Chapter 2
Biological Effects of Ionizing Radiation

The study of the biological effects of ionizing radiation started practically at the same time as the discovery of X-rays in 1895. Since the techniques and methods accepted today to quantify radiation dose were absent at that time, first findings and studies were barely qualitative. Nonetheless, harmful effects to man and to laboratory animal species from ionizing radiation were already observed in the early years of the 20th century, when lack of data was a shared concern and there were no radiological protection standards. The first quantitative studies in experimental radiobiology developed during the 1920s, with the results of the epidemiological studies on the survivors of the atomic bombing of Hiroshima and Nagasaki, and data obtained from studies on patients exposed to radio therapeutic treatments, currently provide a large amount of information on the health effect of ionizing radiation which is the base of safety standards for occupationally exposed individuals.

Biological effects of ionizing radiation are a consequence of the ionization of atoms of biomolecules, which might cause chemical changes and alter or eradicate its functions. As illustrated in Fig. 2.1, energy transmitted by radiation may act directly causing ionization of the biological molecule or may act indirectly through the free radicals resulting from the ionization of the water molecules that surround the cell.

Due to ionization, proteins can lose the functionality of its amino groups and modify its behavior, thus increasing its chemical responsiveness; enzymes would be deactivated; lipids will suffer peroxidation; carbohydrates will dissociate; and nucleic acids chains will experiment ruptures and modifications of structure. But from all possible combined alterations, DNA is the primary target for radiation because it contains genes/chromosomes that hold information for cell functioning and reproduction that are critical to cell survival.
As a result of radiolytic decomposition of water by ionization and excitation, hydrogen, and hydroxyl radicals could combine to form toxic substances as hydrogen peroxide (H$_2$O$_2$), which can also contribute to the destruction of cells.

The deposition of energy by ionizing radiation is a random process. Even at very low doses there is some probability that enough energy may be deposited into a critical volume within a cell to result in cellular changes or cell death. But thanks to the remarkable ability of cells to repair damage, enzymatic, and repair mechanisms would lead in many instances to the correct DNA repair and the cell will survive without any modification to its function or genetic structure. If the repair of DNA damage is incomplete, signaling pathways leading to cell death through apoptosis, terminal differentiation, and senescence are activated. Physical processes of energy absorption and induced ionization and excitation, as well as biochemical processes triggered by the living organism response, would occur within fraction of seconds. Repair of cellular damage, such as DNA repair, may take from minutes to hours after exposure depending on the type of damage.

Another possible result is mutation. The cell will survive but with modification in the DNA sequence of the cell’s genome. Mutated cells are capable of reproduction and thus perpetuate the mutation. If the mutated cell is a somatic cell,

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A signaling pathway describes a group of molecules in a cell that work together to control one or more cell functions, such as cell division or cell death. After the first molecule in a pathway receives a signal, it activates another molecule. This process is repeated until the last molecule is activated and the cell function is carried out.
mutation could lead to a malignant tumor. If the mutated cell is a germ cell, it may cause a hereditary effect. These are stochastic effects and their consequences (cancer or hereditary effects) may be statistically observed long after exposure.

If damage cannot be repaired cell death occurs. Cell death means the loss of a specific function for differentiated cells which do not replicate, such as nervous cells, muscle cells, or secretory cells. For proliferating cells, such as primary blood-forming (hematopoietic) cells or cell growing in a culture media (stem cells), cell death means the permanent loss of their proliferating capacity or the loss of their reproductive integrity. If many cells die, there will be tissue and organ damages which may cause a rapid, whole body response. Figure 2.2 shows both paths by which radiation may affect the whole body system.

Cellular sensitivity to radiation has been better studied in proliferating cells. For proliferating cells, radiosensitivity depends on a number of factors of which the most important are cell proliferation capacity; cell differentiation degree; phase of the cell cycle at which the irradiation occurs; radiation quality; dose rate; and dose fractioning. In general, cellular sensitivity to radiation is directly proportional to the rate of cell division and inversely proportional to the degree of cell differentiation (Law of Bergonie and Tribondeau 1906). This explains why tissues with a high turnover rate are more radiosensitive than those that do not have a continuously turnover. Related to the cell cycle, cells are more sensitive to radiation during mitosis (cell division) than through the preceding substages when the cells are not

![Radiation effects on the whole body system](image)

Fig. 2.2 Radiation effects on the whole body system
dividing and the mechanisms of repair (cell cycle checkpoints\(^2\)) are activated. Some cells, like nerve cells, do not undergo much division. Most cells have a moderate cell rate division. For human organism, it might be considered that lymphocytes, stem cells in the bone marrow, cells of the lens of the eye, and epithelial cells of gastrointestinal tract are the most radiosensitive; surface of the stomach walls, esophagus, mouth, and skin are moderate radiosensitive; while muscle cells, bone cells and nerve cells are low radiosensitive.

Ionizing radiation is more effective at producing biological damage when its LET (linear energy transfer) is high, the dose rate is high and the period of time between consecutive exposures is short.

### 2.1 Classification of Biological Effects

Biological mechanisms can act in favor of tissues to maintain its functionality with a loss of certain number of cells. But when the radiation damage is of such magnitude that the cell killing cannot be compensated by the cellular turnover, tissue functionality is not possible further, leading ultimately to acute organ damages or death. Another concern is the role radiation induced mutations have in carcinogenesis. The risks of cancer after high and moderate doses of radiation are relatively well understood from detailed epidemiological studies of the Japanese atomic bombing survivors and others. Although only down to about 100 mGy\(^3\) risk of mortality and morbidity is proportional to radiation dose [1].

According to the last ICRP recommendations [2], adverse health effects from radiation exposure are grouped in two general categories, i.e., harmful tissue reactions (deterministic effects) and stochastic effects (of random or statistical nature).

Harmful tissue reactions (deterministic effects) resulting from the killing/malfunctioning of cells is characterized by a certain dose called “threshold.” The reason for the threshold is that a serious malfunction or death of a critical population of cells in a given tissue should be sustained before injury is expressed in a clinically relevant form. As shown in Fig. 2.3, the frequency of the injury increases with dose as the number of affected cells is directly proportional to the severity of the effect. The graphic on the right in Fig. 2.3 illustrates the way in what the severity of the damage above the dose-threshold, including the impairment of the capacity for tissue recovery, increases with dose. It has been presently recognized that tissue reactions can be modified after irradiation by the use of some biological response modifiers. Some examples are antioxidants, radical scavengers, apoptosis inhibitors, anti-inflammatory drugs, growth factors, etc.

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\(^2\)Cell cycle checkpoints are control mechanisms which ensure proper cell division. Each checkpoint serves as a potential halting point along the cell cycle, during which the conditions of the cell are assessed, with progression through the various phases of the cell cycle occurring when favorable conditions are met.

\(^3\)Gy (gray) is the unit of absorbed dose used in radiation biology, clinical radiology, and radiation safety. It describes the energy imparted to matter by all kinds of ionizing radiation.
Tissue reactions are also characterized by different periods of latency, so it could be distinguished between early tissue reactions detected in a few days or weeks (on a timescale of hours to weeks), and late tissue reactions, detected months to years after the irradiation. Early tissue reactions may be of the inflammatory type, resulting from cell permeability changes and histamine release (e.g., erythema), or they may be reactions resulting from cell loss (e.g., mucositis, and desquamatory reactions in epithelial tissues) [1]. Late tissue reactions are called “generic” if they arise as a direct result of damage to that tissue, for instance a vascular occlusions leading to deep tissue necrosis after protracted irradiations or “consequential” if they occur as a result of an early cellular damage, e.g., a dermal necrosis as a result of severe epidermal denudation and chronic infection [3].

In view of different individual radiosensitivity, it is accepted that the dose-threshold for a specific tissue reaction is the dose that produces the same tissue reaction in the 1–5 % of the total exposed individuals. Updated information on dose thresholds corresponding to doses that result in about 1 % incidence of morbidity and mortality for various organs and tissues is available in the ICRP 2007 Recommendations [2]. Some examples are temporary sterility in 3–9 weeks from an acute absorbed dose of ~0.1 Gy in the testes; depression of blood forming process in 3–7 days from an acute absorbed dose of ~0.5 Gy in the bone marrow; and cataracts (visual impairment) in several years from an acute absorbed dose of ~0.5 Gy [3]. Ordinarily, fractionated doses or protracted doses at low dose rates are less damaging than acute absorbed doses.

The most severe tissue reaction is death. Mortality after irradiation is generally the result of severe cell depletion in tissues or other major dysfunction of one or more vital organs of the body. Enough high acute doses to the whole body in very short periods of time may lead to lethal disorders. Although there is great uncertainty in the lethal dose-threshold on account of the general health of individuals, the immediate medical assistance received and other specific factors, absorbed doses
between 3–5 Gy may cause death in 50% of exposed individuals in a lapse of 1–2 months. ICRP 2007 Recommendations indicate a LD$_{50/60}$, i.e., within 60 days, for a normal human healthy adult of around 4 Gy midline dose [2]. In between 6–10 Gy, the mortality % increases and the time period to the death decreases. In this dose range (3–10 Gy) the cause of death is hematopoietic failure, i.e., hematopoietic syndrome, primarily from a lack of bone marrow progenitor cells, as well as from hemorrhages without the replacement of radioresistant red cells. Acute doses approaching 10 Gy causes severe gastrointestinal damage (see Fig. 2.2), which when combined with hematopoietic damage causes death in 1–2 weeks with little possibilities of survival. Local acute doses above 10 Gy to the lungs produce an acute inflammation (pneumonitis) that may lead to death. Renal damage occurs in the same dose range if the kidneys are irradiated. Neurovascular syndrome appears at even higher doses toward 50 Gy and above, and induces the death in 48 h [4].

The main concern of radiation safety at low doses has been radiation induced cancer and hereditary diseases, meaning by low doses $\approx$ 100 mGy and less. In the very early days of radiation protection standards it was assumed that “genetic damage” from radiation (meaning hereditary effect), would accumulate across generations and eventually have a marked impact on the health of human populations.

Since recommendations adopted in the late 1970s (ICRP 26) until present, it has been assumed that, stochastic or probabilistic effects may occur at low doses, and are generally considered to be cancers (including leukemia) and genetic defects in the progeny. This assumption has implied that there is a linear no-threshold increase in genetic cell damage as a function of radiation dose, and that each unit of radiation would increase the risk. This approach is consistent with the so-called linear, no-threshold (LNT) hypothesis, accepted because it is still not actually known today what level of risk is associated with very low-dose exposure to radiation. LNT hypothesis was considered to be a prudent judgment for public policy aimed at avoiding unnecessary risk from exposure.

However, in this half-century a lot of research has been done and many advances in modern molecular biology and instrumentation have taken place. As explained before, in the current conventional interpretation of radiation carcinogenesis, ionizing radiation acts primarily by damaging nuclear DNA (much of which is repaired by repair systems), and inducing targeted DNA mutations in stem cells thus initiating the cancer development process. Secondary mutations ultimately accumulate, leading to a malignant neoplasm development [5]. [“From Biological Mechanisms of Radiation Actions at Low Doses. A white paper to guide the Scientific Committee’s future programme of work, by UNSCEAR, ©2012 United Nations. Reprinted with the permission of the United Nations”].

Now it is recognized that acute doses or doses experienced in a protracted form of up to $\approx$ 100 mGy (low LET or high LET), produce no tissue functional impairment [2]. Besides, according to more recent advances in studies of biological effects of ionizing radiation, it is evident that there are much more data describing how biological systems respond to low doses of radiation.
The major areas of study are related to three unique biological responses: bystander effects, adaptive responses, and genomic instability [5]. Because of bystander effects, tissues respond as a whole to ionizing radiation and not as single cells; they demonstrate that even though the energy is deposited in random defined sites, radiation effects are not limited to the individual cells where the energy is deposited. Bystander effects at low doses of low-LET radiation appear to induce biochemical and functional cell and tissue responses that express both damage and adaptive cell protection. Also, a consensus is emerging that low-LET irradiation below 0.5 Gy does not cause transmissible genomic instability. Genomic instability suggests the accumulation of multiple changes to convert a stable genome of a normal cell to an unstable genome characteristic of a tumor, and recent reviews indicate and confirm a likely threshold for the induction of such transmissible instability.

Protective response categories involve cellular defenses, such as radical detoxification; cell removal by immune response—cells with altered phenotype may be detected and killed by the immune system—and cell removal through intracellular signaling, such as by cell differentiation and apoptosis. It has also been suggested the existence of a protective adaptive response [6]. Adaptive response is defined as the temporary modulation (usually reduction) by prior small doses of the response to subsequent high radiation doses [5] ("From Biological Mechanisms of Radiation Actions at Low Doses. A white paper to guide the Scientific Committee’s future programme of work, by UNSCEAR, ©2012 United Nations. Reprinted with the permission of the United Nations").

Supported by the fact that uncertainties regarding the role of these processes in cancer risk are currently too great for the development of practical judgments, the Commission recommended again that the practical system of radiological protection continue to be based upon the assumption that at doses below about 100 mSv4 a given increment in dose will produce a directly proportionate increment in the probability of incurring cancer or heritable effects attributable to radiation [2].

Relation between dose and risk based on the conservative approach and LNT hypothesis is shown in Fig. 2.4. This relation implies that when the dose increases the risk of late health effects like cancer, noncancer and heritable diseases increases, but in the absence of other modifying factors, the severity of the effect is not expected to increase.

Health effects of low doses of radiation continue to be a concern and are a priority so as to take its result into official standards. A bill was passed recently to revitalize the existing DOE low-dose radiation research program so as to increase the understanding of the health effects of low doses of ionizing radiation. The bill calls for a study by the National Academies and seemingly a new Biological Effects of Ionizing Radiation (BEIR) report—the BEIR VIII report—is on its way.

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4Sv (Sievert) is a special unit for the quantities equivalent dose, effective dose, and operational dose, used in radiation safety to reflect the amount of radiation detriment likely to result from the dose, or the amount of harm caused by the dose to a tissue or organ.
Heritable diseases mean induction of cancers from irradiated somatic cells and genetic diseases in offspring following parental germ cell irradiation. They are not to be confused with health effects resulting from an exposure during prenatal development. Even though there is no direct evidence of heritable effects to humans, experimental observations in animal and plant species exposed to relatively high doses, give good reasons to include such risk for future generations as part of the system of protection. However, the BEIR VII report [7] concluded that with low dose or chronic exposures to low-LET irradiation, the risk of adverse heritable health effects to children conceived after their parents have been exposed is very small compared to baseline frequencies of genetic diseases in the population.

2.2 Radiation Effects During Prenatal Development

Prenatal development is the process in which an embryo or fetus gestates during pregnancy. As cells are rapidly dividing and forming the new tissues and organs, the embryo/fetus is considered to be at the most radiosensitive stage of human development, particularly in the first 20 weeks of pregnancy. The effects of radiation exposure during prenatal development depend on the time period when the exposure occurred. Time period of pregnancy is estimated in days or weeks after last menstrual cycle and reflects the developmental stage of the embryo/fetus.

During the first week after the menstrual cycle, radiation exposure to the uterus is not dangerous provided that ovum fertilization occurs about the 14th day after. The embryonic period in humans begins at the moment of fertilization and continues until the 20th day (about 3 weeks). The only effect that could be expected in this period is failure to implant. Typically, it is estimated that 30–50% of pregnancies are lost spontaneously precisely during this period.

Fig. 2.4 Relationship between probability and severity of stochastic effects with dose
From the study of atomic bomb survivors, it was learned that between 3 and 8 weeks of pregnancy the more common expected effect after irradiation is the induction of malformations. A true dose-threshold of around 0.1 Gy has already been considered for this effect, so risk of malformations is not expected after an in-uterus exposure to doses well below 100 mGy.

Japanese atomic bomb data on the induction of severe mental retardation (SMR) after irradiation at the most sensitive prenatal period (weeks 8–15) support a true dose-threshold of 0.3 Gy for this effect, and the total absence of risk at lower doses.

The major concern after 15 weeks of pregnancy is the cancer risk following in-uterus irradiation provided that some evidences suggest an excess of cancers and leukemia. Indeed, the largest studies of in-uterus medical irradiation found an increment of all types of childhood cancer by approximately the same degree, with a relative larger risk for leukemia than for solid tumors [8]. Early indications are that, between the 16th week of pregnancy and birth, cancer risk from prenatal radiation exposure is similar to, or slightly higher (~2%) than, the normal expected cancer risk from exposure in childhood (which is 48–50%). The increased risks will depend on the amount of radiation to which the baby was exposed and the amount of time that it was exposed.

Termination of pregnancy owing to radiation exposure is an individual decision affected by many factors. Nevertheless, absorbed doses below 100 mGy to the embryo/fetus should not be considered a reason for terminating a pregnancy.

### 2.3 Biological Indicators of Radiation Damage

Even though safety is assured through a conscious fulfillment of requirements and regulations, the potentiality of an accident could not be excluded. In events like an accident, it is possible that some workers and, occasionally, members of the public would be receiving doses above the established limits, i.e., experience overexposure. If an overexposure occurs, biological indicators play an important role in defining the severity of the damage, to estimate the exposure retrospectively in some cases, and to help anticipate the occurrence of late effects. They can improve the diagnosis and treatment of injured individuals as well.

There are two main types of biological indicators, i.e., clinical indicators and laboratory test indicators. Clinical indicators of acute radiation damage are categorized according to damaged organs/tissues and symptoms in three different subgroups hematopoietic, gastrointestinal, and neurovascular.

Hematopoietic and gastrointestinal symptoms in advance of acute radiation disease are nausea, vomiting and anorexia within a few hours at the higher dose levels or after 6–12 h at the lower dose levels. Neurovascular indicators of acute radiation disease are severe tiredness (weakness/fatigue), apathy, disinterest, sweating, fever, headache, and ataxia.

As shown in Table 2.1, the severity of an overexposure (and thence the probable dose) might be predicted by the time elapsed from the exposure and onset of
symptoms. Generally, as more stable the symptom and lesser the elapsed time, the higher the dose and life-threatening to the injured person.

At about 3 Gy or over, depending on the particular radiation energies involved, general symptoms and signs also include erythema (reddening of the skin due to inflammation) within hours or days, and epilation (removal of hair) after about 2 to 3 weeks, which is a confirmatory finding.

Laboratory test indicators are cytogenetic, hematological and biochemical indicators.

Cytogenetic indicators—also known as cytogenetic dosimetry or biological dosimetry based on cytogenetic methods—are currently the more effective biological dosimeter. They have eventually become a routine component of radiation safety programs. From four possible cytogenetic methods currently available, the most consistent method is based on the significant increase of the frequency of chromosomal aberrations, in particular dicentrics, in peripheral blood lymphocytes as a result of irradiation. Relevant calibration curves for aberrations can be obtained using human lymphocytes in vitro, then, it is possible to use the frequency of aberrations measured in lymphocytes to estimate radiation dose. A dicentric aberration is an unstable aberration whose frequency decreases with time after exposure. The dicentric aberration frequency depends on cell turnover rate and can be relatively long in nonproliferating cells. The background concentration of dicentric cells in unirradiated persons is low, as little as 1–2 dicentrics per 1,000 cells in T-lymphocytes, and there is little variability among individuals, so that small radiation-induced increases can be quantified. It must be kept in mind that the frequency of aberrations decrease with time, so this method is useful only for individuals recently exposed to radiation. This indicator can be used to estimate doses as low as 0.1 Gy, as well as to establish the homogeneity of exposure and if it is not homogeneous, estimate the part of the body irradiated. Below 0.1 Gy, the sample size required for statistically reliable results is so large that it is impractical to obtain [10].

Methods for estimating radiation dose using biological indicators have made rapid progress during the recent years. While dicentric chromosome method still plays a central role, it is no longer the only quantitative approach in biological dosimetry. Evaluation of the frequency of stable chromosome aberrations (those

Table 2.1 Prognosis of acute radiation damage by clinical indicators [9]

<table>
<thead>
<tr>
<th>Dose (Gy)</th>
<th>Initial symptoms</th>
<th>Critical period</th>
<th>Post exposure prognosis</th>
<th>Lethality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Incidence</td>
<td>Time until onset</td>
<td>1–48 h</td>
<td>Percent</td>
</tr>
<tr>
<td>&gt;50</td>
<td>100</td>
<td>minutes</td>
<td>1–48 h</td>
<td>Hopeless</td>
</tr>
<tr>
<td>10–15</td>
<td>100</td>
<td>30 min</td>
<td>5–14 days</td>
<td>Really bad</td>
</tr>
<tr>
<td>5–10</td>
<td>100</td>
<td>0.5–1 h</td>
<td>2–6 weeks</td>
<td>Poor</td>
</tr>
<tr>
<td>2–5</td>
<td>50–90</td>
<td>1–2 h</td>
<td>2–6 weeks</td>
<td>Poor</td>
</tr>
<tr>
<td>1–2</td>
<td>0–50</td>
<td>&gt;5 h</td>
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<td>0–1</td>
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<td>Excellent</td>
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that do not decrease with time) has been made possible by techniques that measure translocations between chromosomes. This is done by evaluating banded chromosome preparations or by using a less accurate but more rapid size-grouping method. Such techniques were useful in measuring aberrations in the survivors of the atomic bombing of Hiroshima and Nagasaki at long times after radiation exposure occurred [10, 11].

A promising cytogenetic method—particularly when receiving protracted exposures or if the exposure occurred a long time ago—is the “fluorescent in situ hybridization” (FISH) (commonly called the chromosome painting technique), which can be used to further define stable chromosome aberrations and obtain full genomic translocation frequencies. Since this method allows scoring both dicentrics and translocations in the same cell, it is considered of improved efficiency for detecting exchange aberrations and for enabling a higher confidence in estimating radiation dose [12]. Theoretically, it could detect lower doses than is possible using conventional cytogenetic methods.

Further cytogenetic indicators in process of study include MN (micronuclei) analysis and premature chromosome condensation (PCC) analysis [11]. The first is based on the measurement of micronuclei in populations of exposed cells. Micronuclei are formed when cells with broken chromosomes divide; the evaluation of micronuclei is then much easier to perform than is of chromosome. To ensure that only dividing cells are scored, cells are treated with cytochalasin B, which blocks cytokinesis—a process whereby the cytoplasm of a single cell is divided to spawn two daughter cells—and results in binucleated cells. Only the binucleated cells are evaluated for the formation of micronuclei. The PCC analysis consist of the fusion of human lymphocytes with Chinese hamster ovary mitotic cells in the presence of a fusing agent, polyethylene glycol, to enable the measurement of chromosomal aberrations immediately following irradiation without the perturbing influence of processes associated with cell cycle progression to mitosis (cell division) [11].

A common hematological indicator as peripheral blood count can be especially valuable for an early diagnostic and prognosis of the severity of the damage after exposure; it might also serve to obtain a gross dose estimate. Hematopoietic system is known to be one of the most radiosensitive and the reason why blood cell count has maintained a time-honored position as a screening indicator for various disease states. From 0.5–1 Gy it will be well-observed identified changes in the peripheral blood cell counts, like an increase in neutrophil count, severe lymphopenia, and a decrease in the platelet count (thrombocytopenia). Lymphocytes are especially sensitive to radiation, and they may succumb to interphase death after exposure to a dose of only 0.05–0.15 Gy.

There are also some biochemical indicators that play an important role in diagnostic and prognosis of acute radiation damage. Conventional biochemical indicators can be used based on the fluctuation of certain metabolites in human urine and blood after overexposure. They include creatine/creatinine, taurine, and alpha amylase, as well as ALAT (serum alanine aminotransferase), ASAT (aspartate aminotransferase), and γGT (γ-gluamyl transpeptidase), but none of
these parameters is specific to radiation-induced damage with the exception of alpha amylase in serum, which is specific for radiation damage to the parotid glands.

Management of more recent accidents has demonstrated that there are two more biological indicators specific to certain vital importance organs. They are blood citrulline level as an indicator of radiation damage to the intestinal epithelium [13], and Flt3 blood ligand concentration as an indicator of radiation damage to the bone marrow [14]. Flt3 ligand is a ligand for the FLT3 tyrosine kinase receptor and belongs to a small group of growth factors that regulate proliferation of early hematopoietic cells.

These indicators are not extremely practical as biological dosimeters but they offer the physician valuable qualitative information on the condition of the injured individual. In addition, when the dose received is distributed heterogeneously at organ and organism level, the knowledge of the global irradiation dose is important but not sufficient by itself.

References

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