Radiation effects in pine forest

Influence of IR on forest ecosystems most clearly revealed itself near the Chernobyl NPP (ChNPP), where magnitudes of absorbed doses reached 'lethal' values, as applied to conifers. Main contribution to absorbed dose was due to beta-radiation of short-living radionuclides. To largest extent the radiobiological effects appeared at injured plantations of pines and firs. Nevertheless, during the first year maximum absorbed doses influenced also on leaf-bearing trees (birch, alder, asp) which then rehabilitated themselves completely.

Depending on degree of radiation effect, 5 zones of injury were marked out (Table 1). Criteria for that were as follows: absorbed dose, degree of affection of tree crowns, phytomass increase, as well as category of plantation condition as a whole. Weak influence took place at exposure dose (in May - June, 1986) not less than 20 mR/h, the cumulative dose being more than 0.1 Gy (during the period of sharp irradiation). Visible damages of trees were not observed. In some cases the effects of radiostimulation of growth became apparent, but not very expressive. The situation had been normalized here already during the first year.

In the zone of weak effect one could note delay of sprout&needle growth during the first after-accident year and morphological abnormalities in vegetative organs. Action of radiation factor revealed during two years, then growth of trees normalized completely.

In medium injured stands the cumulative absorbed dose was 1 to 10 Gy. Considerable inhibition of growth of sprouts and needles was noted, as well as a drop in radial growth, damage of crowns, numerous morphoses and death of a part of the trees (mostly of low classes of growth). That caused pronounced worsening in the condition of the tree stands. Damage in regenerative sphere of pine was marked. Processes of reparation in those plantations proceeded for three years. Irradiation accelerated differentiation of trees in the stand. It led to some decrease in completeness of plantations. By now normal growth and development of trees have restored here too.
Table 1. Dynamics of condition of pine forests exposed to radiation

<table>
<thead>
<tr>
<th>Degree of injury, absorbed dose, Gy</th>
<th>Condition in 1986</th>
<th>Period of restoration, years</th>
<th>Current condition (1995)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No signs of injury, &lt; 0.1</td>
<td>Changes in growth</td>
<td>1</td>
<td>Complete restoration</td>
</tr>
<tr>
<td>Weak, 0.1 to 1</td>
<td>Inhibition of growth, morphosis</td>
<td>2</td>
<td>Complete restoration</td>
</tr>
<tr>
<td>Medium, 1 to 10</td>
<td>Death of separate trees, inhibition of growth, morphoses</td>
<td>3</td>
<td>Complete restoration</td>
</tr>
<tr>
<td>High, 10 to 60</td>
<td>Death of groups of trees, complete inhibition of growth, decrease in radial increment</td>
<td>Not complete. Change of vegetation community goes on.</td>
<td>Needle/leaf-bearing plantations are forming.</td>
</tr>
<tr>
<td>Total death, &gt;60</td>
<td>Yellowed needles, death of pine stands, invasion of vermin</td>
<td>Change of vegetation community</td>
<td>Leaf-bearing plantations are forming.</td>
</tr>
</tbody>
</table>

Radiobiological effects found their most clear expression in strongly injured plantations. Practically complete oppression of sprout & needle growth, death of buds and of a part of the crown, defects in the structure of wood, death of considerable part of the tree stand and complete inhibition of reproductive functions took place. General inhibition of the stands led to death of the most part of the trees. But survived ones, with at least 5 to 6 growth points remained, gave a good growth next year, having increased significantly the size and mass of needles (Table 2 and 3).

In succeeding years two processes counteracted each other here: post-radiation restoration of survived trees and degradation of tree stands. Deterioration of tree stands led to mass reproduction of vermin. Two years after injury, when radiation factor already did not define survivability of trees, the condition of strongly damaged plantations depended on factors of forestry and

Table 2. Reaction of 30-year old pine trees to acute radiation

<table>
<thead>
<tr>
<th>Degree of plantation injury</th>
<th>Quantity of buds on medium tree, %</th>
<th>Increase in crown phytomass of one tree, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>&lt;5</td>
<td>55 - 75</td>
</tr>
<tr>
<td>Medium</td>
<td>5 - 25</td>
<td>&gt;200</td>
</tr>
<tr>
<td>Weak</td>
<td>25 - 75</td>
<td>&gt;200</td>
</tr>
<tr>
<td>No signs of injury</td>
<td>&gt;75</td>
<td>&gt;200</td>
</tr>
</tbody>
</table>
on development of vermin focal points and root sponge. They determined further
degradation of tree stands. It continues now too. Under the cover of almost
perished forest, a large self-seed of leaf-bearing trees appeared. In the future a
birch tree stand will form here, having some separate survived specimens of pine
on its territory.

On plantations where absorbed dose was more than 60 Gy the loss of trees
succeeded in very short period of time. These areas served as a base for invasion
of secondary vermin that propagated then to adjacent areas. Now these stands of
trees already do not exist. In their place some “meadow communities” have
formed (partly), and on the major part a self-seed of leaf-bearing trees appeared,
and formation of new stand of trees began.

At the present time there are no morphological signs of injury of pine
stands observed on the whole territory of the zone. Separate breaches in the
growth of highly injured tree stands, in particular big sizes of needles (Table 3),
were caused by rather factors of forestry than of radiation influence.

Table 3. Relative size of needles in stands of trees having injuries of different
degrees

<table>
<thead>
<tr>
<th>Degree of injury</th>
<th>Size of needles, % of norm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1986</td>
</tr>
<tr>
<td>High</td>
<td>66</td>
</tr>
<tr>
<td>Medium</td>
<td>65</td>
</tr>
<tr>
<td>Weak</td>
<td>91</td>
</tr>
<tr>
<td>No signs of injury</td>
<td>96</td>
</tr>
</tbody>
</table>

At the same time an increase in quantity of the morphoses in young pine
trees transplanted on the former place of ‘red forest’ was fixed in 1995. The cause
of the phenomenon is unclear yet, and growth of the plantations on the areas
with high density of contamination needs more careful control.

The test cultures were created in different regions of Ukraine in 1988 to
1994 from the seeds gathered on damaged plantations.

Higher frequency of morphoses was observed during the first year of
growth of seedlings but thereafter the deviations in evolution were absent, and
no dependance between the degree of damage and the growth of test cultures was
found.

IR exerted more deep and prolonged influence upon the reproductive
sphere. In early years the plantations having high absorbed doses demonstrated
significant destructive processes in plastides being reflected in damage of
chlorophyll-protein complex, decrease of chlorophyll and increase of carotinoid quantities. The plantations exposed to low degree of irradiation, on the contrary, demonstrated an increase in the content of chlorophylls and in the size of structural elements of the pigment system. Now direct dependance between IR absorbed dose and accumulation of pigments is not observed. Sensitivity of vegetative and generative tissues of a pine tree to irradiation depending on the phase state of cells appeared to be different: one of the cells in the rest phase is lower than one of them in the phase of active cell division. On being irradiated with sublethal doses, the tissues of vegetative organs recover in 1 to 2 years after irradiation, and to normalize the processes of formation of generative organs, it is needed from 2 to 6 years and more, depending on the dose of irradiation.

Damaged plantations demonstrated a delay in beginning the reproductive processes in early years after the accident. In 1995 the beginning of meiosis was noted simultaneously on all areas. Only the plantations with high density of contamination show some delay of separate phases of meiosis. Investigation of the breach frequency in separate phases of meiosis showed that the quantity of anomalies in metaphase - 1 and - 2 at high density of contamination was 3 to 4 times more than in the control group.

The level of chromosome breaches in 1995 appeared to be practically the same as in 1987, but the frequency and types of aberrations considerably changed. In 1987 the aberrations of the chromosome stick-together type were observed most frequently. They were caused by a great number of ruptures when the objects have been exposed to acute external irradiation. In 1995 this type of aberrations was not revealed, but the bridge-type ones have been seen very often. The delays of chromosomes in anaphases were met with near-identically in frequency in 1987 and 1995.

It is interesting that the lowering of the level of breaches in meiosis took place in 1988 (2 to 3 times). In 1995 the original level of breaches was restored, but at the same time the frequency of different types of breaches changed.

A decrease in the quantity of breaches in 1988 can be explained by restoration of intercellular protective system due to disappearance of acute irradiation. Further, as the plants accumulated radionuclides, inner irradiation evidently became even more important factor in the origin of chromosome breaches.

In the whole, at present time the viability of pollen is practically the same on all plots with slight decrease in plantations having high density of contamination. But the length of pollen tubes in these cases is obviously less and
correlates with reduced quantity of starch and high specific activity of pollen. Most likely, the disturbances revealed in the process of formation of microgametophytes did not lead to their considerable loss but disrupted normal metabolism of pollen grains.

Researches of last years showed that the ecological factors (especially climatic ones) could significantly affect development of reproductive sphere of pine and modify the influence of radiation. In 1995 a decrease of relative/absolute gametophyte survivability took place (particularly on plots having high density of contamination) due to summer drought observed now in its second year. It was the worsening of weather conditions that caused an additional loss of seed-buds in the amount of 13 to 15 per cent.

**Effects of irradiation in minks**

Animals being under chronic influence of ionizing radiation (small doses) showed that clinical/laboratory characteristics of most of species, describing functional state of different organs and systems, are within their physiological standards. However, experimental researches (carried out at tissue/cell/subcell/molecular levels) allowed to reveal a number of forming radiobiological effects.

A complex of pathological changes revealed in minks which were kept 2 to 3 years near the ChNPP and have received a dose of internal irradiation (from incorporated radionuclides) of about 29 to 39 Rem.

Using the electronic microscopy in analysis of brain cell elements and of ultrastructural organization of vessels showed that the changes in neurons were rather various, and both initial (reversible enough) and non-reversible ones. In this case the changes in neurons, glial cells and walls of intra-brain capillaries are closely linked together and lead to disturbances in functional state of the complex ‘neuron-glia-capillary’.

Particularly expressed are the changes in neurons in the area of hypotalamus. Due to intracellular oedema, the quantity of dystrophically destructive neurons in hypotalamus and nuclear/cytoplasmatic index of hypotalamus neurons in experimental animals are almost twice as large, and ones of cortex neurons are only 1.5 times as large against the control. Because of this a decrease in quantity of unchanged neurocytes is observed.

Against the background of morphological changes decrease of protein level in hippocamp, striatum, middle brain and cortex has been reliably established. This witnesses that there are non-reversible disturbances in a number of central mechanisms, regulating critical functions of organism.
Animals that were for a long time (2 to 3 years) in the zone of the ChNPP accident showed significant shifts in neurochemical regulation of endocrine system, as well as ultrastructural evidences that there existed stimulation of secretory activity of cortico-tireotrope cells of adenohypophysis at simultaneous deceleration of secretory function of gonadotropocytes. Disturbances of central regulation of endocrine system reveal at the level of periphery glands in the following way: in thyroid glands one can observe distinct activation of secretory process against the background of hypertrophy and hyperplasia of follicular and parafollicular cells; in the cortex of adrenal glands a growth in functional stress of adrenocorticocytes is traced and (in parallel) destructive changes develop in these cells and in microcirculatory flow that leads to weakening of adaptation reserves and of hormonal activity of adrenal glands. One observes weakening of testosterone generation and disturbances in the processes of formation of gametes, influencing negatively on reproductive function of males. Increased number of molecules of products of lipide peroxidation ‘sewn together’ with proteins of cell membranes has been found in liver, thyroid, skin and testicles of minks. That witnesses exhaustion of reserves and of antioxidant protective systems in these organs.

At the study of genotoxicity of radionuclides incorporated in the cells of bone marrow and periphery blood lymphocytes of minks it was established that the irradiated animals are characterized by decrease of a part of cells being in the G0/G1 phase of the cell cycle, followed by compensatory increase of a part of lymphocytes being in the S- and G2/M- phases. This testifies less intensity of reparatory processes in animal tissues being under internal irradiation than in ones of control animals.

The experimental animals demonstrated the worsening of strength characteristics of bone/cover tissues. Experimental group of animals has a strength limit index of bone tissue of limb skeleton elements more variable than one of control group. Histologic researches found (in bone tissues) as follows: a decrease in specific volume of spongy bone in metaphyses and epiphyses; intensification of resorptive processes; the areas where a bone matrix was substituted for fibrous tissue; differences in correlation between the processes of osteopoiesis and resorption at the formation of a compact.

The revealed changes in the amino-acid composition of bones and of skin of minks being 2 and 3 years old are monodirected but older animals have these changes more expressed. Changes in the content of oxilysin, lysin and oxiprolin are due to formation of intra- and intermolecular ‘sewn pieces’, and changes in the content of asparagin acid and of glutamin one are due to the changing
charge of a molecule. Quantitative changes in a number of other aminoacids have an effect on stiffness of collagen spiral and also on the quantity of domains 'arg-gli-asp' that cause adhesion of cells on a collagen fibrilla. Apparently, these disturbances are at the bottom of weakening, first, of bone tissue stiffness and, second, of fixation of hairs in hair bulbs.

Taking into account a great number of signs we are able to make a conclusion that the state of hyperadaptasis appearing in animals is similar to that one developing in the organism in aging.

Consequence of irradiation in cattle

Since 1988 radiobiological investigations are carried out at an experimental vivarium (the 5-km zone ChNPP) on cows. The herd consists of some groups:

- A - four animals, which inhabited in nearest territory around ChNPP during 1986-1987 and were caught in November of 1987; B - cows brought in the vivarium from settlement Polesskoe (abroad 30-km zone); C - first generation from group A; D - first generation from group B.

According with our calculation in 1986-1987 group A received dose about 2.5 Gy on whole body, 10-11 Gy - on gastrointestinal tract and 130 Gy on thyroid gland. During subsequent years accumulated dose didn’t exceed 0.02 Gy/year on whole body and 0.1-0.3 Gy/year on gastrointestinal tract.

In 1989 a first generation from group A was got and more 40 calves of first-third generation were got by 1996. Physiological state of the experimental cows was investigated by different hematological, biochemical and cytogenetic methods.

The main hematological indices (quantity of erythrocytes, leukocytes, hemoglobin) were in physiological limits. Nevertheless, lymphocytes quantity was higher norm (up to 60-65% before 1993). Granulocytes contained eosinophils (13-20%) and basophils (up to 1.5%). Compound of neutrophils testifies about some inhibition of blood-forming. Hard identificated and abnormal cells are observed, such as: limphocyte-similar oone with obvious basophilia of cytoplasm and exocytos of little granules; apoptose of cells; aglomiration oof nucleus segments of eosinophils. Some of these peculiarities are known as usual for chronic irradiation. By 1996 the amount of abnormal and hard identificated cells has decreased. The decrease of lymphocytes quantity has been noted too. Basophilia has disappeared. Expressed eoosinophilia are observed for all animals.
The most remarkable radiation effects were found out when we investigated prooxidant and antioxidant systems. Methabolitic indices of the animals blood (group A) characterised by following percuiarities: higher activity of antioxidant systems on background of weakening of pro-oxidant one, accumulation of secondary and final products of lypide peroxide oxidation (POL), hemolitic resistance of erythocytes (HRE). First generation of the group A had most expressed deviation from norm: highest level of POL activity, strain of antioxidant system, lowest HRE. The animals of group B had relative stable indices, and internal protective mechanisms of group D provided them a most low level of POL and highest HRE.

During last 3 years processes of POL in groups A, B and D normalized. First generation of group A differed by continuation of accumulation of POL products and lowest HRE (blood-forming system had signs of inhibition). Antioxidant system has normalized in groups A and B and reached equilibrium with activity of prooxidant system. In group C the antioxidant system kept strained. In first generation of group B the some higher activity of antioxidant system was observated on background of relative low level of POL. This group has most high HRE.

During all years high quantity of serum protein and low immunoglobulin was usual in all groups.

Chronic irradiation of the cows organisms reflected on cytogenetic percuiarities of them. So, quantity of aberrant cells (lymphocytes) varied: 0-20% in 1991 and 0-5% in 1995. In first years we observed different types of abirrations: chromosome, chromatid and genom one. The chromosome type dominated in 1991 (3.45%) (chromatid - 2.67%). Genom aberrations were represented by tri- and tetraploids. All types of aberrations had bigger frequency than control. The most higher level of aberrations was in group C (7.4%) and group A (6.1%). For most groups such kind of abirrations as dicentric was noted before 1993. In 1995 it kept only in lymphocytes of group C. The frequency of aberration has decreased in 1995 and chromatid aberrations dominated.

Thus, the performed investigations shown, that type and degree of radiobiological effects depended on value and duration of irradiation and onthogenetic stage of animals, when the dose were received. The most deeper changes took place in organism of first generation of cows, which inhabited in nearest zone of ChNPP in 1986-1987. Protection systems of organism of their parents allowed more successfully to maintain homeostasis. Long strain of different systems caused pathological specific and non-specific aberration. Nevertheless, during 8 years of the observation no one death was noted and general state of the animals normalized constantly.