IRPA 11

CURRENT TRENDS IN RADIATION PROTECTION

Edited by H. Métivier, L. Arranz, E. Gallego and A. Sugier

EDP SCIENCES

Current Trends in Radiation Protection

On the occasion of the 11th international congress
of the international radiation protection association
23-28 May 2004, Madrid, Spain

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The editing of this book has been supported by :

- Sociedad Espanola de Proteccion Radiologica

and

- Comunidad de Madrid,
Consejería de Sanidad y Consumo

- Consejo de Seguridad Nuclear

- Empresa Nacional de Residuos Radiactivos, S.A.

- World Health Organisation

Cover Design : Eric Sault

ISBN 2-86883-725-5

Printed in France

FOREWORD

With the objective of spreading the Radiation Protection cultural context, and to facilitate its understanding by the public, this book contains a compilation of the main lectures pronounced between May 23 and 28, 2004, with the occasion of the 11th International Congress of the International Radiation Protection Association (IRPA11).

This volume contains a summary of the advances in the Radiological Protection field and its main application areas, which undoubtedly will have a direct impact to the most immediate future. When introducing it, I wish to devote an emotive remembrance to the memory of the eminent scientist Dan Beninson, who unfortunately did not live enough to participate in this Congress that he enthusiastically supported since its initial project.

I also wish to express mi deepest and sincere gratitude to all the authors, to Henri Métivier, the genuine promoter of this publication, and to all those who helped me towards the success of this great scientific meeting.

Leopoldo Arranz
IRPA Vice-President for Congress Affaires
Chairman of IRPA 11 International Congress Organising Committee

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INTRODUCTION

The IRPA congresses constitute unique opportunities to gather all actors of radiological protection, irrespective of their origin or their field of action in the vast world of radioprotection. The IRPA11 Congress in Madrid will perpetuate this tradition at the very moment where all stakeholders, whether researchers, radioprotectionists, regulators, private industrial operators or national institutions, feel a need for widening the scope of this profession.

Radiological protection falls now under the public's active scrutiny. Its perception of risks and the measures taken to reduce them as much as possible, whilst maintaining the activities necessary to the well being of humankind need now to be explained. This congress directly fulfils this new line of action, a perfect illustration lying in the title that has been chosen: "Widening the radiation protection world".

However, this widening should also become apparent on the libraries shelves and the fruitful debates of IRPA congresses should evolve beyond the restricted circles of specialists. This is why we have launched this new series of books "Trends in radiation protection" which will spread the state of the art in radiological protection to the entire world, researchers, regulators, experts. In order to guarantee an information of high quality and devoid of passion, we have called on board renowned experts in their various fields of activities. Their immediate agreement upon our invitation constitutes the first success of this book. We are very grateful to all of them without whom this book would not exist.

While keeping a single objective, radioprotection, the book offers a variety of topics.

For us, it was clear that a synthesis of biological knowledge deserved to start the book with, but as this was traditionally oriented towards humans, we have deliberately wanted also to review the status of the necessary evolution towards environmental protection.

ICRP recommendations being endorsed throughout the world, a critical analysis of their impact on current life was necessary: how are the recommendations applied, what are the physical tools necessary for their application, how professionals must be educated and especially the medical world that is the big responsible for the collective irradiation of populations.

But beyond such recommendations and the application of rigorous safety rules, incidents with radioactive sources and nuclear accidents remain possible. It is thus necessary to reconstruct such difficult situations in terms of exposure, to properly evaluate the contamination of the affected territories, and to promote coherent and practical proposals for remediation. The experimental feedback from such situations is a tremendous source of progress for their prevention.

In addition, radiological protection also addresses non-ionizing radiation: citizens are increasingly concerned about mobile telephones, and less by the nevertheless omnipresent lasers. This aspect also captured our attention when constructing this book.

We believe that this book will answer a number of questions that are asked by the various actors of our society who are not necessarily experts in our field. The pedagogical efforts of the authors would allow to meet our objective: "Widening the radiation protection world".

H. Métivier, L. Arranz, E. Gallego, A.Sugier
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Current trends in radioprotection

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H. Métivier, L. Arranz, E. Gallego and A. Sugier (eds.)

Non-Targeted Effects of Ionizing Radiation: Implications for Radiation Protection

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Abstract. It is widely accepted that damage to DNA is the critical event in irradiated cells, and that double strand breaks are the primary DNA lesions responsible for the biological effects of ionizing radiation. This has led to the long standing paradigm that these effects, be they cytotoxicity, mutagenesis or malignant transformation, occur in irradiated cells as a consequence of the DNA damage they incur. Evidence has been accumulating over the past decade, however, to indicate that radiation may induce effects that are not targeted to the irradiated cell itself. Two "non-targeted effects will be described in this review. The first, radiation-induced genomic instability, is a phenomenon whereby signals are transmitted to the progeny of the irradiated cell over many generations, leading to the occurrence of genetic effects such as mutations and chromosomal aberrations arising in the distant descendants of the irradiated cell. Second, the bystander effect, is a phenomeon whereby irradiated cells transmit damage signals to non-irradiated cells in a mixed population, leading to genetic effects arising in these "bystander" cells that received no radiation exposure. The model system described in this review involves dense monolayer cultures exposed to very low fluences of alpha particles. The potential implications of these two phenomena for the analysis of the risk to the human population of exposure to low levels of ionizing radiation is discussed.

1. INTRODUCTION

Ionizing radiation has many unique characteristics as a mutagen and carcinogen. These derive from its ability to penetrate cells and tissues and to deposit energy within them in the form of ionizations; that is, the ejection of orbital electrons from atoms or molecules. This event may lead to irreversible damage in the molecule involved, or the resultant free radical (an atom or molecule containing an unpaired electron) may initiate a chain of chemical reactions mediated through cellular water with the ultimate biologic damage occurring in another molecule in the cell. Ionizing radiation is thus non-selective in the damage it produces, depositing

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energy at random by means of ionizations within all cells and tissues. Unlike most chemical agents, it is not organ specific in its effects. Its toxicity does not depend upon absorption, excretion, or localization within the body. It does not depend upon the presence of specific binding sites or receptors in cells, nor on mechanisms of activation or detoxification common to genotoxic chemical agents.

Ionizing radiation also has unique characteristics as a genotoxic agent in terms of the DNA damage it produces. Most chemical carcinogens and mutagens produce specific damage to DNA bases, often as a consequence of the formation of DNA adducts or alkylation products. This is also the case for ultraviolet light radiation. Such base damage is readily restored by metabolic nucleotide or base excision repair processes whereby the damaged base is excised and resynthesized using the complementary DNA strand as a template. While such damage can lead to mutations owing to inaccurate repair, base damage is not very cytotoxic to cells and appears to play a minor role in mutagenesis induced by ionizing radiation [1]. For example, an exposure to 254 nm ultraviolet light that kills 63% of the cells (D_{37}) will produce approximately 400,000 pyrimidine dimers in each cell whereas, for a similar level of cell killing, ionizing radiation will induce only 40 DNA double strand breaks.

The double strand break (DSB) is now considered to be the characteristic DNA lesion responsible for the biologic effects of ionizing radiation [1, 2]. In some experimental systems, it has been estimated that a single unrepaired DSB may lead to cell cycle arrest [3, 4], whereas a single complex DSB in a specific gene has a high probability of producing a mutation in that gene [5]. Double strand breaks can arise from opposed single strand breaks (SSB) arising from either random ionizations or free radical attack leading to breaks in the individual strands. It has been estimated that DSB can arise from SSB occurring in opposite strands within a distance of about 13 base pairs of each other. There is now considerable evidence, however, to indicate that most DSB are a consequence of the specific nature of the energy deposition and distribution of ionizations within DNA caused by radiation. This results in what has been termed "clustered damage", multiple closely associated DNA lesions including single strand breaks and base damage [1, 6]. Such clustered damage occurs after low as well as high radiation doses, and has a high probability of producing complex DNA double strand breaks which may be difficult for the cell to restore accurately by metabolic DNA repair processes [6].

1.1. DNA Repair Processes

There has been intense interest over the past decade in the metabolic processes by which cells repair DNA damage, specifically DSB. It has become evident that mammalian cells possess complex enzymatic pathways for the recognition, signaling and repair of DNA DSB. A detailed description of these pathways is beyond the scope of the present paper. The ATM (ataxia telangiectasia mutated) protein plays a central role in the damage recognition process [7] by detecting DSB and undergoing rapid autophosphorylation converting it to an active monomer [8], leading to phosphorylation of histone H2AX and subsequent signaling to a variety of downstream transducer and effector proteins. These include the Mre11-Rad50-NBS1 complex, which may also act as a damage sensor [9], as well as being involved in both non-homologous end joining (NHEJ) and homologous recombination, the two mechanisms specifically associated with the repair of DSB [10]. NHEJ repair occurs throughout the cell cycle, whereas homologous recombination takes place primarily in cells in the late S and G_2 [11]. Other proteins involved include the breast cancer susceptibility proteins BRCA1 and BRCA2, the latter being particularly associated with homologous recombination, as well as modifiers of DNA topology including the BLM (Bloom's Syndrome) and WRN (Werner's Syndrome) helicases.

The NHEJ pathway involves a specific group of proteins, the DNA-PK complex, which recognize broken ends and catalize their joining [12, 13]. This joining occurs with little or no requirement for sequence homology. The DNA-PK complex consists of the catalytic subunit DNA-TKcs, and two proteins called Ku70 and Ku80. While the molecular mechanisms by which these proteins effect endjoining are not fully

understood, recent evidence suggests that DNA-TKcs undergoes autophosphorylation in response to DNA damage and colocalizes with H2AX [14]. The phosphorylated form of DNA-TK_{cs} may be involved in recruiting the Ku70/Ku80 proteins to the broken ends, initiating their rejoining. Although the joining of broken ends is carried out efficiently by NHEJ repair, this process is error prone. Mammalian cells unable to carry out NHEJ are highly sensitive to the induction of large-scale mutations and chromosomal aberrations by ionizing radiation.

Non-Targeted Effects of Radiation

All of the above findings point to the DNA molecule as the critical target in the cell, and DSB as the critical radiation induced lesion. In reality, this has been an accepted paradigm for several decades. Early studies with microbeam irradiation identified the cell nucleus as the important target for the cytotoxic effects of radiation [15], and later studies showed that radiosensitivity was markedly influenced by DNA repair processes [16]. Radiation exposure confined to the DNA molecule by incorporation of the Auger electron emitting radionuclide Iodine-125 incorporated into Iododeoxyuridine was extremely cytotoxic and mutagenic [5]. The intense release of energy occurring within a few base pairs of the site of decay of the Iodine-125 in DNA leads to the production of complex DNA double strand breaks which are difficult to accurately repair.

A corollary assumption following on this paradigm was that the biological effects of radiation in cells, be they cytotoxicity, mutations or malignant transformation, would occur in the irradiated cells themselves presumably as a consequence of the DNA damage they incurred. Evidence has been accumulating over the past decade, however, indicating that this may not always be the case. It has become evident that radiation can induce a type of genomic instability in irradiated cells that is transmitted to their progeny over many generations of cell replication, leading to enhanced rate at which genetic effects such as mutations and chromosomal aberrations arise in the distant descendants of the irradiated cell. It has also been discovered that irradiated cells may transmit damage signals to non-irradiated cells in a mixed population leading to the occurrence of such genetic effects in these "bystander" cells that receive no radiation exposure. These two phenomena have been termed "non-targeted" effects of radiation. Discussion will be limited in this review to bystander effects observed in dense monolayer cell cultures. There is also an extensive literature on effects arising in normal cells incubated in conditioned medium from irradiated cells, owing to factors released into the medium.

Radiation-Induced Genomic Instability

Early evidence for this phenomenon arose from an examination of the kinetics of radiation-induced malignant transformation of cells in vitro [17, 18]. Transformed foci did not appear to arise from a single radiation damaged cell; rather, radiation appeared to induce a type of instability in 20-30% of the irradiated cell population which had the effect of enhancing the probability of the occurrence of a second neoplastic transforming event. This second event was a rare one and involved the actual transformation of one or more of the progeny of the original irradiated cells after many rounds of cell division. This transforming event occurred with a constant frequency per cell per generation, and had the characteristics of a mutagenic event [18]. These findings were consistent with the hypothesis that radiation induces transmissible genetic instability in cells that enhances the rate at which malignant transformation or other genetic effects arise in the descendants of the irradiated cells after many generations of cell replication.

This hypothesis was subsequently confirmed for the induction of specific gene mutations [19, 20] and chromosomal aberrations [21]. This phenomena is usually studied by examining the occurrence of such genetic effects in clonal populations derived from single cells surviving radiation exposure. In terms of mutagenesis, approximately 10% of clonal populations derived from irradiated single cells showed a significant elevation in the frequency of spontaneously arising mutations as compared with clonal populations derived

from non-irradiated cells [20, 22]. This increased mutation rate persisted for approximately 30 generations post-irradiation. The molecular structural spectrum of these late-arising mutants resemble those of spontaneous mutations in that the majority of them are point mutations [22, 23], indicating that they arose by a different mechanism from that of direct x-ray-induced mutations which involve primarily deletions. An enhancement of both minisatellite [24] and microsatellite [25] instability has also been observed in the progeny of irradiated cells selected for mutations at the *thymidine kinase* locus, further evidence that a subpopulation of genetically unstable cells arises in irradiated populations. It is of interest that instability as measured in minisatellite sequences of x-ray-transformed mouse $10T\frac{1}{2}$ cells was markedly enhanced when the cells were grown *in vivo* as compared to prolonged cultivation *in vitro* [26].

An enhanced frequency of non-clonal chromosomal aberrations was first reported in clonal descendants of mouse hematopoietic stem cells examined 12–14 generations after exposure to alpha radiation [21]. Persistent radiation-induced chromosomal instability was subsequently demonstrated in a number of other cellular systems [22, 27–32]. Susceptibility to radiation-induced chromosomal instability differs significantly among cells from different strains of mice [31, 33], and similar differences in genetic susceptibility to radiation-induced chromosomal instability have been observed in different strains of human diploid fibroblasts [34]. The fact that Dugan and Bedford [35] found no evidence for induced chromosomal instability in a normal human diploid fibroblast strain may be related to such genetic factors [34]. Furthermore, delayed reactivation of p53 and a persistent induction of reactive oxygen species have been reported in normal human fibroblasts [36] as well as in human fibrosarcoma cells [37].

A persistently increased rate of cell death also occurs in cell populations many generations after irradiation [38–40]. This phenomenon has been variously referred to as "lethal mutations" or "delayed reproductive failure", but has been measured as a reduction in the ability of cells to attach and form macroscopic colonies in a classic clonogenic survival assay. In some cellular systems, an increased rate of apoptotic cell death has been shown to accompany this phenomenon [40–42]. Persistent reproductive failure has been linked to chromosomal instability [42] and malignant transformation [43, 44], and evidence presented to suggest that DNA is at least one of the critical targets in the initiation of this phenomenon [45]. Instability was attenuated by treating the irradiated cells with free radical scavengers or allowing potentially lethal damage to be repaired by confluent holding prior to analysing the subsequent development of chromosomal instability [46]. It has been proposed that oxidative stress perhaps consequent to enhanced, p53-independent apoptosis may contribute to the perpetuation of the instability phenotype in these populations [42, 44].

The transmission of chromosomal instability *in vivo* has been reported in several distinct experimental models [47–50], though not in others [51]. Evidence for transmissible instability in irradiated human populations is at present weak [52, 53]. While it has been suggested that instability induced in X-irradiated mouse hematopoietic stem cells may be related to the occurrence of the non-specific genetic damage found in radiation-induced leukemias in these mice [54], other work from this laboratory indicates that susceptibility to radiation-induced leukemia/lymphoma is generally separable from sensitivity to induced genomic instability [55].

One interesting model involves the induction of mouse mammary tumors by radiation. Sensitivity to tumor induction was found to be strain specific and to correlate with the induction of chromosomal instability in mammary epithelial cells irradiated *in vivo* [50]. The induction of such instability was dose dependent. It was subsequently shown that reduced expression of the DNA repair protein DNA-PKcs occurred in the sensitive, cancer-prone mouse strain (BALB/c), leading to inefficient end-joining of DNA double strand breaks induced by radiation [56]. This finding is of interest in relation to the evidence for the involvement of chromosome telomeres in radiation sensitivity and genomic instability [57]. DNA-PKcs has been shown to play an essential role in telomere function and capping [58, 59]. A high frequency of telomere fusions occur in DNA-PKcs deficient cells [59]; the loss of telomeres has been associated with

the development of chromosomal instability in cancer cells [60]. Transmissible instability might thus be a consequence of successive bridge-breakage-fusion cycles resulting from telomere loss.

The Bystander Effect in Irradiated Cell Populations

The experimental model employed in the studies of the bystander effect to be discussed here involves the exposure of dense monolayer cultures of mammalian cells to very low fluences of alpha particles, fluences whereby only a very small fraction of the nuclei in a cell population will actually be traversed by an alpha particle. This may be accomplished by irradiation from an external source of alpha particles [61] or by use of precision microbeam irradiators whereby specific cells can be targeted [62–64]. The first evidence for this phenomenon was derived from studies of the induction of sister chromatid exchanges (SCE) in monolayer cultures by very low fluences of alpha particles from an external source [65]. An enhanced frequency of SCE was observed in 20–40% of the cells exposed to fluences whereby only about 1/1000 to 1/100 cell nuclei were actually traversed by an alpha particle. This finding was later confirmed and evidence presented to suggest that the phenomenon involved secretion of cytokines or other factors by irradiated cells leading to the upregulation of oxidative metabolism in bystander cells [66, 67].

An enhanced frequency of specific gene mutations also occurs in bystander cells in populations exposed to very low fluences of alpha particles [68]. As a result, the induced mutation frequency per alpha particle track increases at low fluences where bystander as well as directly irradiated cells are at risk for the induction of mutations. This leads to hyperlineality of the dose-response curve in the low dose region, and thus a greater effect than that predicted by a linear extrapolation from higher doses. Studies with various sources of microbeam irradiation have provided evidence for an enhanced frequency of micronucleus formation, cell killing and apoptosis in bystander cells [64, 69–71], as well as an enhanced frequency of mutations [72, 73] and malignant transformation [74].

Changes in gene expression also occur in bystander cells in monolayer cultures; the expression levels of p53, $p21^{Waf1}$, CDC2, cyclin-B1 and rad51 were significantly modulated in non-irradiated cells in confluent human diploid cell populations exposed to very low fluences of alpha particles [75]. These experiments were carried out by western blotting and *in situ* immunofluorescence staining techniques utilizing confocal microscopy; although only about 1–2% of the cell nuclei were actually traversed by an alpha particle, clusters of cells showed enhanced expression of $p21^{Waf1}$. This phenomenon involved cell-to-cell communication via gap junctions [75, 76], as has also been shown for micronucleus formation [77] and mutations [73]. It appears that radiation exposure itself can enhance intercellular communication as evidenced by an upregulation of Connexin 43 [78]. Evidence for DNA damage in bystander cells was provided by examining micronucleus formation, a surrogate measure of DNA damage; that the upregulation of the p53 damage response pathway in bystander cells was a consequence of this DNA damage is supported by the observation that p53 was phosphorylated on serine 15 [76].

DNA damage occurring in bystander cells, however, appears to differ from that induced in directly irradiated cells. Mutations induced in directly irradiated cells are primarily partial and total gene deletions, whereas over 90% of those arising in bystander cells were point mutations [79]. This finding would be consistent with the evidence that oxidative metabolism is upregulated in bystander cells [67, 80], and has led to the hypothesis that the point mutations are a result of oxidative base damage occurring in bystander cells [79]. A similar mechanism has been proposed for the observation that localized cytoplasmic exposure from a microbeam irradiator led to a significant increase in the frequency of point mutations which appeared to involve the generation of reactive oxygen species [81].

Bystander cells defective in the NHEJ DNA repair pathway including mouse knockout cell lines for Ku80, Ku70 and DNA-PKcs are extremely sensitive to the induction of mutations and chromosomal aberrations [82, 83]. The mutations in these repair deficient bystander cells were primarily the result of partial and

total gene deletions [83], whereas those in wild type bystander cells were predominantly point mutations. The marked sensitization of repair-deficient bystander cells to the induction of large-scale mutations and chromosomal aberrations may be a consequence of unrejoined DNA double strand breaks occurring as a result of clustered damage arising from opposed oxidative lesions and single strand breaks.

In earlier studies, it was reported that alpha particle irradiation could induce the intracellular generation of reactive oxygen species (ROS) including the superoxide anion and hydrogen peroxide [67]. The role of oxidative stress in modulating signal transduction and micronucleus formation in bystander cells was examined in confluent monolayer populations of human diploid cells exposed to low fluences of alpha particles [80, 84]. The results support the hypothesis that superoxide and hydrogen peroxide produced by flavin containing oxidase enzymes mediate the activation of several stress inducible signaling pathways as well as micronucleus formation in bystander cells. These include the p53 damage response pathway as well as the MAP kinase family of signaling pathways. It has also been reported that nitric oxide may initiate intercellular signal transduction pathways influencing the bystander response to radiation [85, 86]. It thus appears that ROS may be the primary mediators of the bystander response, reminiscent of the effect associated with radiation-induced genomic instability [44, 46]. The activation of MAP K proteins and their downstream effectors in bystander cells [80] is of particular interest in terms of the observation that membrane signaling is involved in the bystander effect in monolayer cultures [87].

Overall, these results support the hypothesis that an upregulation of oxidative metabolism occurs in bystander cells in monolayer cultures. This conclusion is consistent with findings in other model systems [64, 88, 89], and suggests that oxidative metabolism is intimately involved in the bystander response for mutations and chromosomal aberrations. Over 90% of the mutations occurring in bystander cells were point mutations [79] as are classically associated with oxidative base damage. ROS can induce DNA double strand breaks, particularly as a result of opposed oxidative lesions and single strand breaks. However, most of these DSB should be restored in normal cells by recombinational repair, leaving oxidative base damage as the primary mutagenic lesions. When the NHEJ pathway is inactivated, however, DSB repair is compromised and a markedly increased bystander effect was observed; that is, many more bystander cells in the population were susceptible to the induction of these genetic effects.

This hypothesis is consistent with the finding that mutations occurring in repair deficient bystander cells were primarily partial and total gene deletions [83], as would result from mis-repaired or non-repaired DSB. The marked increase in the fraction of cells with gross chromosomal aberrations [82] is also consistent with this finding. The relatively small bystander effect for mutagenesis and chromosomal aberrations in wild type cells is thus a consequence of oxidative base damage to DNA. When the bystander cells in the population cannot repair DNA double strand breaks, however, they become much more sensitive to the induction of these genetic effects as manifested by deletion mutants and gross chromosomal aberrations.

The results of all of these studies indicate clearly that damage signals can be transmitted from irradiated to non-irradiated cells. In confluent monolayer cultures, this phenomenon involves gap junction mediated cell to cell communication, and appears to involve both the induction of reactive oxygen species and the activation of extra-nuclear signal transduction pathways. Multiple biological effects may occur in bystander cells including cell killing, the induction of mutations and chromosomal aberrations, and the modulation of gene expression. Some evidence suggests that regulation of the p53 damage response pathway may be central to this phenomenon. Finally, preliminary studies with co-culture models both *in vitro* [90–92] and *in vivo* [93], as well as with tissue explant models [94] and a mouse bone marrow stem cell transplant system [49], suggest that a bystander effect may occur *in vivo*.

Implications for Radiation Protection

Loeb *et al* [95] and others have postulated that early in the process of carcinogenesis a mutation may arise in a gene that is important in maintaining genomic stability, yielding a cell lineage with a mutator phenotype.

This phenotype would enhance the frequency with which spontaneous mutations arise in these cells, and thus facilitate the accumulation of the requisite number of genetic events necessary to produce an invasive cancer. Such an example involves hereditary non-polyposis colon cancer which is associated with a germline defect in DNA mismatch repair. While genomic instability is a hallmark of tumor cells, most types of cancer have not been associated with specific DNA repair defects.

The finding that radiation itself may induce an instability phenotype has thus attracted considerable interest. It would suggest that the initial radiation-induced event may be a frequent one involving as many as 10 – 20% of the population, rather than a rare mutagenic event. This increased level of instability which is transmissible over many generations of cell replication would thus enhanced the rate at which multiple genetic events important to the development of cancer would arise in the cell population. However, the degree to which this radiation-induced phenomenon may be of importance in carcinogenesis remains unknown. The fact that it appears to saturate at fairly low doses (of the order of 10-50 cGy) implies that it could influence the extrapolation to low dose effects. Additional research is clearly needed to determine the mechanisms involved in radiation-induced genomic instability, in terms of both the initiating event and how the effect is transmissible for many generations of cell replication, before its implications for the assessment of the carcinogenic risk of low dose, low dose-rate exposure to ionizing radiation can be clarified.

Another area where this phenomenon could well be of significance involves potential transgenerational effects of irradiation. The sum of the available evidence suggests that such instability is induced in the germ cells of irradiated parents and is transmitted to the offspring born to them [96]. If exposure to low levels of ionizing radiation thus induces the instability phenotype in germ cells of the offspring of irradiated parents, it is entirely feasible that this instability could increase their susceptibility to cancer or other genetic effects. For example, Pils *et al* [97] reported that genomic instability manifested by lethal and teratogenic effects may be passed on to two successive generations of offspring in mice after irradiation of the zygote, while Niwa and Kominami [98] and Dubrova and his colleagues [99, 100] presented evidence for transmissible germline instability at mouse minisatellite loci. There is preliminary experimental evidence to suggest that an increased susceptibility to the induction of tumors may occur in the offspring of irradiated mice [101, 102]; the induction of transmissible genomic instability by radiation in germ cells would provide a mechanism for such transgenerational effects.

The bystander effect has clear implications in terms of human exposures to very low fluences of high LET particulate radiation, such as alpha particles from environmental radon or densely-ionizing galactic cosmic rays in space [103]. In the case of radon, for example, only a small fraction of a person's bronchial epithelial cells, the presumed target for lung cancer, will be hit each year by an alpha particle arising from residential radon exposure. In the past, the genetic or carcinogenic risk has been assumed to be related directly to the number of cell nuclei actually traversed by an alpha particle, thus yielding a linear dose response relationship. The evidence that irradiated cells may transmit damage signals to neighboring non-irradiated cells that result in genetic alterations in these "bystander" cells would invalidate this assumption. Rather, it would suggest that the dose-response curve may be non-linear at low mean doses yielding a greater effect than that predicted on the basis of the dose received by individual cells at low alpha particle fluences.

Evidence for the convergence of these phenomenon is also of interest [104, 105]. Studies involving both *in vitro* and *in vivo* assays have shown, for example, that transmissible genomic instability may arise in bystander cells [106, 107]. Defects in the NHEJ DNA repair pathway have been associated with both radiation-induced genomic instability [56] and the bystander effect [82]. It has been reported that conditioned medium from certain (but not all) unstable clones harvested many cell generations post-irradiation is highly cytotoxic to unirradiated cells [108]. Finally, oxidative stress manifested by enhanced levels of reactive oxygen species has been implicated in both phenomena.

When considered as a whole, the emerging results suggest that the risk of low level exposure to ionizing radiation remains uncertain; a simple extrapolation from high dose effects may not always be justified. In some cases, such as the induction of mutations by exposure to very low fluences of high LET particles, or as reported for the cytotoxic effects of very low doses of x-rays [109], the effect may be greater than predicted by a linear extrapolation from higher doses. On the other hand, certain studies of malignant transformation have revealed a reduced effect for very low doses [110, 111]. Overall, however, these findings imply that the biological effects of radiation in cell populations may not be restricted to the response of individual cells to the DNA damage they receive, but rather that tissues respond as a whole. A better understanding of the mechanisms for these phenomenon, the extent to which they are active *in vivo*, and how they are interrelated is needed before they can be evaluated as factors to be included in the estimation of potential risk to the human population of exposure to low levels of ionizing radiation.

ACKNOWLEDGMENTS

Supported by Research Grant DE-FG02-98ER62685 from the U.S. Department of Energy.

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take into account the complexity of the situation and as a consequence, in the absence of public involvement, they generate a phasing out of the personal initiative among the population and a general feeling of abandonment and fatalism. They also generate a dependency culture within the affected population, social distrust and loss of confidence in authorities and experts. The ETHOS Project has demonstrated among other that, because exposures are mainly driven by individual behaviour and family modes of living, and because collective countermeasures fail to take into account the individual situations, the active involvement of the population and the local authorities and professionals in the assessment and management process of the radiological situation, is feasible and necessary to break the vicious circle of exclusion, loss of control and fatalism.

From the methodological point of view, the ETHOS project, like other types of public participation approaches reveals the recurrent following features in the stakeholder involvement process which are also the keys for success [5]:

- Participation of a wide panel of stakeholders. This is especially important to avoid possible exclusion of persons or groups which can reveal to be in fact key actors in the process. The structure developed to involve the stakeholders must clearly allow their possible and easy withdrawal in order to favour their voluntary commitment;
- Empowerment of local people. This is a means to encourage the appropriation by stakeholders of the local situation and to favour their autonomy in the involvement process;
- Flexibility. It is a necessary feature to avoid crushing the initiative of local people, which could be prejudicial to their commitment first, and to the success of a project further on. It is also important to accept to change the strategies when identifying deadlocks and paying attention to "turning points" and "opportune moments" all along the intervention;
- Individual relationships between involved stakeholders. This must also concern the experts involved in the process. It is an important aspect to enable all those involved in a project to increase their self-confidence and to confront situations and personal interests;
- Working with all levels of authority and functions linked to the problem. In order to develop solutions to complex problems with multiple dimensions (health, environment, social, economic, etc) and authorities, experts and professionals at the local, regional, national and international levels must be involved and bridges must be build between these different levels.

Finally, the stakeholder involvement experience in the ETHOS Project has illustrated new forms of governance for the rehabilitation of contaminated territories based on actions developed in a common good perspective by all concerned parties. The classic form of scientific rationality, and particularly the basic concepts and principles of radiological protection, have been mobilised and appropriated by the involved actors to conduct an inclusive democratic process aiming at the construction of individual and collective choices adapted to the concerns of the population. The ETHOS experience has also demonstrated that to be sustainable the management of the situation by the stakeholders must rely on the dynamic of economic development grounded primarily on the individual initiatives of the local actors.

Following this perspective, the Belarus government initiated in October 2003 an international programme (Cooperation for Rehabilitation — CORE) to develop these new rehabilitation approaches during 5 years in 4 contaminated districts of the country based on a partnership between local, national and international stakeholders [11]. This programme includes the development of an inclusive and pluralist radiological monitoring at the local level to support the various initiatives of the population and the local authorities and professionals and to improve the health status as well as the social and economic situation in the territories. It also comprises an educational dimension to ensure the transmission to the future generations of the necessary know-how to live in a contaminated territory as well as the memory of the Chernobyl accident.

ACKNOWLEDGMENTS

The author wishes to particularly thank Samuel Lepicard from CEPN for his invaluable help in the preparation and drafting of this paper.

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