworms had bred in this period since cocoons were found in the soil (Krivolutsky and Pokarzhevsky, 1992).

In the first year after the accident, recovery of the resident populations of soil fauna was very slow, but size of populations was increasing due to migration of insects from surrounding areas. The abundance of earthworms was about 15% of the control but the presence of their cocoons suggested that they were reproducing even in the most contaminated areas (Sokolov et al., 1994). The total abundance of soil invertebrates in the forest litter was 45% of that of the reference site. Mesofauna was represented mainly by insect larvae, i.e. forms which could populate the damaged regions from outside by flight of adult insects. Within 2.5 years after the accident the population of mesofauna was almost completely restored in size but the species diversity of communities in the affected regions, even 10 years after the accident, was only 80% compared to that before the contamination event (Krivolutsky, 1996).

Some changes related to the succession of abandoned agrocenoses were observed in the insect phytophages, which are pests in agricultural crops. In an abandoned alfalfa (*Medicago sativa* L.) fields, the abundance and species diversity of plant-eating insects typical for this crop were drastically reduced (Sokolov et al., 1994). The same situation was observed in winter wheat fields. The abundance of bush crickets (*Tettigoniidae*) and locusts (*Acrididae*) markedly increased, suggesting floristic succession from agrocenoses to the meadow.

Increased levels of fluctuating asymmetry of morphological structures related with radioactive contamination were observed (Sokolov et al., 1994) in both Colorado beetle (*Leptinotarsa decemlineata* L.) and leaf beetle (*Chrysomela vigintipunctata* Scopoli). Enhanced levels of wing venation asymmetry in different species of dragonflies (*Odonata*) were reported as well (Sokolov et al., 1994). Comparison of dragonflies collected in sites differing in radioactive contamination has shown that the maximum level of fluctuating asymmetry of venation was observed in populations inhabiting sites with a middle level of contamination. In some dragonfly populations differences were found in the asymmetry of wing venation in males and females. In 1996, stag beetles (*Lucanus cervus* L.) from the contaminated area had a significantly increased level of fluctuating asymmetry in the secondary sexual character (length of horns) compared with both animals from the control area and collected before 1986 (Moller, 2002). Male stag beetles found with a female had significantly lower asymmetry than males found alone. While mated males did not differ in asymmetry between areas, unmated males from contaminated site were much more asymmetric than unmated males from the control area. Therefore, even 10 years after the accident the radiation exposure in some contaminated areas was strong enough to disrupt developmental processes, thereby affecting the mating status of free-living invertebrates.

3.2. Amphibians

In seven populations of brown frogs (*Rana temporaria* L.) inhabiting the contaminated regions of the Republic of Belarus the rate of aberrant metaphases and number of aberrations per aberrant cells in bone marrow were significantly higher compared with animals from the control area (Eliseyeva et al., 1994; Eliseyeva et al., 1996). The aberration spectrum suggests that radiation was responsible for an increase in the frequency of abnormalities. Radioactive contamination in the examined areas varied from 177 to 2331 kBq/m^2 for ^{137}Cs and 3.7 to 284 kBq/m^2 for ^{90}Sr. No decrease in the level of cytogenetic disturbances was found in 1989 compared to 1986. Only in the first years after the accident a relationship was observed (Voitovich and Afonin, 2000) between the level of cytogenetic disturbances in bone marrow cells and osteotropic radionuclides concentration in the body of animals. Till 1991, in all the populations of brown frogs from the contaminated regions the number of micronuclei was higher (Voitovich and Afonin, 2000) than in the control, in some cases up to 30 times. The decrease with time in the level of radioactive contamination was accompanied (Voitovich and Afonin, 2000) by a 5–6-fold increase in the aberration yield per unit dose, i.e. over 1990–1994 the decline in the proportion of aberrant cells in bone marrow did not correspond to the decrease of radiation exposure. Animals inhabiting the contaminated areas differed from the control in both the initial level of bone marrow cells with chromatin aberrations and the rate of apoptosis after an additional radiation exposure (Afonin et al., 1999; Afonin, 2002). The analysis of more than 2,500 frogs from 13 biotopes in 1988–1991 revealed (Voitovich, 2001) 7 animals with bone neoplasms, 5 being from the same habitat (environs of the Cherikov town, Mogilev Region, Belarus). In later years no tumors were registered. Judging by the size of animals with tumors, all of them at the moment of the accident were at the age of 1 year and in the phase of accelerated growth.

3.3. Hydrobionts

When assessing effects of radioactive contamination on hydrobionts fish are of greatest interest, they represent the most radiosensitive organisms of the cold-blooded hydrobionts. More than 30 different fish species inhabited the Chernobyl NPP cooling pond at the moment of the accident. Overall, lithophilous fish (spawning on rocky ground) are to be recognized as the critical group, among which the highest dose is received by predators. In the cooling pond these are pike-perch (*Lucioperca lucioperca* L.) and asp (*Aspius aspius* L.). According to Sokolov et al. (1994), the doses accumulated for 1990–1995 by some fish species amounted to 10–17 Gy.

Analysis of young pike-perch of the 1986 generation by the number of rays in thoracic fins demonstrated a level of fluctuating asymmetry by a factor of 30 higher than in the control. In the Kiev reservoir where exposure doses were noticeably lower, the phenotype variability of this fish species was within the species range (Ryabov, 1992).

Shortly before the accident a fish farm was established in the cooling pond of the Chernobyl NPP. Radiation exposure had resulted in increased occurrence of alterations in the reproduction system of fish inhabiting the cooling pond. By 1988, young silver carps (*Hypophthalmichthys molitrix* Valenciennes) which were in the live boxes of the cooling pond at the moment of the accident were reaching sexual maturity. The dose accumulated by silver carp during the period from 1986 to 1991 amounted to 9–11 Gy (Belova et al., 1993; Makeeva et al., 1994). 5.6% of silver carps were totally sterile (in the control — 0.25%), 15.4% demonstrated partial sterility (Belova et al., 1993). Asymmetric gonad development was observed in 11.2% of individuals (2.9% in the control). Amongst other alterations the following may be mentioned: testicular tubule wall destruction, accretion of connective tissue, destructive changes of sexual cells. The amount of fertilized spawn was 94%, abnormally developing spawn — 11%, female fertility was by 40% higher than the control but 8% of sires were sterile (Makeeva et al., 1994). ^{137}Cs concentration in ovulated spawn was 15 kBq/kg. Offsprings of silver carp exhibited growth retardation, enhanced variability in linear and weight indices, increased number of individuals with aberrant morphology of genital glands and cells, non viable abnormalities in oocytes, spermatogonia and spermatocytes, occurrence of bisexual and sterile individuals. In males the reproductive system disorders were more pronounced (Makeeva et al., 1994). The level of variability in the morphological parameters in offsprings of carp (*Cyprinus carpio* L.), white bream (*Blicca bjoerkna* L.) and silver carp were significantly higher than in parents (Pechkurenkov, 1991). In phytophilous fish, whose spawn received the major dose from aquatic plants that accumulate radionuclides, failure in the blood system (carp), reproductive system (perch and carp), as well as cytogenetic abnormalities (carp) (Polikarpov and Tsytsugina, 1995) and aneuploid-like patterns in the DNA histograms (catfish) were observed (Dallas et al., 1998).

Taking into account the long-term character of radioactive contamination of the bottom sediments, mollusks, playing an important role in the biological self-cleaning of water bodies, may be one of the critical hydrobionts in the Chernobyl NPP cooling pond. The maximum level of radionuclides concentrations was observed in mollusks from the warm zone of the reservoir, 410 Bq/g (Sokolov et al., 1994). In the populations of these organisms in 1986–1987 depression was revealed but by 1991 the situation was stabilized (Ryabov, 1992). On the other hand, investigations of the genetic and morphological differences among seven *Dreissena polymorpha* (Pallas) populations in the Chernobyl NPP cooling pond and adjacent water basins have shown that radiation failed to cause any significant effect on the population structure (Fetisov et al., 1992). Populations of the pond snail *Lymnaea stagnalis* L. inhabiting the Perstok lake, heavily contaminated by radionuclides (over the period from 1997 to 1998 the γ-activity of bottom sediments in the littoral region reached up to 2186–2996 kBq/m^2) were characterized (Golubev et al., 2005) by increased frequency of cells with micronuclei in hemolymph in comparison with populations of the pond snail from moderately contaminated inlet of the Pripyat river (over the period from 1997 to 1999 the γ-activity of bottom sediments in the coastal zone varied within 17.6–38.6 kBq/m^2). The embryonic mortality in the progeny of snails from the Perstok lake was lower and radioresistance of snails much higher than that in the Pripyat river. On the other hand, radioresistance of populations from the both reservoirs was considerably higher (Golubev et al., 2005) than that of *L. stagnalis* populations from noncontaminated lakes out of the 30-km ChNPP zone. So, clear evidence of radioadaptation was revealed in *L. stagnalis* populations from the both reservoirs.

Tsytsugina and Polikarpov (2003), when studying the oligochaeta (*Dero obtuse* Undekem, *Nais pseudobtusa* Piguet, *Nais pardalis* Piguet) reproduction in a reservoir of the Chernobyl affected zone near the settlement of Yanov (dose rate on the surface of bottom sediments was 0.34 mGy/day), evidenced a statistically significant relationship between the severity of cytogenetic damage in the worm population and the number of individuals switching to sexual reproduction. It is known that sexless reproduction, while maintaining valuable heterozygous types and thereby ensuring a high vitality, restricts the capacities of genetic variability and reduces potencies of populations in the changing environment. Adverse environmental conditions activate the sexual mode of reproduction which is associated with the increase of adaptive possibilities of the populations. Species with sexual reproduction have a higher rate of adaptation to ionizing radiation than species with prevalent asexual reproduction (Tsytsugina and Polikarpov, 2006).

3.4. Mammals

3.4.1. Mouse-like rodents

Mammals are the most radiosensitive group compared with other terrestrial animals. The level of radiation exposure in the most contaminated sites near the Chernobyl NPP in the spring of 1986 suggested that animals inhabiting this area most likely extirpated (Sokolov et al., 1993; Hinton et al., 2007). Indeed, during the fall of 1986, the number of small rodents on highly contaminated plots decreased by a factor of 2 to 10 (Taskaev and Testov, 1999). The numbers of animals had recovered by the spring of 1987, mainly due to migration from less affected areas.

Mouse-like rodents are the most abundant group of mammals occurring in the vicinity of the Chernobyl NPP. Populations of mouse-like rodents, because of their large numbers and fertility, rapid rotations of generations, as well as inhabiting top horizons of the soil where the highest doses are formed, are a suitable model to study radioecological effects. Moreover, in the absence of data on humans, small mammal populations may be one of the best models for evaluating the radiation risks for human populations. Therefore it is not surprising that most comprehensive information about effects of radiation on mammals was obtained on mouse-like rodents.

Small native mammals have a tremendous reproductive potential, and although there may be a shortened life expectancy and other health-related problems, a high population density could be maintained. The effects observed in populations of mouse-like rodents in areas with high levels of radioactive contamination resulted from the two different processes — decrease in the embryonic survival and increase in potential fertility of females due to an increase of ovulating ovicells. In the spring of 1986, decreased litters caused by embryonic mortality at contaminated plots averaged 15% of the control, which seemed to be directly dependent on the contamination level. In October 1986, embryonic mortality in bank vole (*Clethrionomys glareolus* Schreber) was on the average 34% (control 6%) (Sokolov et al., 1993). If in the autumn of 1986 in plots with an increased radiation background the birth rate was about 30% less than in the control plots, in the spring of 1987 this difference was only 12% (Sokolov et al., 1994). In subsequent years (1994–1995) no decrease in numbers or taxonomic diversity of small mammals even in most radioactive habitat was reported (Baker et al., 1996). Moreover, no valid data on an increased occurrence of teratogenesis in offsprings of small mammals were obtained in this study.

Materiy (1996) described the results of a long-term (1986–1992) study of the dynamics of damage and recovery of the hemopoietic system in tundra voles (*Microtus oeconomus* Pall.) from the 30-km ChNPP zone. By the period of animal catching in autumn 1986, the dose rate of γ-radiation on plot 4 (5 km southwards from the ChNPP) was 0.84–1.25 mGy/day and dose absorbed from external γ-irradiation was 1 Gy. Plot 6 was located 20 km to the south-west from the ChNPP; there the dose rate was 0.17 mGy/day, and the dose absorbed by animals — 0.02 Gy. The contribution of β-radiation to the total absorbed dose on these experimental plots was 2–5 times higher than that of γ-radiation (Ermakova, 1996). The contribution of incorporated radionuclides to the absorbed dose was by 1–2 orders lower than of external exposure. In 1987–1992, the levels of external exposure dropped considerably and the contribution of incorporated radionuclides to the absorbed doses increased. The mean values of ^{134}Cs, ^{137}Cs and ^{90}Sr concentrations for mammals in unremediated habitats in most affected by Chernobyl accident areas are among the highest ever recorded for free-ranging animals (Chesser et al., 2000).

The examined animals, despite a visual clinical well-being, exhibited numerous and diverse changes in both the state of red and white blood and internal organs. Noticeable alterations in haematogenic system were observed (Materiy, 1996) 6 months after the accident. These alterations persisted and even worsened during the following years, i.e. in several generations of animals. More than 20% of tundra voles showed pronounced symptoms of hyperchromic anemia. Animals had reduced content of haemoglobin and reticulocytes which is a typical manifestation of depression of erythropoiesis. The number of leukocytes per unit tissue volume in tundra voles from plot 4 exceeded that of the control almost twice in 1986 and by a factor of 1.5 in 1987 (Materiy, 1996; Sokolov et al., 1994). In later years (1988–1992) new generations showed a decrease in formed elements of white blood, up to 60% of normal. A similar moderate leukopenia was typical for small mammals from plot 6.

A hystologic analysis of bone marrow did not reveal any structural alterations capable of influencing its hemopoietic function (Ermakova, 1996; Materiy, 1996); however, the frequency of micronuclei was significantly increased compared to the control. The content of lymphoid cells in the white pulp was by 45–50% lower when compared with the physiological norm. A comparison of this finding with results of radiobiological experiments suggests that hystomorphological state of the spleen of tundra voles from the 30-km zone corresponds to the moderately severe radiation disease. Likewise, barn swallows from Chernobyl had depressed levels of several types of leukocytes and immunoglobulins, and reduced spleen mass compared with individuals from control areas, suggesting a reduced ability to cause an efficient immune response (Camplani et al., 1999).

Liver controls chemical hemostasis of the body, being thereby responsible for its resistance to unfavorable effects. Besides, liver cells are capable to accumulate latent damages which manifest themselves under activation of proliferation. The parenchyma in animals from the 30-km ChNPP zone had a mosaic pattern (Materiy and Goncharov, 1996). Investigations of tundra voles have shown simultaneous changes typical for the primary response on acute irradiation and tissue destruction characteristic for chronic radiation pathology. The increased number of binuclear hepatocytes in intact sites of tissue evidenced in this study indicates an enhancement of physiological regeneration processes. As a rule, in tundra voles, depending on age, binuclear hepatocytes amount normally to 12–18%, in animals from the 30-km zone this parameter amounted to 71–100% in 1989, later on — 65–92% (Materiy and Goncharov, 1996). Pathologic mitoses were observed in the liver of tundra voles. Till 1989, these abnormalities were mainly connected with chromosome aberrations, in later years — with pathology of the mitotic apparatus.

The morphological status of hepatocytes, the magnitude of antioxidation activity of lipids, the composition of phospholipids and the activity of dehydrogenases in the liver of field mice (*Apodemus agrarius* Pallas) and tundra voles caught in 1987 on 7 plots with different contamination level in the Chernobyl zone were studied by Shishkina et al. (1992). The animal habitats in terms of the ecological conditions were the same but varied in the level of external and internal exposure to γ- and β-radiation. The exposure dose rate varied depending on the site from 4×10^{-3} to 42 mGy/day. The contribution of β-emitters exceeded that of γ-emitters 34–37 times. Multiple destructive injuries, antioxydant exhaustion of lipids, drop in the fraction of phospholipids in the total lipid content and inhibition of dehydration processes were observed in the liver. There was no relation between the changes observed in the biochemical and biophysical parameters, as well as the severity level of degenerative changes in hepatocytes, and exposure dose rate. Even 5 years after the accident, the observed changes in the composition of liver phospholipids in tundra voles caught on the same plots persisted, albeit less pronounced (Kudyasheva et al., 2000).

Based on the analyses of the endocrine organs of 943 tundra voles from the 30-km zone, Ermakova (1996) demonstrated an increase in the general width of the adrenal cortex due to hypertrophy of the *zona fasciculata* generating glucocorticoid hormones and decrease in the size of the *zona glomerulosa* producing mineralcorticoid hormones. The appearance of cells was reported with pyknotic nuclei, increased mitotic activity of both *zona fasciculata* and *zona glomerulosa* cells, increased number of diploid and polyploid cells. Hypertrophy of the adrenal cortex was observed in the tundra voles from Chernobyl 30-km zone during 5 generations after the accident (Ermakova, 1996).

In the early years after the accident necrotic changes, mainly destructive, were observed in the thyroid as well as in adrenals. There were (Ermakova, 1996) mosaic sites of destructions, dystrophic changes, discomplexation of different tissue sites, local accumulations of lymphoid elements, hypertrophy and hyperplasia of cells, pyknosis of nuclei, i.e. signs of local damaging effect. In the first post-accident generation of tundra voles the number of degenerating follicles in the thyroid was increased. While in the early years after the accident the thyroid exhibited a high functional activity, in later years a trend was observed towards the organ division into actively- and hypofunctioning zones accompanied by the destruction of follicles and partial destruction of thyreocytes. No statistical relation could be drawn between the observed morphological alterations and the dose rate of external exposure on various plots in different years (Ermakova, 1996).

3.4.2. Agricultural and domestic animals

Following the Chernobyl accident the radiation damage to agricultural animals was caused by affection of the thyroid because of accumulation in it of radioactive iodine. Thus, 240 days after the accident, in cows from the Gomel region (Belarus) the ratio of absorbed doses from all sources of exposure between the thyroid, gastro-intestinal tract mucosa and whole body was 230:1.2:1 (Alexakhin et al., 1992). Doses received by farm animals were dependent on the density of radioactive contamination in their locations and residence time in the contaminated regions.

Doses to the gastrointestinal tract mucosa over the first month in cattle could reach 10 Gy in a few animals grazing in the 30 km zone of the ChNPP for 2–4 months after the accident, 7 Gy in tens of thousands of the evacuated animals and about 1 Gy in the rest livestock (Alexakhin et al., 2004).

An impaired thyroid function in cattle was related to the dose received (69% reduction in function with a thyroid dose of 50 Gy, and 82% reduction in animals which received a dose of 280 Gy) (Astasheva et al., 1991). In the evacuated cows 5–8 months after the accident increased lethality was observed. Others showed impaired immune responses, lowered body temperatures and cardiovascular disorders. The dissection revealed partial atrophy or total destruction of the thyroid, liver degeneration, increased amount of visceral fat, gall bladder and spleen enlargement, myocardium dystrophy (Alexakhin et al., 2004). Changes in the concentration of thyroid hormones and adenylatcyclase activity observed in cattle in the first post-accident year were of a reversible nature. The results obtained indicate the existence of a compensatory mechanism for the activation of cAMP system in animals with reduced secretion of thyroid hormones in case of thyroid damage (Shevchenko et al., 1990). The severity of radiation damage to the thyroid is connected with the content of stable iodine in the diet of animals. Thus, in sheep from the Belorussian Poliessie with a reduced level of iodine nutrition the thyroid entrapped much of radioiodine, thereby contributing to 2–2.5 times higher doses to this organ than in the control (Budarkov et al., 1992).

Five months after the accident sheep evacuated from the 30-km zone developed serious hematological alterations in the peripheral circulation (Alexakhin et al., 2004). Leukopenia was reported in 89% of animals and lymphopenia in 90%; 54% of sheep exhibited initial and marked anemia and 34% — serious inhibition of hemopoiesis.

Noticeable blood changes, mainly in the form of leukopenia and hyper- and normochromic anemia, were registered in pigs, dogs and cats captured in the 30-km zone in the summer and autumn of 1986 (Alexakhin et al., 2004). The dogs abandoned in the 30-km zone developed alterations typical for a chronic radiation disease in the visceral organs and tissues. Pathologies such as reduced mass of muscular and fat tissue, changes in the liver, kidneys, gut, stomach with hemorrhages and local necroses (liver, kidneys) appeared.

Offspring of highly exposed cows had reduced weight, decreased daily weight gains, disruptions of the hormonal status (Astasheva et al., 1991). Reproduction returned to normal in the spring of 1989. No valid data on an increased occurrence of teratogenesis in offsprings of the evacuated from the 30-km zone animals have been obtained.

3.4.3. Genetic effects

The yield of chromosome aberrations in bone marrow cells of bank voles and, to a lesser extent, embryonic lethality was correlated (Ryabokon and Goncharova, 2006) with the radionuclide contamination of the five monitoring sites in Belarus (^{137}Cs contamination of soil from 8 to 8500 kBq/m^2). The increased levels of chromosome aberrations and frequency of embryonic lethality appeared to remain relatively constant over 22 animal generations within 10 years following the Chernobyl accident (1986–1996), although in the same period of time the whole-body absorbed dose rate decreased exponentially with a half-time of about 2.5–3 years (Ryabokon et al., 2005). Offsprings of females captured in these sites, grown up under contamination-free laboratory conditions, showed equal enhanced level of chromosome aberrations. This suggests that the increased level of cytogenetic disturbances depends not only on the actual exposure level. Before the accident, only chromatid-type aberrations were observed, and the cells never contained multiple aberrations. In contrast, in the post-accident period, the chromosome anomalies consisted of both chromatid- and chromosome-type aberrations as well as Robertsonian translocations (Ryabokon and Goncharova, 2006).

All populations studied demonstrated unexpectedly high frequencies of polyploid cells in the bone marrow of bank voles, 1–3 orders of magnitude above the pre-accident level (Ryabokon, 1999). A relationship was established between their frequency and the concentration of incorporated in the body of bank voles radionuclides. A statistically significant increase with time was found in the rate of genome mutations up to 1991, inclusive, i.e. up to the 12th post-accident generation of animals. In addition, an increased frequency of micronuclei in polychromatid erythrocytes of bank voles caught on these plots in 1996 (i.e. in the 21st–22nd generations of animals) was observed. In contrast to this study, Rogers and Baker (2000) reported that frequency of micronuclei in polychromatid erythrocytes of bank voles living in the highly contaminated areas around the Chernobyl nuclear power plant were not significantly different from that in reference populations. These observations demonstrate a need to document more thoroughly the frequencies of chromosomal damage in chronically exposed animals from the vicinity of the Chernobyl nuclear power plant.

The comparative analysis of the frequencies of genetic disturbances (embryonic mortality, frequency of abnormal sperm heads, reciprocal translocations and heterozygotes for recessive lethal mutations) in several generations (1986–1991) of house mice (*Mus musculus* L.) inhabiting sites with different levels of radioactive contamination within the 30-km Chernobyl NPP zone was carried out (Pomerantseva et al., 1997). The dose rates of γ-radiation on the soil surface ranged from 0.05 to 48 mGy/day. The total radionuclide content per mouse immediately after the capture in 1986 in the most affected site reached 6000 Bq (Pomerantseva et al., 2006). The dose of internal radiation did not exceed 10% of the dose of external radiation. Maximum total doses absorbed by gonads did not exceed 3–4 Gy per month in 1986–1987 and gradually decreased as the time passed.

No apparent symptoms of acute radiation sickness were observed (Pomerantseva et al., 1997). From 17 males caught in the most contaminated plot, only two were irreversibly sterile. For part of the males from this plot, a period of temporary sterility was observed. The testes mass in the animals from the most contaminated plot was significantly reduced (56.7 mg when compared with 117.3 mg in males from the same plot but examined later). No significant differences in the testes mass were observed in the males from other plots. On plots with a high level of radioactive contamination the yield of chromosome aberrations and cells with micronuclei in polychromatid erythrocytes (PCE) of the bone marrow was higher; however no dose–effect relationship was statistically established. Frequency of cells with micronuclei in PCE was approximately by an order lower than that with chromosome aberrations. The frequency of cells with micronuclei in all the studied types of somatic cells was of the same order, though when analyzing normochromatic erythrocytes of the peripheral blood one could expect accumulation of damages over the entire exposure period, as opposed to PCE cells of the bone marrow where effect was caused by the dose received over one cellular cycle. Differences in the level of plot contamination were markedly higher than those in the effects observed.

Embryonic mortality was increased only in the offsprings of males caught on the most contaminated plot (total doses absorbed by gonads of house mice in this plot in 1986–1987 did not exceed 3–4 Gy per month). No noticeable differences were reported in the rate of abnormal sperm cell heads in males depending on the contamination level and time (Pomerantseva et al., 1997). The frequency of reciprocal translocations in mouse spermatocytes was relatively low, but increased with the dose rate. The frequency of heterozygotes for recessive lethal mutations decreases with the time after the accident. This fact suggests that the relatively high level of heterozygotes for recessive lethal mutations observed in 1986 and 1987 is partly due to mutations induced by a drastically increased radiation background. Heterozygotes for recessive lethal mutations are not accumulated in the populations with time, despite chronic exposure to radiation. This fact may be explained by selective elimination of cells with genetic disturbances that can purify the population of excessive mutations.

Among tundra voles, bank voles and field mice caught on four plots of the 30-km zone (in 1986, dose rate of external γ-radiation in these plots amounted to 0.006, 0.84, 8.4 and 63 mGy/day) the maximum frequency of abnormal sperm cell heads was observed in the first two years after the accident (Zainullin et al., 1994; Rakin and Bashlykova, 1996). The frequency of micronuclei in 1986–1989 was also significantly higher than in the following years. Only in that period abnormalities such as multiple micronuclei (up to 10–14 per cell), nucleus pulverization and focal injury of cells were observed. The maximum frequency of these disturbances was registered in tundra voles, the minimum — in field mouse. Within 5–6 years after the accident the frequency of genetic disturbances in germ and somatic cells fell to a spontaneous level.

A comparison of mutation spectra in three vole species captured in the 30-km ChNPP zone has revealed that the evolutionary younger species, common vole (*Microtus arvalis* Pallas), was characterized by a higher occurrence of aneuploid cells, bank vole — metaphases with Robertsonian translocations (Kostenko et al., 2001). The evolutionary ancient species, tundra vole, demonstrated a relative stability of the chromosomal apparatus. On the other hand, Baker et al. (1996) did not observe in 1994–1995 an abundance of atypical chromosomes in small mammals even in most contaminated habitat. In 1997–1998, genetic diversity of bank vole populations from highly contaminated sites of the 10-km zone was investigated (Matson et al., 2000). According to dose estimates, bank voles inhabiting the Red Forest site received dose rates as high as 86 mGy/day. The number of unique haplotypes and the mtDNA gene diversity (0.72 ± 0.024) in the Red Forest population were significantly higher than in the reference population (0.62 ± 0.068). However, without further information, it is difficult to tell whether this difference was due to environmental contamination or some other selective pressure.

The analysis of the progeny of a bull and three cows found in the 30-km zone one year and a half after the accident and later kept on an experimental farm located 10 km from the sarcophagus in the area with a contamination density of 7400 kBq/m^2 for ^{137}Cs has shown significant deviations in the transmission of allele variants of some genes from parents to offsprings (Glazko, 2001). The prevailing allele variants were not typical for highly specialized Holstein breed but for a more primitive, however more tolerant to unfavorable reproduction conditions, breeds such as Gray Ukrainian. That means that, under these conditions, radiation exposure manifested itself as a selection factor causing changes in the frequency of occurrence of non-mutant genes rather than by the induction of new mutations. An adaptive character of genetic alterations in populations of common vole and bank vole inhabiting severely contaminated plots (185; 7400; 18,500 and 37,000 kBq/m^2 for ^{137}Cs) in the ChNPP Exclusion Zone was confirmed (Glazko et al., 2006). Populations inhabiting plots with the maximum level of radioactive contamination mainly consisted of animals in which frequency of occurrence of cytogenetic disturbances did not exceed the control level. Intensity of selection for radioresistance depended on the contamination level and was not observed in populations inhabiting sites with the contamination density below 7400 kBq/m^2 for ^{137}Cs. An increased numbers of radioresistant animals were first discovered only in 1999, i.e. in the 26th generation of voles survived in the Chernobyl accident.

4. Lessons learned

An assessment of the state of plant and animal populations inhabiting polluted territories and the analysis of mechanisms of their adaptation to adverse environmental conditions undoubtedly has general biological importance. Only information from natural settings can improve our ability for evaluating of the consequences of climatic change or environmental perturbations resulting from severe man-caused disasters. Consequently, studies that examine biological effects on non-human biota in natural settings provide a unique opportunity for obtaining information about the potential biological hazard associated with radioactive contamination. Nevertheless, up to now there is a clear lack of quantitative data on the real long-term biological consequences of chronic radiation exposure (Brechignac et al., 2003). Actually, few studies exist that are directly relevant to understanding the response of plant and animal populations to radionuclides in their natural environments.

A characteristic feature of large radiation accident is the presence of two periods — intensive short-term radiation impact and subsequent long period with a slow decline in the dose rate. The most severe ecological impact is caused by exposure in the early period after the deposition. In the radiation affected ecosystems two groups of effects are identified (Alexakhin et al., 2004). Irradiation of plants and animals with lethal and sublethal doses (primary effects) results in the disruption of ecological relations between the components of ecosystems and in further (secondary) disturbances. The disturbances of ecological interrelations are induced by the following factors: (1) changes in microclimatic and edaphic conditions (in affected coniferous forests, because of improvement of both light and mineral nutrition condition, more radioresistant deciduous species actively develop); (2) disturbances in the synchronism of seasonal phases in the development of ecologically connected groups of organisms (shifts in the time of leaves blossoming and eggs of leaf worms hatching); (3) imbalance in food interrelations between consumers and producers (decrease in food resources as a result of irradiation); (4) changes in biological pressure as a result of species differences in radioresistance (changes towards prevalence of more radioresistant species in meadow phytocenoses; disturbances in both host-parasites and predator–pray relationships); (5) induced by ionizing radiation changes in affected communities make open ecological niches for immigration of new species.

It follows from the above analysis that effects induced by radiation in natural and agricultural ecosystems are dependent on the radiosensitivity of dominant species. Coniferous trees should be mentioned among the most radiosensitive plant species, mammals are the most radiosensitive representatives of animal species while coniferous forests are the most sensitive communities at the ecosystem level. Radiation effects at a biocenotic level begin from the dose that induces disappearance of the most radiosensitive species (e.g., conifers death). Such an ecological change (mass mortality of pine trees) is reported at an absorbed dose of 60–100 Gy over the "acute" period (Arkhipov et al., 1994; Tichomirov and Shcheglov, 1994). Death of weakened coniferous trees and mass yellowing of needles are observed under exposure to lower doses: spruce — 4–5 Gy (Kozubov and Taskaev, 2007), pine — 8–12 Gy (Arkhipov et al., 1994; Kozubov and Taskaev, 2007). Doses at which the recovery processes were observed amounted to 50–60 Gy for Scots pine and 10–12 Gy for Norway spruce (Kozubov and Taskaev, 2002). Death of shrubs and deciduous trees occurs at doses above 200 Gy (Smirnov and Suvorova, 1996).

Reduced numbers of plants per m^2 and species diversity were observed in herbaceous phytocenosis in 1987 starting from γ-exposure dose rate of 17 mGy/day (Suvorova et al., 1993), enhancement of vegetative mass of heather (*Calluna vulgaris* L.) and gigantism of some plant species were observed at external dose rates more than 24 mGy/day and 36 mGy/day, respectively (Smirnov and Suvorova, 1996). Decrease in the population density and species composition of forest litter mesofauna is observed at dose of about 9 Gy (Krivolutsky and Pokarzhevsky, 1992). On the other hand, no decrease in population size or taxonomic diversity of small mammals even in most contaminated habitat was reported (Baker et al., 1996). This may be partly due to a tremendous reproductive potential and rapid rotation of generations in populations of small mammals.

Inhibition of reproductive capacity in pine is observed at doses 1–5 Gy (Fedotov et al., 2006). Higher occurrence of the reproduction system alterations as well as reduced viability of progeny of silver carp is observed at dose of 9–11 Gy over 5 years (Belova et al., 1993; Makeeva et al., 1994). The significantly reduced testes mass as well as irreversibly or temporary sterility in part of males from mouse-like rodents populations was observed (Pomerantseva et al., 1997) at absorbed doses by gonads of 3–4 Gy per month.

Morphological changes in pine needles and underwood of deciduous trees in 1987 were observed starting from dose of 0.1–1.0 Gy (Arkhipov et al., 1994; Kozubov and Taskaev, 2002) and in herbaceous plants from dose of 1.5–2.3 Gy (Suvorova et al., 1993). Destruction of thyroid as well as chronic radiation disease in agricultural animals is observed at thyroid doses above 200 Gy (Budarkov et al., 1992; Alexakhin et al., 2004). Doses of 3–6 Gy resulted in multiple destructive alterations in both the hemopoietic system and the internal organs of mouse-like rodents (Materiy, 1996; Ermakova, 1996; Materiy and Goncharov, 1996).

In Fig. 1 effects of non-human species irradiation observed in the conditions of the 30-km Chernobyl exclusion zone were compare with the estimated no effect values recommended by the International Atomic Energy Agency (IAEA, 1992) and the Canadian Nuclear Safety Commission — CNSC (Bird et al., 2003). It is easy to see that at doses below those recommended by the IAEA as safe for plants (3.65 Gy/year), within the

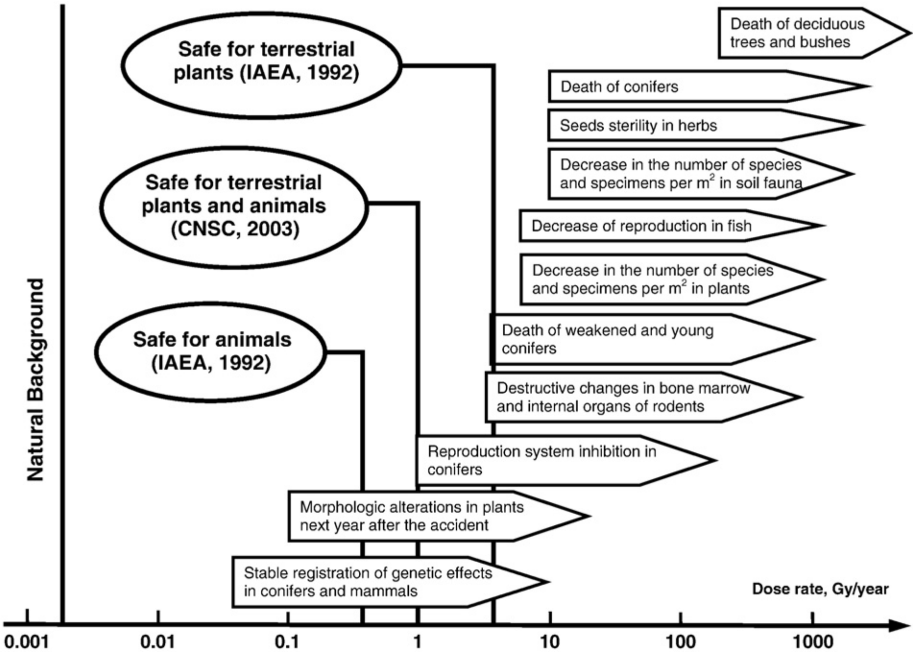

Fig. 1. A comparison of effects observed on non-human species in the conditions of the 30-km Chernobyl Exclusion Zone with estimated no effect values recommended by International Atomic Energy Agency and Canadian Nuclear Safety Commission.

Chernobyl exclusion zone, some morphological changes in different plant species (0.1–2.3 Gy/year) and the reproductive function inhibition in conifers are observed. In this sense a dose limit to plants of 1 Gy/year suggested by the CNSC seems to be more valid. Exposure to lower doses in the conditions of the area affected by the Chernobyl NPP accident results only in morphological changes in the most sensitive plant species and persistent genetic effects in the most sensitive biota species.

Table 1 briefly summarizes the minimum doses at which effects on non-human species were observed in the Chernobyl NPP surroundings. In addition, in the last column of the Table 1 estimated doses at which effects should not be observed for the conditions specific for the Chernobyl area are presented. These doses are established by the method of expert estimates. This approach used to estimate dose thresholds that do not cause reliable registered biological effects of radiation exposure has been described in more details in our previous publication (Fesenko et al., 2005).

It should be noted that even analysis of such an extensive information on biological effects observed in close vicinity of the ChNPP in the early years after the accident gives no way to estimate with a satisfactory accuracy the minimum (maximum) doses at which effects were (were not) observed. The reasons are:

1. Extreme heterogeneity of radioactive contamination of the territory, long period of intensive release of radionuclides and variable meteorological conditions at that time, wide spectrum of deposited radionuclides, noticeable “spottiness” (in both amount and spectrum of radionuclides) of deposition. These factors were responsible for essential heterogeneity of dose in both space and time.
2. The uncertainty in dosimetric data given for the observed biological effect. In the course of the crucial (in terms of biological effects forming) early period radiation monitoring was insufficient. Therefore, and because of rapid changes of doses to biota species in that period (decay of the short-lived radionuclides, radionuclide redistribution in the ecosystems, changes in contributions of exposure pathways, impact of the hot particles), estimations of doses absorbed by biological objects were mainly rough.
3. Estimation of radiation effects is also complicated by the lack of verified methods for reconstruction of absorbed doses to living organisms due to a complexity of organization and nonstationary of biological systems. Biological objects are characterized by a huge variety of sizes, shapes, rations, behavioral responses and occupied ecological niches, which result in orders of magnitude differences in resulting doses and in the types of affected tissues and organs in the same radiological situations (Fesenko et al., 2005). Living beings in the course of individual development undergo different stages, the sensitivity of which varies by orders of magnitude (Sarapult’zev and Geras’kin, 1993). During ontogenesis the geometry and conditions of irradiation vary significantly, which also makes dose estimation difficult and makes necessary to consider ecological and physiological peculiarities of organisms’ development.

Radiation levels in the 30-km zone are still much higher than those considered safe for human habitation. Because of their particular use of the habitat, plants and animals inhabited affected by ChNPP accident areas may receive doses of radiation exposure that substantially greater than those of humans occupying the same area (Fesenko et al., 2005). Nevertheless wildlife appears to have been relatively unaffected by the radiation and has therefore benefited from the absence of human disturbance and damage through agriculture, forestry, hunting,

Table 1
Doses or dose rates to biota that do not induce the considered effects, estimated based on the Chernobyl NPP accident studies

Species Effects	Minimum doses (or dose rates) at which effect was observed	Estimated maximum doses (or dose rates) at which effect should not be observed
Forest trees		
Scots pine. Death of weakened trees	8–12 Gy (Arkhipov et al., 1994; Kozubov and Taskaev, 2007)	5 Gy
Mass death of young cones and anthers	10–12 Gy (Sokolov et al., 1994)	5 Gy
Mass yellowing of needles, trees 35–40 years old	8–12 Gy (Kozubov and Taskaev, 2007)	5 Gy
Inhibition of reproductive capacity (reduced number of seeds per cone and increased fraction of hollow seeds)	1–5 Gy (Fedotov et al., 2006)	0.5 Gy
Morphological disturbances one year after the accident	0.1–1.0 Gy (Arkhipov et al., 1994)	0.05 Gy
Significant increase in cytogenetic effects in seedlings and needles	0.5 Gy (Fedotov et al., 2006)	0.05 Gy
Frequency of mutations of enzyme loci in seed endosperm	0.07 Gy (Fedotov et al., 2006)	0.01 Gy
Spruce. Death of trees 10–15 years old	4–5 Gy (Kozubov and Taskaev, 2007)	1 Gy
Trees 25 years old. Dying-off of young sprouts. Mortality of much of the trees within 2–3 years	8–10 Gy (Kozubov and Taskaev, 2002)	5 Gy
Trees 40 years old. Noticeable reduction in sprout mass	2.5–3 Gy (Kozubov and Taskaev, 2002)	1 Gy
Mass yellowing of needles	3.5–5 Gy (Kozubov and Taskaev, 2002)	2 Gy
Herbaceous plants		
Reduced numbers of plants per m^2 and species diversity in the next year after the accident	17 mGy/day (Suvorova et al., 1993)	10 mGy/day
Morphological changes	4.2–6.3 mGy/day (Suvorova et al., 1993)	2 mGy/day
Enhanced vegetative reproduction	24 mGy/day (Smirnov and Suvorova, 1996)	10 mGy/day
Gigantism of some species	36 mGy/day (Smirnov and Suvorova, 1996)	15 mGy/day
Sterility of seeds	40 Gy — vetch; 10 Gy — dandelion and arabidopsis (Suvorova et al., 1993)	5 Gy
Decrease in the number of peas in pods of wild vetch, increase in both fraction of sterile pods and fraction of embryonic lethals	0.4 mGy/day (Smirnov and Suvorova, 1996)	0.1 mGy/day
Animals		
Soil mesofauna. Drastic decrease in the population density and species composition of forest litter mesofauna	Dose absorbed on the soil surface 9 Gy (Krivolutsky and Pokarzhevsky, 1992)	1 Gy
Amphibians		
Brown frogs. Increased yield of chromosome aberrations and damage severity in aberrant cells	Dose rate, mGy/day: 0.01 from ^{90}Sr to bone tissue, 0.038 from other sources to the whole body, 0.013 from external γ-radiation (Eliseyeva et al., 1994; Eliseyeva et al., 1996)	0.01 mGy/day
Hydrobionts		
Silver carp. Higher occurrence of the reproduction system alterations, reduced viability of progeny	9–11 Gy for 5 years (Belova et al., 1993; Makeeva et al., 1994)	1 Gy/year
Mammals		
Mouse-like rodents. Inhibition of reproductive capacity (the significantly reduced testes mass as well as irreversibly or temporary sterility in part of males)	Doses absorbed by gonads was 3 Gy per month (Pomerantseva et al., 1997)	1 Gy/year
Pathologic changes in hemopoietic system, liver, adrenals and thyroid	Dose absorbed from external γ-radiation from the moment of accident till animal catching in autumn 1986 was 1 Gy. Contribution of β-radiation was 2–5 times higher than γ-radiation, internal irradiation from incorporated radionuclides was 1–2 orders lower than from external radiation (Materiy, 1996; Ermakova, 1996)	0.5 Gy
A dose-dependent increase in the frequencies of chromosome aberrations in bone marrow cells and embryonic losses in bank voles, high frequency of polyploid cells and genome mutations	Whole-body absorbed dose rate in 1986: 6.44–605.46 μGy/day (Ryabokon and Goncharova, 2006)	5 μGy/day
Cows. Destruction of thyroid, chronic radiation disease	Doses absorbed by thyroid>200 Gy, with dose to the whole body being no more than 0.2 Gy (Budarkov et al., 1992; Alexakhin et al., 2004)	20 Gy to thyroid. Effect in the early days after the accident was mainly determined by ^{131}I action and depended greatly on content of stable iodine in animal ration

fishing and so on. Moreover, for some years after the accident, the agricultural fields still yielded domesticated produce, and many animal species, especially rodents and wild boars, consumed the abandoned cereal crops and potatoes as an additional food source. It has resulted in a considerable growth in the populations of wild animals in the 30-km ChNPP zone. By the spring of 1988 the population of wild boars in the 30-km ChNPP zone was 8 times in excess that of the pre-accidental level, the populations of elk, deer, stork, wolf, fox and mouse-like rodents increased manifold (Sokolov et al., 1994; Baker and Chesser, 2000). Similar trends are characteristic for other species. To date, the Chernobyl exclusion zone has become a breeding area for white-tailed eagle, spotted eagle, eagle owl, crane and black stork. In this respect, the most severe nuclear power plant accident in the history had positive ecological consequences.

On the other hand, physiologic degradation and loss of immunity in plants and animals in the affected area has activated a nidus of infectious diseases such as tularemia, encephalitis and fungal pathogens. Forest and fruit trees in the 30-km ChNPP zone were seriously injured by pests (Krivolutsky, 2000). Within this zone accelerated development of new phytopathogenic forms, races with enhanced virulence, accelerated horizontal transfer of genes among different species of microorganisms became possible (Dmitriev et al., 2007). These pathogens could be easily exported out of the contaminated areas. Therefore, the 30-km ChNPP zone may be an environmental hazard and the ecological processes within the zone need to be monitored.

To date, plant and animal populations inhabiting the 30-km ChNPP zone demonstrate high levels of mutagenesis (Baker and Chesser, 2000; Kovalchuk et al., 2004; Golubev et al., 2005), morphologic anomalies (Sorochinsky, 2003) and fluctuating asymmetry (Oleksyk et al., 2004; Moller, 2002), which can lead to a decline in adaptation and reproductive potential in organisms comprising the populations (Ellegren et al., 1997; Moller and Mousseau, 2003). Despite the increase in many mammalian population numbers and diversity, estimated dose rates in some areas of the 30-km ChNPP zone (about 10 mGy/day) exceed those reported to impede reproductive success in mammals (Chesser et al., 2000). These data suggest that some areas of the 30-km ChNPP zone may be reproductive sinks for mammal populations, and densities are maintained by immigration from nearby habitats with lesser levels of contamination. In a similar way, studies of the barn swallow have shown (Moller et al., 2005) significant negative relationships between the dose rate level and the fraction of non-breeding females, clutch size and hatching success. Reduced adult survival and reproduction suggests that extant populations of these bird species in this area clearly cannot be sustained without immigration. Actually, measurements of stable isotope composition of feathers have shown (Moller et al., 2006) that the rate of immigration of barn swallow was considerably greater into Chernobyl zone than into control areas, but only after 1986.

The removal of human residents complicates any examination of population-level effects to wild life at the 30-km ChNPP zone. Nevertheless, there are well-known facts of adaptation to radiation of plant and animal populations to the changed conditions of the 30-km ChNPP zone (Kovalchuk et al., 2004; Golubev et al., 2005; Fedotov et al., 2006; Glazko et al., 2006; Tsytsugina and Polikarpov, 2006). Moreover, an opinion exists (Glazko, 2001), that under conditions of the 30-km ChNPP zone the selection plays in favor of the least specialized primeval genotypes. Overall, the above data suggest that adaptation to chronic radiation exposure is a complex long-time process which includes interconnected changes at different levels of biological organization.

Far less evident are the genetic consequences for biota inhabiting areas with enhanced level of radioactive contamination. Levels of contamination and dose rate estimates reported (Chesser et al., 2000; Ryabokon et al., 2005) would be expected to confer considerable insult to genetic material by ionizing radiation. Whether the observed levels of genetic anomalies in plants and animals inhabiting areas affected by Chernobyl accident have any detrimental biological significance to populations is still not known. A study of the population-genetic consequences of the Chernobyl accident is directly connected with the assessment of the adaptive capacities of biota and comparison of these with the pace of environmental changes. The experience of ecological research points to the increase of the phenotypical variability in natural populations under technogenous influence of which the genetic nature as well as dynamics within generations remain poorly known. Mutations with slightly negative fitness effects could be easily exported out of the contaminated areas via the organisms' migration, with consequences for the populations that have not been directly exposed to radiation from the accident. Although these studies are well documented, the role of microevolutionary processes in natural populations' response to low-level chronic exposure is still not clearly understood. Further long-term observations are required in order to understand exactly the dynamics of a mutation burden to natural populations inhabiting areas with enhanced levels of radioactive contamination. Much has yet to be learned before we will be able to give an objective and comprehensive assessment of the genetic consequences of the Chernobyl catastrophe for natural plant and animal populations.

Finally, large-scale radioecological studies performed in the areas affected by the largest nuclear power plant accident in the history have made it possible to obtain unique information on responses of living nature at different levels of biological organization, from molecular–cellular to ecosystem, in conditions of wide-ranging and extremely heterogeneous radioactive contamination of wide areas. These findings make a valuable contribution to scientific and public understanding of the environmental risks of ionizing radiation and to debates on the environmental costs, benefits and risks of nuclear energy. Within the 30-km Chernobyl NPP zone, where humans are absent, unique ecosystems are developing, where representatives of numerous taxonomic groups of biota are reproducing against the background of drastic changes in the range of ecological factors. The investigations described in this review raise a number of key questions regarding the long-term effects of anthropogenic pollution on natural populations; their findings widen our knowledge of the nature of adaptation processes. The 30-km ChNPP zone has become a unique test site, where in natural conditions long-term ecological and biological consequences are studied of a dramatic change in the complex of environmental factors, trends and intensity of selection.

Acknowledgements

The authors are very grateful to anonymous reviewers for their patient reading of the manuscript and their invaluable comments. This work was partly supported by the ISTC project No. 3003.

References

Abaturov YuD, Goltsova NI, Rostova NS, Girbasova AV, Abaturov AV, Melankholin PN. Some peculiar features of pine radiation damage in the Chernobyl affected region. Russ J Ecol 1991;22(5):28–33.

Abramov VI, Fedorenko OM, Shevchenko VA. Genetic consequences of radioactive contamination for populations of *Arabidopsis*. Sci Total Environ 1992;112:19–28.

Abramov VI, Rubanovich AV, Shevchenko VA, Shevchenko VV, Grinikh LI. Genetic effects in plant populations in the zone of the Chernobyl accident. (in Russian)Radiation Biology. Radioecology 2006;46:259–67.

Afonin VYu. A comparative analysis of apoptosis and cytogenetic disturbances in heterogeneous cellular populations of hemopoietic tissue of animals from regions of Belarus different on ecological conditions (in Russian). Candidate thesis. Minsk; 2002.

Afonin VYu, Voitovich VYu, Eliseyeva KG. Extra γ-radiation induced cellular apoptosis of bone marrow in amphibians inhabiting areas contaminated by radionuclides. (in Russian). Bull Nat Acad Sci Belarus 1999;4:131–2.

Alexakhin RM, Sarapultsev IA, Spirin EV, Udalov DB. Formation of dose burdens to farm animals after the Chernobyl accident and influence of their evacuation on the absorbed doses. Proc USSR Acad Sci 1992;323:576–9.

Alexakhin RM, Buldakov LA, Gubanov VA, Drozhko YeG, Ilyin LA, Kryshev II, et al. Large radiation accidents: consequences and protective countermeasures. Moscow: IzdAT Publisher; 2004.

Arkhipov NP, Kuchma ND, Askbrant S, Pasternak PS, Musica VV. Acute and long-term effects of irradiation on pine (*Pinus sylvestris*) stands post-Chernobyl. Sci Total Environ 1994;157:383–6.

Astasheva NP, Lazarev NM, Khramtsova LK, Drozdenko VP, Chmirev MA, Zigarenko VN. Influence of radiation released during the Chernobyl NPP accident on clinical and physiological status of agricultural animals. Problems of agricultural radiology. Kiev: UIAR 1991. p. 176–80. (in Russian).

Baker RJ, Chesser RK. The Chernobyl nuclear disaster and subsequent creation of a wildlife preserve. Environ Toxicol Chem 2000;19:1231–2.

Baker RJ, Hamilton MJ, Bussche RAVanDen, Wiggins LE, Sugg DW, Smith MH, et al. Small mammals from the most radioactive sites near the Chernobyl nuclear power plant. J Mammal 1996;77:155–70.

Belova NV, Verigin BV, Emelianova NG, Makeeva AP, Ryabov IN. Radiobiological analysis of silver carp *Hypophthalmichthys molitrix* in the Chernobyl NPP cooling pond in the post-accident period. I. The reproductive system status in the survived fish. (in Russian). J Ichthyol 1993;33:814–28.

Bird GA, Thompson PA, MacDonald DR, Sheppard SC. Ecological risk assessment approach for the regulatory assessment of the effects of radionuclides released from nuclear facilities. Protection of the environment from ionizing radiation. Report CSP-17. Vienna: International Atomic Energy Agency 2003. p. 241–7.

Brechignac F, Polikarpov GG, Oughton DH, Hunter G, Alexakhin RM, Zhu YG, et al. Protection of the environment in the 21st century: radiation protection of the biosphere including humankind. Statement of the International Union of Radioecology. J Environ Radioact 2003;70:155–9.

Budarkov VA, Zenkin AS, Arkhipov NP, Junosova RM, Mayakov EA, Amirkhanian AR, et al. Effects of ^{131}I on sheep dependent on the stable iodine content in the ration. (in Russian)Radiobiology 1992;32:451–8.

Camplani A, Saino N, Moller AP. Carotenoids, sexual signals and immune function in barn swallows from Chernobyl. Proc R Soc Lond B 1999;266:1111–6.

Chesser RK, Sugg DW, Lomakin MD, Bussche RAVD, DeWoody JA, Jagoe CH, et al. Concentrations and dose rate estimates of ^{134,137}Cesium and ^{90}Strontium in small mammals at Chernobyl, Ukraine. Environ Toxicol Chem 2000;19:305–12.

Dallas CE, Lingenfelser SF, Lingenfelser JT, Holloman K, Jagoe CH, Kind JA, et al. Flow cytometric analysis of erithrocyte and leukocyte DNA in fish from Chernobyl-contaminated ponds in the Ukraine. Ecotoxicology 1998;7:211–9.

Dmitriev A, Krizanovskaya M, Guscha N, Grodzinsky D. Effects of low dose chronic radiation on plant disease resistance and fungal pathogen virulence. Current problems of radiation research. Proceedings of the 35th annual meeting of the European Radiation Research Society. Kiev, Ukraine 2007. p. 109–17.

Eliseyeva KG, Voitovich AM, Ploskaya MV, Smal SE. Genetic monitoring of brown frog populations inhabiting radiocontaminated areas of Belarus. (in Russian). Radiation Biology. Radioecology 1994;34:838–46.

Eliseyeva KG, Kartel NA, Voitovich AM. Chromosome aberrations in different tissues of mouse-type rodents and amphibians in regions of Belarus contaminated by radionuclides. Cytol Genet 1996;30:20–5.

Ellegren H, Lindgren G, Primmer CR, Moller AP. Fitness loss and germline mutations in barn swallows breeding in Chernobyl. Nature 1997;389:593–6.

Ermakova OV. Compensatory-recovery processes in the endocrine system of voles in conditions of radioactive contamination of the environment. (in Russian). Effects of radioactive contamination on terrestrial ecosystems in the Chernobyl affected area. Proc. Komi Sci. Center, Urals Branch of RAS. No. 145Syktyvkar 1996. p. 58–76.

Fedotov IS, Kal'chenko VA, Igonina EV, Rubanovich AV. Radiation and genetic consequences of ionizing irradiation on populations of *Pinus sylvestris* L, within the zone of the Chernobyl NPP. (in Russian). Radiation Biology. Radioecology 2006;46:283–8.

Fesenko SV, Alexakhin RM, Geras'kin SA, Sanzharova NI, Spirin YeV, Spiridonov SI, et al. Comparative radiation impact on biota and man in the area affected by the accident at the Chernobyl nuclear power plant. J Environ Radioact 2005;80:1–25.

Fetisov AN, Rubanovich AV, Slipchenko TS, Shevchenko VA. The structure of *Dreissena polymorpha* populations from basins adjacent to the Chernobyl atomic power station. Sci Total Environ 1992;112:115–24.

Geras'kin SA, Dikarev VG, Zyablitskaya YeYa, Oudalova AA, Spirin YeV, Alexakhin RM. Genetic consequences of radioactive contamination by the Chernobyl fallout to agricultural crops. J Environ Radioact 2003;66:155–69.

Glazko VI. A note on genetic structure of cattle breed within increased ionizing zone at the Chernobyl accident area. Anim Sci Pap Rep 2001;19:95–109.

Glazko TT, Grodzinsky DM, Glazko VI. Chronic low dose ionizing irradiation and polyfactorial adaptation. (in Russian). Radiation Biology. Radioecology 2006;46:488–93.

Golubev A, Afonin V, Maksimova S, Androsov V. The current state of pond snail *Lymnaea stagnalis* (Gastropoda, Pulmonata) populations from water reservoirs of the Chernobyl nuclear accident zone. Radioprotection 2005;40:S511–7.

Grodzinsky DM, Gudkov IN. Radiation injury of the plant in the zone of influence of the accident on Chernobyl Nuclear Power Plant. (in Russian). Radiation Biology. Radioecology 2006;46:189–99.

Gudkov IN, Kitsno VE, Grisyuk SN, Tkachenko GM, Ivanova EA, Saenko KV, et al. Anti-radiation protection of plants by metal under radioactive contamination of the area. (in Russian). Radiation Biology. Radioecology 1999;39:349–53.

Hinton TG, Alexakhin R, Balonov M, Gentner N, Hendry J, Prister B, et al. Radiation-induced effects on plants and animals: findings of the United Nations Chernobyl Forum. Health Phys. 2007;93:427–40.

IAEA. International Atomic Energy Agency. Effects of ionizing radiation on plants and animals at levels implied by current radiation protection standards. Technical Reports Series N.332. Vienna: IAEA; 1992.

IAEA. International Atomic Energy Agency. Environmental consequences of the Chernobyl accident and their remediation: twenty years of experience. Report of the UN Chernobyl Forum Expert Group "Environment" (EGE). Vienna: IAEA; 2006.

Khromova LV, Romanovsky MG, Dukharev VA. Partial pine sterility in 1986 and 1987 within the zone of Chernobyl accident. (in Russian). Radiobiology 1990;30:450–7.

Kostenko SA, Buntova EG, Glazko TT. Species specifity of karyotype instability under conditions of radionuclide contamination (the Chernobyl

NPP) in voles *Microtus oeconomus, Microtus arvalis, Clethrionomys glareolus*. Cytol Genet 2001;35(2):11–8.

Kovalchuk O, Burke P, Arkhipov A, Kuchma N, James SJ, Kovalchuk I. Pogribny I. Genome hypermethylation in *Pinus sylvestris* of Chernobyl — a mechanism for radiation adaptation? Mutat Res 2003;529:13–20.

Kovalchuk I, Abramov V, Pogribny I, Kovalchuk O. Molecular aspects of plant adaptation to life in the Chernobyl zone. Plant Physiol 2004;135:357–63.

Kozubov GM, Taskaev AI. Radiobiology investigations of conifers in region of the Chernobyl disaster (1986–2001). Moscow: PPC; 2002 (in Russian), "Design. Information. Cartography".

Kozubov GM, Taskaev AI. The features of morphogenesis and growth processes of conifers in the Chernobyl nuclear accident zone. (in Russian). Radiation biology. Radioecology 2007;47:204–23.

Krivolutsky DA. Radioecology of terrestrial animal communities. Moscow: Energoatomizdat; 1983 (in Russian).

Krivolutsky DA. Dynamics of biodiversity and ecosystems under conditions of radioactive contamination. Dokl Bolg Akad Nauk 1996;347:166–8.

Krivolutsky DA. Problems of sustainable development and ecological indication in radioactively contaminated areas. Russ J Ecol 2000;31(4):257–62.

Krivolutsky DA, Pokarzhevsky AD. Effects of radioactive fallout on soil animal populations in the 30 km zone of the Chernobyl atomic power plant. Sci Total Environ 1992;112:69–77.

Kudyasheva AG, Shishkina LN, Zagorskaya NG, Shevchenko OG, Ivashevskaya EV. The composition of liver phospholipids in tundra vole (*Microtus oeconomus*) inhabiting areas with different state of radiation background. (in Russian). Radiation biology. Radioecology 2000;40:327–33.

Makeeva AP, Emelianova NG, Belova NV, Ryabov IN. Radiobiological analysis of silver carp *Hypophthalmichthys molitrix* in the Chernobyl NPP cooling pond in the post accident period. II. Development of the reproductive system in offsprings of the first generation. (in Russian). J Ichthyol 1994;34:681–96.

Materiy LD. Dynamics of morphological manifestations of the affection and recovery processes in the hemopoietic system of small mammals from the 30 km ChNPP zone. (in Russian). Effects of radioactive contamination on terrestrial ecosystems in the Chernobyl affected area. Proc. Komi Sci. Center, Urals Branch of RAS. No. 145 V.1. Syktyvkar 1996. p. 12–40.

Materiy LD, Goncharov MI. Mobilization of the compensatory-recovery processes in the affected liver of small mammals from the 30-km ChNPP zone. (in Russian). Effects of radioactive contamination on terrestrial ecosystems in the Chernobyl affected area. Proc. Komi. Sci. Center, Urals Branch of RAS. No. 145 V.1. Syktyvkar 1996. p. 41–57.

Matson CW, Rogers BE, Chesser RK, Baker RJ. Genetic diversity of Clethrionomys glareolus populations from highly contaminated sites in the Chernobyl region, Ukraine. Environ Toxicol Chem 2000;19:2130–5.

Mikheev AM. The geterogenity of ^{137}Cs and ^{90}Sr distribution and dose loading on critical tissues of main seedling root. (in Russian). Radiation Biology. Radioecology 1999;39:663–6.

Moller AP. Developmental instability of plants and radiation from Chernobyl. Oikos 1998;81:444–8.

Moller AP. Developmental instability and sexual selection in stag beetles from Chernobyl and a control area. Ethology 2002;108:193–204.

Moller AP, Mousseau TA. Mutation and sexual selection: a test using barn swallow from Chernobyl. Evolution 2003;57:2139–46.

Moller AP, Mousseau TA. Biological consequences of Chernobyl: 20 years on. Trends Ecol Evol 2006;21:200–7.

Moller AP, Mousseau TA, Milinevsky G, Peclo A, Pysanets E, Szep T. Condition, reproduction and survival of barn swallows from Chernobyl. J Anim Ecol 2005;74:1102–11.

Moller AP, Hobson KA, Mousseau TA, Peklo AM. Chernobyl as a population sink for barn swallows: tracking dispersal using stable-isotope profiles. Ecol Appl 2006;16:1696–705.

Oleksyk TK, Novak JM, Purdue JR, Gashchak SP, Smith MH. High level of fluctuating asymmetry in populations of *Apodemus flavicollis* from the most contaminated areas in Chernobyl. J Environ Radioact 2004;73:1–20.

Pechkurenkov VL. The influence of Chernobyl disaster on fish populations in a cooling pond. (in Russian). Radiobiology 1991;31:704–8.

Polikarpov GG, Tsytsugina VG. After-effect of the Kyshtym and Chernobyl accidents on hydrobionts. (in Russian). Radiation biology. Radioecology 1995;35:549–56.

Pomerantseva MD, Ramaiya LK, Chekhovich AV. Genetic disorders in house mouse germ cells after the Chernobyl catastrophe. Mutat Res 1997;381:97–103.

Pomerantseva MD, Ramaiya LK, Rubanovich AV, Shevchenko VA. Genetic consequences of an increased radiation background at mouse-like rodents. (in Russian). Radiation biology. Radioecology 2006;46:279–86.

Popova ON, Taskaev AI, Frolova NP. Genetic stability and variability of seeds from herbaceous phytocenosis inhabiting regions radioactively contaminated as a result of Chernobyl accident. (in Russian). St. Petersburg: Nauka; 1992.

Rakin AO, Bashlykova LA. Results of cytogenetic monitoring of mouse-like rodents from the Chernobyl contaminated region. (in Russian). Effects of radioactive contamination on terrestrial ecosystems in the Chernobyl affected area. Proc. Komi Sci. Center, Urals Branch of RAS. No. 145 V.1. Syktyvkar 1996. p. 113–22.

Rogers BE, Baker RJ. Frequencies of micronuclei in bank voles from zones of high radiation at Chernobyl, Ukraine. Environ Toxicol Chem 2000;19:1644–8.

Ryabokon NI. Biological effects in natural populations of small rodents in radiocontaminated areas. The frequency of bone marrow polyploid cells in bank voles in different years following the Chernobyl accident. (in Russian). Radiation biology. Radioecology 1999;39:613–8.

Ryabokon NI, Goncharova RI. Transgenerational accumulation of radiation damage in small mammals chronically exposed to Chernobyl fallout. Radiat Environ Biophys 2006;45:167–77.

Ryabokon NI, Smolich II, Kudryashov VP, Goncharova RI. Long-term development of the radionuclide exposure of murine rodent populations in Belarus after the Chernobyl accident. Radiat Environ Biophys 2005;44:169–81.

Ryabov IN. Effect of radioactive contamination on hydrobionts within the thirty-kilometer zone of Chernobyl NPP. (in Russian). Radiobiology 1992;32:662–7.

Sarapult'zev BI, Geras'kin SA. Genetic basis of radioresistance and evolution. Moscow: Energoatomizdat Publishers; 1993 (in Russian).

Shershunova VI, Zainullin VG. Monitoring of natural populations of *Dactylis glomerata* L., growing within zone of Chernobyl NPP. (in Russian). Radiation biology. Radioecology 1995;35:690–5.

Shevchenko VV, Grinikh LI. Cytogenetic effects in *Crepis tectorum* populations from Bryansk region observed in the 7-th year after the Chernobyl accident. (in Russian). Radiation biology. Radioecology 1995;35:720–5.

Shevchenko AS, Vakulenko AD, Isamov NN. Increase in activation by prostaglandin E_1 of adenilatcyclase in blood cells of animals found in the Chernobyl affected region. (in Russian). Reports of All-Union Academy of Agricultural Sciences, vol. 11. 1990. p. 55–8.

Shevchenko VA, Abramov VI, Kalchenko VA, Fedotov IS, Rubanovich AV. Genetic consequences of radioactive contamination of the environment caused by the Chernobyl accident for plant populations. (in Russian). Radiation biology. Radioecology 1996;36:531–45.

Shevchenko VV, Grinikh LI, Abramov VI. Cytogenetic effects in natural *Crepis tectorum* populations growing at the East-Ural radioactive track. (in Russian). Radiation biology. Radioecology 1998;38:330–6.

Shishkina LN, Materiy LD, Kudyasheva AG, Zagorskaya NG, Taskaev AI. Structural and functional disturbances in the liver of wild rodents living in the regions affected by Chernobyl disaster. (in Russian). Radiobiology 1992;32:19–29.

Sidorov VP. Cytogenetic effect in *Pinus sylvestris* needle cells as a result of the Chernobyl accident (in Russian). Radiation biology. Radioecology 1994;34:847–51.

Smirnov EG, Suvorova LI. Estimation and prediction of biological effects of radioactive contamination on the plant cover in the Chernobyl affected area. (in Russian). Effects of radioactive contamination on terrestrial ecosystems in the Chernobyl affected areas. Proc. Komi Res. Center Ural Branch of RAS 1996. p. 27–37.

Sokolov VE, Ryabov IN, Ryabtsev IA, Tichomirov FA, Shevchenko VA, Taskaev AI. Ecological and genetic consequences of the Chernobyl atomic power plant accident. Vegetatio 1993;109:91–9.

Sokolov VE, Ryabov IN, Ryabtsev IA, Kulikov AO, Tichomirov FA, Shcheglov AI, et al. Effect of radioactive contamination on the flora and fauna in the vicinity of Chernobyl nuclear power plant. Sov Sci Rev F Physiol Gen Biol Rev 1994;8:1–124.

Sorochinsky BV. Molecular-biological nature of morphological abnormalities induced by chronic irradiation in coniferous plants from the Chernobyl exclusion zone: emphasis on a possible role of the cytoskeleton. Cytol Genet 2003;37:49–55.

Suvorova LI, Spirin DA, Martyushov VZ, Smirnov EG, Tarasov OV, Shein GP. Assessment of biological and ecological consequences of radioactive contamination of biogeocenoses. (in Russian). In: YuAIzrael YuA, editor. Radiation aspects of the Chernobyl accidentvol. 2, vol. 2. 1993St. Petersburg: Hydrometeoizdat 1993. p. 321–5.

Taskaev AI, Testov BV. Number and reproduction of mouse-like rodents in the Chernobyl accident area. (in Russian). Bioindication of radioactive contamination. Moscow: Nauka 1999. p. 200–5.

TaskaevA.I. AI, FrolovaN.P. NP, PopovaO.N. ON, ShevchenkoV.A. VA. The monitoring of herbaceous seeds in the 30-km zone of the Chernobyl nuclear accident. Sci Total Environ 1992;112:57–67.

Tichomirov FA, Shcheglov AI. Main investigation results on the forest radioecology in the Kyshtym and Chernobyl accident zones. Sci Total Environ 1994;157:45–57.

Tichomirov FA, Shcheglov AI, Sidorov VP. Forests and forestry: radiation protection measures with special reference to the Chernobyl accident zone. Sci Total Environ 1993;137:289–305.

Tsytsugina VG, Polikarpov GG. Radiological effects on populations of Oligochaeta in the Chernobyl contaminated zone. J Environ Radioact 2003;66:141–54.

Tsytsugina VG, Polikarpov GG. The criteria of identification of “critical” populations in aquatic radiochemoecology. (in Russian). Radiation biology. Radioecology 2006;46:200–7.

UNSCEAR. United Nations Scientific Committee on the Effects of Atomic Radiation. Sources and effects of ionizing radiation. Scientific annex within 1996 UNSCEAR report to the general assembly. New York: United Nations; 1996.

Voitovich AM. Bone tumor in frog (*Rana temporaria* L.) in radionuclide contaminated environment. (in Russian). Proc Natl Acad Sci Belarus 2001;45:91–4.

Voitovich AM, Afonin VYu. Natural populations of small vertebrates in the ecologic-genetic monitoring system. (in Russian). Ecology and rational land use at the centuries interface, vol. 2. 2000. p. 35–6. Tomsk.

WilsonE.O. EO. The diversity of life. London: Penguin; 1999.

Zainullin VG, Rakin AO, Taskaev AI. The dynamic of cytogenetic aberrations in mouse-like rodents inhabiting the region of the Chernobyl accident. (in Russian). Radiation biology. Radioecology 1994;34:852–7.

Zelena L, Sorochinsky B, Arnold vonS, Zyl vanL, Clapham DH. Indications of limited gene expression in *Pinus sylvestris* trees from the Chernobyl region. J Environ Radioact 2005;84:363–73.