AGENDA FOR RESEARCH ON CHERNOBYL HEALTH

TECHNICAL REPORT

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INTRODUCTION

It is now 25 years since the Chernobyl accident, and, while a number of reviews of the health consequences of the accident have been made (see for example, (*1-3*), there is controversy over its consequences to date, and considerable variability in the assessment of the potential consequences in the future (*4*) (*5, 6*).

There is general agreement on the importance of the demonstrated health effects to date, particularly thyroid carcinoma. Studies of the atomic bomb exposures in Japan show the importance of late and unexpected consequences of radiation exposure, particularly solid cancers, for which a significant increase was not established until more than 25 years after the bombs, and cardiovascular complications which were first recognised more than 40 years later (*7, 8*).

The reported rise in the incidence of breast carcinoma in areas of high fallout after Chernobyl (*9*) suggests that other potential effects may occur in the future. The Agenda for Research on Chernobyl Health (ARCH) project was therefore created to advise on the scientific strategy needed for further research on the health consequences of the Chernobyl accident.

This strategy was developed on the premise that any future research should address the following important objectives:

- health improvement in those exposed to Chernobyl or to future nuclear accidents;
- a realistic assessment of present and future health consequences to aid health planning for those exposed after Chernobyl, and after future accidents; and
- improved understanding of radiation effects and direct future radiation protection measures.

These are wide-ranging objectives and to achieve them ARCH built on existing reviews, new results and the knowledge and experience of experts. With the help of the Expert Group and Advisors, the ARCH Core Group outlined a practical strategy combining epidemiological studies using large-scale surveillance with studies focused on specific issues. This has resulted in two documents:

- the Strategic Research Agenda (SRA) http://arch.iarc.fr/documents/ARCH_SRA.pdf
- and the current report, which complements and supports the SRA, with a review of the state of the art and questions arising from Chernobyl and motivated project proposals which the Expert Group felt were essential for the short, medium and long-term to maximise the information that can be drawn about radiation effects from the Chernobyl accident.

In addition, recent advances in radiobiology and their relevance to the consequences of the Chernobyl accident have been considered by a subgroup of ARCH and this document is also submitted.

While the health impact of the Chernobyl accident has been widely studied, particularly in the first decade after it occurred, it has not been comprehensively studied, as have the effects of the atomic bombings in Japan. Research on the outbreak of thyroid cancer in those exposed as children has been intensive, while claims about possible effects on the immune system have received little scientific attention outside the affected countries. Nevertheless the accident has already proved uniquely illuminating in some aspects such as:

- in providing unique information concerning the high sensitivity of the child's thyroid to radioactive isotopes of iodine and factors which may modify this risk, including iodine deficiency and supplementation (*10-13*);
- suggestions of an increased risk of leukaemia following low dose protracted exposures among liquidators (*14*) (*15*)
- evidence for the non-threshold nature of radiation cataracts (*16*);

- the discovery of the inheritance of mini-satellite mutations by children born after the accident but whose fathers were exposed to Chernobyl fallout (*17, 18*).

The fact that no other radiation-related health effect has yet been clearly demonstrated does not mean that no increase has occurred or will occur in the future. Many of the studies conducted to date provide little information about radiation risks because of a number of methodological limitations. Further, based on the experience of other populations exposed to ionising radiation, a small increase in the relative risk of cancer, at least, is expected, even at the low to moderate doses received. In addition, because radiation-related diseases continue to occur decades after exposure, it is certainly too early to evaluate the full radiological impact of the accident (*19, 20*).

Although ionising radiation is one of the most studied carcinogens in our environment, much of the knowledge about its effects on human beings comes from observations of Japanese atomic bomb survivors who were exposed to very high dose-rate external whole body radiation and of patients who received very high doses for therapeutic purposes. Major questions in radiation protection today relate to the choice of models used to interpolate risk between populations with different background disease rates; for projection of risk over time; for extrapolation of risks following primarily external high dose and high dose-rate exposure to low dose and low dose-rate exposures (*21-23*). This is particularly important for exposures resulting, as they do in the case of the Chernobyl accident, from a mixture of external and internal radiation, as current risk estimates for internally incorporated radionuclides are very uncertain (*24*). The populations exposed after Chernobyl range from the early liquidators, whose exposure was dominantly to high levels of external radiation, through later liquidators whose exposure was a mixture of external and internal exposure, to the general population whose exposure was mainly to internal radiation from fallout.

Major questions vital to the above mentioned uncertainties also remain about the risk of non-cancer diseases following low levels of ionising radiation and concerning non-targeted effects of radiation (*22, 23*)indeed, the mechanistic dogma underlying the effects of ionising radiation has been challenged by the phenomena of genomic instability and bystander effect. At present no consensus has been reached on the underlying mechanisms for these non-targeted effects.

While new technologies, genome wide sequencing for example, have been developed and used to explore fundamental aspects of biology and genetics over the past decade, studies have also stressed the importance of epigenetic processes in determining phenotype and in carcinogenesis (*25*). That radiation induced genomic instability is mediated by epigenetic effects has been proposed (*26*) and endorsed (*27*). There is presently no consensus as to the precise basis for the epigenetic process involved but attempts to unify the cancer and non-cancer effects of radiation based on a purely epigenetic regulatory system for the cell (*28*) have been proposed by the ARCH radiobiology group (*29*). The Radiobiology Report is attached as an addendum to this report.

Careful studies of populations exposed following the accident are therefore potentially able to provide important answers to some of these questions and to test hypotheses generated both in respect of processes underlying radiation action and biology/epigenetics in general. As such, they may have important consequences for radiation protection in general, for action required following future nuclear emergencies, and, potentially, for monitoring and promoting the health of people exposed as a result of the Chernobyl accident.

To make the best use of this unique opportunity to increase our understanding of radiation effects, the ARCH project has developed a long-term strategic plan for research into the health effects of the Chernobyl accident and the specific individual project proposals set out in this document.

During the development of these proposals stakeholders, including the general public, national and international bodies, were invited to contribute to the assessment of the proposed research on the better understanding of effects of radiation, particularly low dose and low dose rate radiation, and implications for public health decision making.

Issues that were addressed include:

- are there sufficient grounds for health monitoring aimed at detecting currently unrecognised effects of radiation?
- what investigations, if any, might provide sufficient information to corroborate or alter our current understanding of radiation effects, including germ-line effects?

Because radiation from the accident travelled all over the Northern Hemisphere and particularly Europe, health consequences of the accident must be assumed to have occurred all over Europe (particularly in Belarus, Ukraine and the Russian Federation), although not necessarily at a level at which they could be detected, and are likely to continue to do so in the foreseeable future. Currently, no multinational organisation is taking responsibility for monitoring disease trends. An impartial review of the present position and a careful and critical assessment of the value of further studies were therefore needed, with the building of a strategic research agenda and that is what the EU-funded ARCH project has achieved.

Finally, a major expansion of nuclear power generation now seems likely in the coming decade, in Europe, as well as in countries with different safety cultures. The risk of accidents similar to the Chernobyl accident cannot be eliminated and there have been a number of close calls since Chernobyl. Nuclear accidents have trans-boundary consequences and thus can lead to social and economic costs in neighbouring countries. National authorities will, in the event of an accident, be required to give assurances about the likely public health impact. Nearly twenty-five years after the accident, unfortunately, the international response to the Chernobyl accident cannot be described as a success. Many will find it surprising that the health effects of a major nuclear catastrophe which occurred in a European country and led to fallout affecting virtually the whole of Europe have not been the subject of a comprehensive ongoing European study. The failure of the scientific community to reach a consensus over the likely extent of health damage has undoubtedly contributed to the psychosocial effect and has undermined the confidence of the general public in the safety of nuclear power generation.

It is with this background that ARCH assembled a group of experts with knowledge on the health consequences of the Chernobyl accident that is dispersed throughout Europe and among the three most affected countries. A 'scoping study' was conducted to advise on future needs for research as well as on potential value of the proposed research for public health decision making in the affected countries. The outcome of this work has led to the development of a Strategic Research Agenda and to the present Technical Report. The Strategic Research Agenda suggests the organisation needed to oversee comprehensive studies of Chernobyl health effects, and recognises the importance of the formation of life-span cohorts.

The present document is the Technical Report which details the specific project proposals, including proposals for the formation of the life-span cohorts to facilitate both general surveillance of the consequences and the individual more focussed studies.

ORGANISATION OF WORK

To meet the aims for which ARCH was supported by the Commission the work was carried out by a Core group and a group of experts and advisors (listed below). Position papers and outlines of a Strategic Research Agenda and a wide range of possible projects suggested by the Expert Group were prepared by the Core group. These were then discussed at meetings with the experts and advisors, where the papers were discussed, modified, new projects proposed and prioritised. The proposals and the prioritisation evolved during several meetings and by correspondence until overall agreement was reached on the SRA and on the projects and their prioritisation.

In more detail the work consisted of:

- Overview of current knowledge, ongoing projects and existing research recommendations and preparation of list of research questions which could in principle be answered by studies of Chernobyl consequences
- Overview of list of research questions agreed by expert group (type and design of study, requirements in terms of dosimetry, follow-up, biological markers and statistical power) and preliminary prioritisation
- Identification of current ("fast-tracked") research priorities, i.e. research that is both urgent and of demonstrated feasibility (prepared as Deliverable 1 and submitted to the European Comission).
- Identification of medium- and long-term research priorities, i.e. important research areas where studies cannot be conducted at present either because they would not be sufficiently informative yet or because feasibility/pilot work is needed before they can start; these may be the object of a further funding application;
- Assessments of strategic resource needs, added benefits over existing work, expected outcomes, timelines, risks, key assumptions about external factors for success;
- Development of project proposals.

The proposals for inclusion in Deliverable 1 were prioritized based on the following criteria:

1) Study of high scientific and social importance which if funded could start shortly;
2) Valuable work in progress which would collapse without urgent support;
3) Infrastructure forming an important resource for current and/or future projects, including those requiring urgent political discussion rather than short term financial support.

The current report includes all projects identified at the first Expert Group meeting and assessed in terms of their feasibility, importance (scientific and social) and priority for implementation.

ARCH MEMBERSHIP

Core group

The Core group was composed of those who conceived the proposal to the Commission. They were responsible for the overall organisation of the study, for writing the documents, in collaboration with members of an Expert group and advisors (see the description below), for modifying them in the light of the comments of the experts and advisors, and then, when priorities had been agreed by the Expert group, making final changes to meet the comments of the external reviewers (see also the description below).

The Core group consisted of the following members:

- Keith Baverstock, radiobiologist, University of Eastern Finland, Kuopio
- Elisabeth Cardis, epidemiologist, CREAL, Barcelona
- Ausrele Kesminiene, epidemiologist, IARC, Lyon
- Dillwyn Williams, pathologist, University of Cambridge.

Expert group and advisors

The Expert group included leading experts with considerable experience in the follow-up of the health consequences of the Chernobyl accident and representing the essential complementary disciplines: epidemiology, radiation biology, medicine (in particular endocrinology), dosimetry, pathology. The names were approved by the EC. They were chosen to cover all health aspects of the consequences of radiation exposure and included representatives of the three most affected countries and the EU. Members:

- Keith Baverstock, University of Eastern Finland (radiobiology and public health)
- Dmitryi Bazyka, Radiation Research Centre, Ukraine (epidemiology)
- Elisabeth Cardis, CREAL, Spain (epidemiology)
- Vadim Chumak, Radiation Research Centre, Ukraine (dosimetry)
- June Crown, UK (public health)
- Yuri Demidchik, Belarusian Medical Academy of Postgraduate Education, Belarus (thyroid treatment)
- Yuri Dubrova, University of Leicester, UK (genetics)
- Victor Ivanov, MRRC, Russia (epidemiology and risk assessment)
- Ausrele Kesminiene, IARC, France (coordination, epidemiology and medicine)
- Semion Polyakov, RSPC MT, Minsk, Belarus (cancer registration and public health management)
- Christoph Reiners, University Wurzburg, Germany (thyroid treatment)
- Margot Tirmarche, IRSN, France (epidemiology)
- Klaus Trott, Gray Cancer institute, UK, (medicine, non-cancer effects)
- Dillwyn Williams, University of Cambridge, UK (pathology and mechanism of cancer)

Scientists with significant experience in radiation research were also included as advisors to ensure harmonization with other existing or planned activities around the world:

- André Bouville, NCI, US (dosimetry)

- David Brenner, Columbia University, US (radiobiology)
- Vladimir Drozdovitch, Belarus, currently at NCI, US (dosimetry)
- Ian Fairlie, UK, (environment)
- Bernd Grosche, Federal Office for Radiation Protection, Germany (epidemiology)
- Sisko Salomaa, STUK, Finland (radiobiology)
- Richard Wakeford, University of Manchester, UK (epidemiology)
- Shunichi Yamashita, University of Nagasaki, Japan (thyroid diseases),

as well as the UNSCEAR secretary, Malcolm Crick and Zhanat Carr, WHO, Geneva.

The members of the Expert group and advisors met on three occasions. They reviewed and completed, when appropriate, in their area of expertise, draft position papers and documents prepared by the Core group and agreed on the priorities.

IMPROVEMENTS OF INTRASTRUCTURES - CHERNOBYL LIFE SPAN COHORT

The Chapter was prepared by E. Cardis, M.T. Do, CREAL, Barcelona, Spain and E.D. Williams, University of Cambridge, the UK

1) Cohorts of children with measured thyroid activity

Background

Two specific general population cohorts have been established in Belarus (the BelAm cohort) and Ukraine (the UkrAm cohort) based on a sample of all individuals aged younger than 18 years who lived in the most contaminated regions (oblasts) of these countries and whose thyroid activity was measured within two months after the accident (1). Sampling included all individuals with measured thyroid activity doses of 1 Gy or more and a random sample from two lower dose groups (0-0.29 and 3.39-0.99Gy). The cohorts, which include about 15,000 subjects from Belarus and the same number from Ukraine who have been traced and have agreed to be screened, have been supported financially by the US NCI and are administered jointly with the country concerned. They have been periodically screened for thyroid disease with ultrasound examination and palpation since 1998 and have provided valuable information on thyroid cancer and thyroid disease risk. The NCI is now discontinuing support for the active follow-up of the Bel-Am and Ukr-Am cohorts and only passive follow-up through cancer and mortality registries are planned.

Objective

To establish and commit to the long-term follow-up of these populations which can be the basis for studies of the long-term biological and health consequences of the accident.

Justification

This is a well defined cohort of 25,000-30,000 children from Belarus and the Ukraine exposed to radiation emitted from the Chernobyl accident. By design, it includes a large proportion of children from the most contaminated territories. It is rich in information necessary to study all types of thyroid diseases including, but not limited to, thyroid cancer. Active follow-up conducted in the past included systematic collection of blood and urine samples, thyroid palpation, and ultrasound examinations to determine structural abnormalities of the thyroid, medical history and analysis of thyroid hormone levels.

The existence of this cohort makes it a unique source of information not only for the study of thyroid diseases but also for a number of other outcomes for which screening is needed in a well-defined population with individual dose estimates. It would also be invaluable for the conduct of clinical and health services research.

Proposal

The potential for scientific contribution from this cohort to the radiation literature is very important. As such, it is proposed that an EC initiative should seek to join forces with the data custodians of this cohort in order to continue active screening of this population.

Study design

Since there are other pressing research questions that could be addressed using data collected by this cohort, we would suggest continuing the active screening of the population, but broadening the scope of the studies - looking not only at thyroid diseases but at a range of other diseases for which this population will be informative (including cataracts, etc.).

Dosimetry

Thyroid doses have been estimated for all subjects from ^{131}I and are underway for external radiation and intake of long-lived radionuclides. The approach used for reconstructing doses from the later sources should be applicable to the estimation of dose to other organs of interest.

Feasibility

The feasibility of conducting periodic active screening of this cohort has been demonstrated. Political boundaries may present an obstacle for collaborative efforts like the one proposed here. These cohorts have been set-up and followed-up under the framework of bilateral agreements with the US and negotiations will be required to explore the potential for possible collaborations.

Next steps and prioritisation

- Official EC contact with the data custodians of this cohort in order to continue active screening of this population.
- If agreement is reached, establishment of a trans-national steering committee to overview harmonisation, plan joint studies and review proposals from other groups for use of data.

It is urgent to start discussions with the governments of Belarus, Ukraine, as well as Japan and the US, to set up a joint funding mechanism in order to maintain existing BelAm Na Ukram cohorts and continue active screening of this population.

2) Cohorts of Chernobyl liquidators

Background

Cohorts of liquidators currently exist in Russia, Belarus, Ukraine, and the Baltic countries in the national Chernobyl Registries. This group consists of approximately 600,000 individuals, of whom about 240,000 worked in 1986 and 1987, when doses were highest, at the reactor site and the surrounding 30 km zone (*1*). The average recorded dose for these liquidators is about 100 mSv, with few individual doses over 250 mSv.

A number of nested case-control studies have been conducted successfully to evaluate the risk of thyroid cancer, leukaemia, and lymphoma associated with exposure to radiation from the Chernobyl accident. (*14, 15, 30*)

Objective

- To establish and commit to the long-term follow-up of this well-defined cohort which can be the basis for studies of the long-term health consequences of the accident.

Justification

This cohort would be invaluable in contributing new knowledge on the effects of low to moderate doses on a number of health outcomes of interest (including cancer and non-cancer outcomes, such as cardiovascular and cerebrovascular diseases). It is a large cohort with a wide range of low to moderate doses and as such it is the population of persons exposed to radiation after Chernobyl which is likely to be most informative, with the greatest statistical power, for the study of cancer and non-cancer effects.

Proposal

We recommend that registries of liquidators be maintained as accurately and completely as feasible and the data across countries be harmonized. Data from these registries would be invaluable to future studies in identifying eligible study subjects and ascertaining outcomes of study interests.

Study design

We recognize that this cohort is too large to be followed-up actively in its entirety and, given difficulties with population registration in the most affected countries, even a complete passive follow-up poses a major challenge. We also recognize that it may not be possible to calculate individual doses for everyone and collecting information on potential confounders and modifying factors would not be feasible for the whole cohort.

However, future case-cohort or case-control studies nested within this cohort would be a viable and powerful epidemiological tool for the study of radiation risk in this cohort. Accurate maintenance of the cohort rosters would allow for unbiased recruitment of cases and controls for studies of specific outcomes of interest.

Dosimetry

Dose-reconstruction to a number of specific organs (red bone marrow and thyroid) from external radiation and from intake of long-lived radionuclides has been successfully conducted in nested case-control studies of liquidators using Realistic Analytical Dose Reconstruction with Uncertainty Estimation (RADRUE) (*21*). This approach could also be used to estimate doses to other organs from these radiation types. A foreseeable challenge is the future ability to continue doses reconstruction efforts using highly specialized techniques such as RADRUE given that the experts are getting older and much time has passed since the accident and hence relying on subject´s memories of their activities 25 years in the past may be problematjc.

It is important to investigate dosimetry on a broader level therefore. Official doses estimates exist in the Chernobyl Registries for a large number of liquidators. Efforts have been made to validate and calibrate these on specific subgroups (*31*). For full cohort follow-up, it is important that such efforts are continued and that the feasibility of improving them and quantifying uncertainties is ascertained (see Dosimetry project proposal).

Feasibility

The feasibility of conducting nested case-control studies of specific outcomes within cohorts of Chernobyl liquidators has already been demonstrated. The challenge in each study would be to define procedures for identifying the cases in a complete and comprehensive way. Difficulties in collecting reliable information on potential confounding or effect modifying factors 23 years or more after the accident would also need to be addressed.

The feasibility of conducting full cohort follow-up in each country depends on the country. An essential aspect will be the improvement of official dose estimates across cohorts.

Next steps and prioritisation

Work unde this project entails the following:

- Arrange a meeting of staff responsible for maintaining Chernobyl registries to review comparability of information and feasibility of harmonising data collected and approaches
- Establish an international steering committee to overview harmonisation, plan joint studies and review proposals from other groups for use of data
- Arrange a meeting of dosimetrists to review adequacy of available dose estimates and feasibility of improving them.

The work to evaluate comparability of information and feasibility of harmonising data collected within various liquidators' registries could start immediately. Further timing will depend on the results of the pilot work.

3) Evacuees and Offspring of Liquidators and Evacuees

Background

In the days after the Chernobyl accident, approximately 116,000 residents living within the 30 km exclusion zone were evacuated. Over 100,000 residents of contaminated territories of Belarus and Ukraine were also relocated in the following months. Whole body doses to the evacuees are estimated to be of the order of 33 mSv on average (*1*) making this, the second most exposed population after the liquidators.

Since then, several tens of thousand children have been born to families of evacuees and of liquidators. About 43,500 are currently registered in the Chernobyl Registries of Russia and Belarus.

Objective

To establish and commit to the long-term follow-up of:

- a well defined cohort of evacuees
- a well defined cohort of offspring of those most exposed as a results of the accident (liquidators and evacuees)

that can be the basis for studies of the long-term biological and health consequences of the accident.

Justification

Of all of the populations exposed to radiation from the accident, the liquidators and evacuees are those with the highest average doses and the widest dose distributions. Many of them are, in principle, registered in the Chernobyl registries, making it theoretically possible to reconstruct rosters of these populations. The offspring of liquidators and evacuees is a particular important source of information on effects of pre-conception and in-utero exposure to radiation.

Proposal

Given the potential amount of information that could be obtained for radiation protection from studies of these populations, it is proposed that the feasibility of assembling these cohorts be assessed.

The feasibility study should focus on the feasibility of establishing representative rosters of the populations, of tracing them 25 years after the accident and of reconstructing individual doses.

Study design

A pilot study should be conducted to evaluate the completeness of available sources (Chernobyl registries, lists of Ministries of Internal Affairs, or of Chernobyl or Emergency Affairs ministries, depending on the country) needed to identify and trace potential study subjects and the feasibility of reconstructing individual doses. This study would be helpful in determining whether future studies based on these cohorts would be logistically possible and informative.

Dosimetry

The feasibility of reconstructing individual doses to evacuees, as well as in utero and postnatal doses of offspring of liquidators and evacuees need to be evaluated.

Feasibility

Data on evacuees and on offspring of liquidators and evacuees are difficult to obtain, and there is anecdotal evidence that, following their relocation, many of the evacuees have subsequently moved and that official registration of their movements does not exist.

The feasibility of setting up and following up these cohorts therefore needs to be assessed.

Next steps and prioritisation

The pilot study should arrange:

- Meeting of staff responsible for maintaining Chernobyl registries to review information registered on evacuees and offspring of liquidators and evacuees.
- Establishment and conduct of a formal feasibility study.
- Meeting of dosimetrists to review existing dose estimates and feasibility of reconstructing doses for entire cohorts.

The pilot work to assess feasibility of setting up and following up cohorts of evacuees and offspring should start immediately. It also should include dosimetriy experts to evaluate feasibility of reconstruction doses for this population. Further timing of formal studies will depend on the results of the pilot work.

TISSUE BANKS

The chapter was prepared by E.D. Williams, University of Cambridge, the UK

Background

The value of storing samples of tumour tissue and paired samples of non-neoplastic tissue or blood, either as intact samples or as nucleic acid extracts is widely recognised (*32-36*). The samples allow study of both somatic and germline changes involved in the carcinogenetic process, and allow the application in the future of techniques yet to be developed which may enable answers to some of the basic questions in carcinogenesis, including radiation carcinogenesis. Storage of such samples is particularly important for major radiation events where large numbers of radiation related tumours occur with a known latency and where both the molecular evolution of the tumours and the molecular epidemiology need study.

The possibility of creating a tissue bank specifically for the Chernobyl related projects that may result from the ARCH strategic review, of encouraging each project to store its own samples, or of collaboration with the existing Chernobyl Tissue Bank (*37*) was discussed by the ARCH expert group. The need to store samples of tumour and blood or buccal mucosa was widely supported, but before deciding on any proposal for the strategic research agenda it was considered that more information was needed on the existence, number, content, and distribution of tumour/tissue banks in the three most affected countries.

Proposal and prioritisation

Creation of a simple questionnaire seeking information on existing tissue banks in the three most affected countries. Circulation to appropriate members of the expert group, together with a request that copies be passed to other centres involved in tissue storage. These would include the major centres involved in treating thyroid cancer, and others dealing with large populations from exposed areas, treating conditions such as breast cancer. Analysis of the responses so that the information can then be used for decision on the proposals in the SRA.

Inventory of existing tissue banks and collections of biological samples (storred in the three most affected countries and elsewhere) should start immediately.

INVENTORY OF DOSIMETRIC INFORMATION FOR POPULATION GROUPS MOST AFFECTED BY THE CHERNOBYL ACCIDENT

The Chapter was prepared by K. Baverstock, University of Eastern Finland, Kuopio, Finland

Introduction

Dosimetry underpins all radiation effect studies. Up to now dosimetric needs have been assessed on a study by study basis but an alternative population based approach would help to plan and assess the feasibility of future studies. The problem is to assess the extent to which dosimetric information is already available in the three affected countries (Ukraine, Belarus, and Russia), and also possibly in less affected countries in Europe, and what resources would be required to ascertain where there are deficits and what would be required to correct the deficits.

Objective

The aim is to design and test a questionnaire to ascertain from study groups in participating countries the available information obtained in the course of past studies, the present status of dosimetry in the ongoing research studies, what future studies are planned, and opinions as to what future studies are required. Studies of workers as well as of members of the public would be considered. The project would have a strong focus on Belarus, Russia, and Ukraine, but the Baltic countries and other European countries could be considered as well.

Rationale

The expert group has recommended various studies for which dosimetry will be essential. Before embarking on major dosimetric studies it would be useful to understand the extent of the existing dosimetric information and the methodologies used to acquire it.

Proposed methodology and prioritisation

The principal investigator (an expert dosimetrist) will compile a list of all Chernobyl related dosimetry and epidemiological research groups in the three participating countries and design a questionnaire to ascertain the required information. The expert dosimetrist will also provide an assessment of the reliability of the dosimetric information obtained in the course of past studies.

In order to assess the feasibility of future epidemiologic studies, the most valuable source of dosimetric information for the members of the public might be the dosimetry catalogs (or “passport doses”) that were established in Belarus, Russia, and Ukraine. For clean-up workers, it would be the registries. If that proves to be true, special efforts will be made to investigate how the doses included in the registries/catalogs were calculated.

Designing and testing of the questionnaire to compile the list of all Chernobyl related dosimetry should start urgently.

THYROID CANCER AND THYROID DISEASES

The Chapter was prepared by E. Cardis and M.T.Do, CREAL, Barcelona, Spain; E.D. Williams, Cambridge, the UK and A. Kesminiene, IARC, France

Background

General

Thyroid cancer is one of the least frequent causes of death from cancer but one of the most important radiation induced malignancies. In the general population, it accounts for approximately 1% of the total cancer incidence (*38*). Thyroid carcinomas are about three times more frequent in women than in men, suggesting a possible role of hormonal factors in thyroid cancer aetiology. Incidence of this disease is particularly elevated in Iceland and Hawaii, where the rate is nearly twice that in North European countries and in North America. In Hawaii, the incidence rate of thyroid cancer in all ethnic groups is higher than in the same ethnic group living in their country of origin, most likely due to differences in environmental, particularly dietary exposures.

Thyroid tumours are rare in children (less than one case per million per year in most developed countries); the age-specific incidence rates increase rapidly with age. In the past three decades, incidence rates have been increasing in most developed countries, while mortality rates have been slowly decreasing

Experimental studies have shown that long-term stimulation of the thyroid gland by thyroid stimulating hormone, such as results from iodine deficiency, can lead to tumour formation with or without addition of a mutagenic agent (*39*). Animal experiments indicate that iodine deficiency is a potent promoter of thyroid carcinogenesis (*40, 41*) and that iodine excess may play a role in tumour promotion (*42*). In humans, the evidence for a relation between thyroid carcinoma risk and iodine status is less clear. Iodine deficiency is thought to be involved in the development of thyroid cancer because thyroid cancer mortality rates are high in mountainous areas, such as the Alps, Andes, and Himalayas, where severe iodine deficiency was common. However, several high-risk populations live on islands (such as Hawaii and Iceland), where iodine intake is generally high. The relationship between iodine intake and risk of thyroid cancer appears to be complex, since both deficiency and excess may inhibit the synthesis of thyroid hormones and cause goitre (*43*). The two main types of thyroid carcinoma (papillary and follicular) may be linked to iodine-rich and iodine-deficient diets, respectively (*44, 45*). Other dietary factors, including cruciferous and goitrogenic vegetables (*46*), may play a role in thyroid carcinogenesis.

The incidence of thyroid carcinoma, in particular PTC, has been shown to increase with external exposure to X- and gamma-rays, both in epidemiological and experimental studies (*47, 48*). The risk of radiation induced cancer is considerably greater in those exposed as young children than as adults (*48*). In studies of atomic bomb survivors and of children exposed to ionising radiation for tinea capitis and other benign disorders, an increased risk is observed ten years after exposure and appears to follow a relative risk model, with a decline starting about 30 years after exposure, though the increased risk was still elevated at 40 years (*49, 50*).

Before the Chernobyl accident, results of epidemiological studies of populations exposed to ^{131}I appeared to indicate a much smaller effect than that of external X- or gamma irradiation (*48*). The number of young people exposed in these studies was, however, very small, ranging between 127 and 3500 in the different studies (*51-54*). Early animal studies also found that ^{131}I was much less effective than external radiation in inducing thyroid tumours, but a later much larger study found no significant difference in effectiveness (*55*).

Thyroid cancer and thyroid disease following the Chernobyl accident

The main health effect of radiation from the accident observed to date is a dramatic increase in the incidence of thyroid cancer in persons exposed as young people. This increase was observed first in

the early 1990s in Belarus and continues until now in the most contaminated areas of Belarus, Ukraine and the Russian Federation (*12, 21, 56, 57*). By 1995, the incidence of childhood thyroid cancer had increased to 4 per 100,000 per year compared to 0.03–0.05 cases per 100,000 per year prior to the accident. As those who were children at the time of the accident have aged (by 2002, even the very youngest had reached adulthood), the childhood thyroid cancer rates have declined to near zero but parallel increases in the incidence of thyroid cancer in adolescents and slightly later in young adults have been seen (*1*).

During the period 1986 to 2002, nearly 4,000 cases of thyroid cancer were diagnosed and treated in Belarus, Ukraine and in the four most contaminated regions of Russia among those who were children (less than 15) or adolescents (*58-60*) at the time of the Chernobyl accident. Of these, 15 are known to have been fatal up to now (*61*). Screening for thyroid cancer – either through formal screening campaigns or through closer attention by medical professionals – is known to have taken place in many European countries, and in particular in the most contaminated areas of Belarus, the Russian Federation and Ukraine (*21*). It is therefore possible that the observed increased thyroid cancer incidence in these countries is at least in part attributable to a screening bias. Analyses of childhood thyroid cases in these countries show that the majority are fairly aggressive, with a large proportion showing extracapsular invasion and distant metastases (*61, 62*), cases that would have been likely to be diagnosed even in the absence of screening. This is consistent with recent analyses that indicate that approximately 60% of the cases diagnosed in Belarus between 1986 and 2001 among subjects who were children or adolescents in 1986 are attributable to radiation (*12*).

A number of epidemiological studies of thyroid cancer following exposure to radioactive iodines from the Chernobyl accident have been reported both in the most contaminated countries and in other European countries (*21*). The most recent and informative studies of persons exposed in childhood and adolescence) (*10-13, 63*) are summarised in table 1. Risk estimates from the large case-control studies in Belarus and Ukraine and from the cohort study in Ukraine are very close and similar, though slightly lower, to estimates from the pooled analysis of studies of external radiation (*49*). The ERR derived in the ecological study (*12*) is higher than those derived from the larger case-control and cohort studies. The reasons for the difference in risk estimates for the two study designs are not yet clear, although uncertainties in dose estimates may be partly responsible. A very large ERR per Sv was estimated in the case-control study in the Bryansk area of Russia, based on small numbers of cases (*63*); doses in this study tended to be low, however and estimates of risk at 1 Sv are therefore relatively uncertain.

Iodine deficiency, age, genetic susceptibility – factors which may modify radiation risks

The large increase in the number of thyroid cancer cases in the contaminated regions suggests that there may be factors either environmental (iodine status), host (age and sex) and/or genetic which modify the risk of radiation induced thyroid cancer (*64*). There is some indication that iodine deficiency at the time of exposure may have increased the risk of developing thyroid cancer among persons exposed to ^{131}I as children (*11, 65*). Conversely, prolonged stable iodine supplementation in the years after exposure may have reduced this risk (*11*). Further studies are needed to replicate these findings. Young children at exposure developed many more thyroid cancers than older children, but they also received a higher mean thyroid dose. The effect of age at exposure on the risk/Gy among those exposed as children is unclear in current studies and needs to be investigated further.

There is evidence for genetic predisposition to papillary thyroid carcinoma both in irradiated and non-irradiated populations (*58, 60, 66, 67*). Among the cases which were studied in Belarus and Russia, a number of families were found in which two siblings were affected (*64*). Given the rarity of this disease in children, this observation suggests that genetic predisposition may be increasing the susceptibility to radiation induced thyroid cancer. While no gene has yet been identified which accounts for familial papillary carcinoma of the classical type, genes have been linked to the thyroid tumours found in association with polyposis coli, with Cowden's syndrome, and with some cases of oxyphil tumours. A recent genome wide association study has identified common variants at two

loci that predispose to thyroid cancer (*68*), and a possible link to several polymorphisms found in a study of thyroid tumours in a population exposed to radiation from nuclear tests (*69*). A study of DNA damage response genes in Chernobyl related papillary carcinomas found a link with a number of genes, several linked to both sporadic as well as radiation related tumours; one particularly associated with radiation (*70*). One of the genes explored in the Sigurdson study is also involved in DNA repair, and in view of the mutations found in the post Chernobyl thyroid carcinomas further study of the genes involved in double strand break repair is clearly needed in this population.

Table 1 Summary of case-control and cohort studies, and of the most recent ecological study of thyroid cancer following the Chernobyl accident.

Study	Observed cases	Controls/ study population	Median dose (Gy)	Excess relative risk at 1 Sv
Case-control studies				
Belarus (*10*)	107	214	0.106	OR ≥1 Gy vs. <0.3Gy: 5.04 (1.5-16.7) to 5.84 (1.96-17.3)
Belarus and Russian Federation (*11*)	276	1 300	0.365 (Belarus) 0.040 (Russia)	4.5 (2.1-8.5) to 7.4 (3.1-16.3)
Russian Federation – Bryansk (*63*)	66	132	0.020	49.7 (5.8 to 1152)
Cohort study Ukraine (*13*)	45	13 127	0.78 (mean)	5.25 (95% CI 1.70, 27.5)
Ecological study Belarus and Ukraine (*12*)	1,089	623 000	0.002-0.5 (mean) depending on region	18.9 (11.1-26.7)

The evolution of the Chernobyl thyroid cancer endemic

Based on many decades of follow-up from studies of populations exposed to external radiation (*49*), it is expected that Chernobyl-related thyroid cancers will continue to occur for many more years, although the long-term magnitude of risk cannot yet be quantified.

Existing studies have shown that over the first 20 years after the accident the Chernobyl related thyroid carcinomas have been almost all papillary carcinomas. The oncogenes involved, and the subtypes of papillary cancer have changed with increasing latency, with a broad correlation with clinical behaviour. There is now anecdotal evidence that follicular adenomas are increasing in frequency, and follicular carcinomas of the thyroid may well increase in the future, either de novo or by progression from follicular adenomas. In the relatively small studies of children treated by external radiation for non thyroid conditions where the radiation field included the thyroid, follicular adenomas showed a particularly long latency. The fact that much of the population exposed to fallout from Chernobyl lived in a relatively iodine deficient area increases the possibility that there will be a considerable increase in the incidence of follicular adenomas and carcinomas in the future. Papillary carcinomas continue to occur, and it is also important to investigate future changes, including the possible occurrence of anaplastic carcinomas.

Treatment of childhood thyroid cancer

The Chernobyl accident led to an unprecedented increase in the incidence of thyroid carcinoma in children between the fourth and fourteenth year after the accident. Between 1985 and 2006 nearly

1000 cases of thyroid cancer aged 0-15 were treated in Minsk. The treatment evolved as circumstances improved, but the experience of the centres in Minsk and Kiev (which saw fewer cases than Minsk) was unprecedented. The pattern of surgical treatment changed, and radioactive treatment became available both for ablation and for the treatment of metastases. A number of studies have already been carried out, particularly by EP and Yuri Demidchik together with Christoff Reiners (*71*), but it is clearly important that such studies are continued for the lifespan of those affected. Questions to be answered include (a) mortality and morbidity of treated radiation associated childhood cancer; so far deaths due directly to radiation associated thyroid carcinoma have been remarkably few (b) the benefits and complications of treatment, including surgery and radiation (c) correlation of the clinical behaviour and response to treatment with the molecular status of the tumour.

Molecular studies

The clear pathological distinction between papillary and follicular cancers is supported by the finding that the majority of papillary carcinomas show either a BRAF point mutation or a RET rearrangement, while the majority of follicular carcinomas show either a RAS point mutation or a PAX8-PPARgamma rearrangement. Anaplastic carcinomas show P53 mutations some also a BRAF point mutation, while nearly all medullary carcinomas possess a RET point mutation. The post Chernobyl thyroid carcinomas have nearly all been papillary in type, but BRAF mutations have been less common than in unexposed populations. It is not clear whether this is because of age or latency, or whether it reflects a real difference in the spectrum of mutations induced by radiation. It does not seem likely that there is a single specific radiation induced oncogene, but it remains possible that radiation preferentially induces mutations dependent on double strand breaks. This possibility is strengthened by the finding that while BRAF point mutations may prove to be relatively less common than in sporadic tumours, BRAF activation by rearrangement has been described in Chernobyl related tumours. Follicular carcinomas can be associated with a rearrangement, but they may arise from adenomas and have a long latent period. Thyroid adenomas also show an increased incidence in radiation exposed populations, and need to be analysed in the Chernobyl exposed population. Molecular and morphological studies of the continuing increased incidence of thyroid tumours should show whether there is a specificity in the type of mutation induced by radiation; studies that cannot satisfactorily be carried out in other radiation exposed populations because of the much smaller numbers of thyroid tumours and the lower attributable fraction.

Several studies have looked at the relationship between the oncogenes found in sporadic thyroid cancers and the clinical behaviour of the tumour. Most find that BRAF is linked to a more aggressive tumour than RET-PTC, and that BRAF but not RET-PTC tumours are liable to undergo the rare transition to an anaplastic carcinoma (*72*). In addition RET-PTC3 is linked to a more aggressive tumour than RET-PTC1. The importance of the correlation of molecular and morphological findings with latency is well shown by studies on Chernobyl related tumours. When RET-PTC rearrangements were analysed, the early short latency papillary carcinomas were largely of the solid type often associated with direct invasion, and showing RET-PTC3 rearrangements. Later tumours showed a high proportion with RET-PTC1 and a classical morphology and less aggressive behaviour. Many of the existing studies of radiation induced tumours are relatively short-term, this project offers the opportunity of a long term study of outcome, its link to latency and to the type of tumour and oncogene present, as well as insight into the specificity of radiation induced mutations.

Exposure of adults

While the increased risk of thyroid cancer in those exposed in childhood and adolescence is well demonstrated, the effect of exposure on adults remains unclear. In the only study that has evaluated the risk for adults living in the contaminated areas (*59*), no dose-response relationship was found. In a recent case-control study of thyroid cancer nested within the cohorts of liquidators from Baltic

countries, Belarus and Russia, in which individual doses were reconstructed, a dose-related increased risk was observed (*73*). The ERR per 100 mGy was 0.38 (95% CI 0.10, 1.09) and the risk estimates were similar when doses from 131I and external radiation were considered separately, although for external radiation the ERR was not statistically significantly elevated. The ERR was similar for microcarcinoma and larger size tumours, and for tumours with and without lymph node involvement, suggesting that screening alone cannot explain this increase. A recent analysis of thyroid carcinoma incidence in those exposed to atomic bomb radiation at an age of 20 or more found no increase in males, but in females the excess relative rate/Gy was 0.7 (90% CI 0.2-1.46) (*74*). Uncertainty remains about the quantification of the risk in adults, and a possible gender difference, and studies of the populations exposed after Chernobyl could make an important contribution.

Other thyroid diseases

Hypothyroidism

Hypothyroidism is a well recognised effect of radiation to the thyroid; it is a deterministic effect, with a threshold dose for intervention in adults generally accepted as 5Gy. Thyroid dose from exposure to fallout from Chernobyl in the most affected countries ranged up to 40Gy, with only small numbers exposed to the highest level.

Knowledge of the effect of radiation on thyroid function comes from a variety of sources. External radiation to the neck can affect thyroid function, usually in the treatment of non thyroid disease – for example childhood cancers, breast cancer and lymph gland cancer, and therapy for 'enlarged thymus', haemangioma, tinea capitis etc. In most of these studies thyroid doses were relatively high, and changes were found indicating thyroid injury – for example high TSH or low thyroid volume. Isotopes of iodine used in the treatment of thyroid diseases, including thyrotoxicosis and thyroid cancer affect thyroid function, often deliberately inducing hypothyroidism. Overt hypothyroidism is common after treatment of thyrotoxicosis, but the onset can occur even decades after treatment. Subclinical hypothyroidism, defined as an elevated TSH was also found in those exposed to the atomic bombs in Japan. In the Marshall Islanders, 2 cases of cretinism occurred following exposure as infants to a dose estimated as over 50Gy. The effects of such high doses to the thyroid could not be studied after the atomic bombs in Japan because of the lethality of whole body radiation. The Marshall Islanders were exposed to fallout, not whole body radiation, but the total number exposed was in the hundreds, compared to the millions exposed after Chernobyl.

Autoimmune thyroiditis

Evidence concerning the relationship between environmental exposure to radioactive iodine and thyroid autoimmunity and autoimmune thyroiditis (AIT) is limited and inconclusive. Studies conducted within 11 years of the accident on children living in contaminated areas in Kaluga, Orel and Tula (Russia), Chernihiv and Kyiv (Ukraine), and Hoiniki (Belarus) found a higher percentage of anti-thyroid positive antibodies (to thyroglobulin, ATG, and/or to thyroid peroxidase, ATPO) relative to children living in uncontaminated control areas (*75, 76*). By contrast, the Chernobyl Sasakawa health and medical cooperation project, after examining about 120,000 children from contaminated areas of Belarus, Russia and Ukraine, did not find a significant relationship between prevalence of antimicrosomal antibodies (an earlier term for ATPO) and/or ATG with exposure to radiation on the basis of ^{137}Cs contamination in the body or soil. The majority of studies have not distinguished between the presence of elevated anti-thyroid antibodies and AIT.

The largest study to date involved 12,240 subjects who resided in an area of mild to moderate iodine deficiency in Ukraine (*13*). All subjects had estimates of thyroid doses due to intake of ^{131}I based on individual thyroid radioactivity measurements performed in May–June 1986. Measurements of circulating antibodies and TSH levels together with ultrasonography of the thyroid gland were taken to determine whether the autoantibodies produced were significantly affecting thyroid function. This study, despite its size, could demonstrate no conclusive evidence of a relationship between thyroid dose and autoimmune thyroid disease, defined by the presence of

both circulating autoantibodies and evidence of thyroid dysfunction by echography and/or TSH elevation.

Objectives

While much has been learned already on the relation between radioactive iodines and the risk of thyroid cancer from the study of the consequences of the Chernobyl accident, much remains to be learned from the accident. Questions concern the evolution of the endemic, the specific aetiology of Chernobyl thyroid cancer, the consequences of the treatment received by the cases, the risk of exposures in adulthood and the relation between radiation exposure and risk of other, non-cancer, thyroid diseases.

Specifically, the main questions to be answered about thyroid cancer risk are:

1. What will be the evolution of the Chernobyl thyroid cancer endemic?
    - a) The magnitude of the thyroid cancer burden due to radiation from the accident in years to come.
        - For those exposed at younger ages, will the ERR/Gy continue at a constant level for years to come?
        - Can we characterise the time trends and age-period-cohort effect of thyroid cancer in the affected areas (inside and outside the most contaminated areas?
    - b) The molecular evolution of thyroid tumours occurring as a result of exposure to fallout from the Chernobyl accident and its correlation with changing patterns of clinical and morphological findings.
2. What can we learn about the aetiology of radiation inducted thyroid cancer?
    - a) Can we confirm the reported effects of iodine deficiency and supplementation?
    - b) What is the contribution of individual susceptibility (in particular genetic susceptibility and epigenetic effects) to the level of consequences observed after the Chernobyl accident? To identify mechanisms involved and, if possible individual genes.
    - c) What other factors, if any (including host and environmental factors) may be modifying the risk of radiation induced thyroid cancer in these populations.
3. To determine the optimum treatment of childhood thyroid cancer, and evaluate the long-term consequences of treatment on risk of cancer and other outcomes (including fertility and outcomes of pregnancy).
4. What can we learn about the effects of radiation exposure in adulthood?
5. What are the effects of radiation exposure on the risk of other, non-cancer, thyroid diseases?

Specific relevance (value-addedness) of Chernobyl population(s)

There is little doubt that the Chernobyl accident presents a unique opportunity to answer the questions listed above. This is the first (and hopefully last) occasion on which population exposure to non-negligible levels of ^{131}I has occurred on such a scale. The number of thyroid cancers diagnosed among those who were children and adolescents at the time of the accident is unprecedented and the future of the endemic is an important public health and health services concern in the most contaminated countries, which must be evaluated.

By far the greatest source of radiation to the thyroid came from ^{131}I with an 8 day half-life, the initial event leading to the development of thyroid cancers must therefore have occurred within a few weeks of April 26th 1986 in the great majority of cases. Because of the very large numbers of thyroid carcinomas that have occurred this provides a unique opportunity to study the molecular

evolution of the tumours, linking oncogene changes to latency, morphology and clinical behaviour. Future changes in any of these parameters cannot be predicted from existing studies.

We have already learnt much concerning the relation between exposure to radiodines in childhood and the risk of thyroid cancer from the accident, but this unique situation provides a unique opportunity to better understand the aetiology of radiation induced thyroid cancer. The setting of the accident, in eastern European populations which carry some important founder mutations in genes (such as BRCA1 and NBS1) involved in DNA damage recognition and repair, is also unique to investigate the possible role of genetic susceptibility in the aetiology of this disease.

Proposed approaches - Objective 1a

The various questions listed above necessitate different study designs, study populations and approaches. These are therefore discussed, below, according to the respective research objective.

What will the magnitude of the thyroid cancer burden due to radiation from the accident be in years to come?

- For those exposed at younger ages, will the ERR/Gy continue at a constant level for years to come?
- Can we characterise the time trends and age-period-cohort effect of thyroid cancer in the affected areas (inside and outside the most contaminated areas?

Population

Continued surveillance of thyroid cancer, particularly among young children of the affected area, is necessary and remains an important priority until the complete burden of this disease caused by the accident is fully characterized. In general, children are more sensitive to exposure to environmental contaminants than adults for a number of reasons. Proportionally, children eat more food, drink more water, and breathe more air relative to their size than adults do. As such, they may be exposed to relatively higher amounts of contaminants in these media. Behaviourally, children's normal activities, such as putting their hands in their mouths or playing on the ground, create additional opportunities for exposures to environmental contaminants that adults do not face. As such, we are more likely to assess the full impact of the accident from studies of those exposed at an early age.

Study Design

Trends in diseases are most efficiently studied using ecological designs, relying on population based cancer registries to continuously monitor trends in those exposed at different ages and evaluate, in particular, whether increased risks in those exposed in childhood continue at the same rate or are starting to level off or diminish. Data from already established cohorts of children from the affected areas could also be used to assess thyroid cancer trend over time. Incidence study is more informative than mortality in capturing the full burden of this disease as thyroid cancer typically has a very good survival. Information on changes in thyroid risk over time can generate etiologic hypothesis that could be tested using analytical means.

Doses

Average doses to the thyroid (so-called "passport doses") have been calculated at the level of individual settlements in Belarus and contaminated regions of Russia and Ukraine and used in ecological studies to date (see for example Jacob et al 2006 (*12*)). These are therefore available for future ecological studies.

Feasibility

Ecological studies are routinely conducted to examine disease trend over time. Given the existence of population based cancer registries in Belarus and in the most contaminated regions of Ukraine and Russia, it is quite feasible to conduct this type of study in the Chernobyl context to determine

future trends. Such studies are generally quite economical and can be conducted periodically in the future as the necessary data are collected routinely.

Although such studies are routinely conducted, there are a number of methodological considerations that needs to be taken into account to ensure appropriate interpretation of study results:

- Screening and improved diagnosis in certain regions or age group could artificially create trends where not exist. It will be important therefore to collect relevant information about screening so that this can be taken into account
- While population based cancer registries exist in the affected regions, it will be important (see section on population based registries) to ensure complete coverage and registration and continued validation of diagnoses to maximise the quality of the data in the registries.
- Population mobility.

Proposed approaches - Objective 1b

The molecular evolution of thyroid tumours occurring as a result of exposure to fallout from the Chernobyl accident and its correlation with changing patterns of clinical and morphological findings

Before the accident, it was generally thought that the latent period for thyroid cancer following childhood exposures was of the order of 10 to 20 years (*49, 77*). However, in Chernobyl the first reports of excess thyroid cancer appeared approximately 5 years after the accident (*21, 56, 57*). The differences in the latent periods may be related to different biological mechanisms involved in thyroid tumour genesis in Chernobyl population. These biological mechanisms would likely manifest through different clinical, morphological, and molecular findings.

This objective can be achieved in two separate but complementary approaches:

- Studies of a defined cohort with known thyroid doses, correlating morphological changes in tumour type and subtype with dose, age at exposure, latency and clinical behaviour. Identification of oncogene findings, and correlation with morphology, dose, age at exposure, latency and clinical behaviour. The BelAm and UkrAm cohorts *(see section on Life-span cohorts)* would be a useful study group if regular screening could be continued. Surveillance through tumour registries would not be adequate because follicular adenoma would not be included, and molecular and morphological studies would be extremely difficult. The distinction between a cellular follicular adenoma and a low grade follicular carcinoma is well known to be difficult, and a uniform standard of diagnosis requires review of all cases.
- An ecologic study of thyroid tumours operated at the major centres in Minsk or Kiev would also be of value, as it would provide many more cases of known latency for the morphologic and molecular studies than could any study of existing cohorts. The success of such an approach, however, would depend on the availability of biological tissues on all of the cases and on appropriate documentation of referral patterns from study regions to ensure that cases are representative of the populations from which they arose and that no bias related to severity and prognosis is introduced by focusing on the cases referred to the participating centres. For greater efficiency, such a study should probably be restricted to those exposed at young ages, where sporadic cancer is still relatively rare.

Population

Children recruited to the BelAm and UkrAm cohorts (*13, 57*) would be a suitable population to address this study objective. In these cohorts, 11,918 children from Belarus and another 13,243 children form the Ukraine were recruited into the study. This cohort was screened every 2 years for thyroid disorders. At each screening, each cohort member provided blood and spot urine samples,

underwent thyroid palpation and ultrasound examination, and information was collected on medical history, and other information that could be useful in estimating thyroid dose.

Study Design

Given that relevant cohorts (e.g., BelAM and UkrAm cohorts) have already been assembled and necessary data has been collected, continued active follow-up of these cohorts would be needed to in addressing these objectives.

Doses

Individual thyroid doses from ^{131}I have been calculated, based on direct thyroid measurements, for all subjects in these cohorts and doses from intake of long-lived nuclides and from external radiation are being calculated. Thus all needed dosimetric information would be available for such a study.

Biological samples

The most important information needed in this study would be blood and tumour tissue which should be available in further active follow-ups of these cohorts. Nucleic acid extracted from these samples and from samples from the ecological study should if possible be stored in a biobank for future study.

Data collection

Individual level information on potential modifiers of risk and on potential risk factors for thyroid tumours as well as the relevant clinical data could be collected during the active screening if information is not already available.

Molecular markers

Two approaches could be used to follow the molecular evolution of the endemic, analysis of known genes and genome wide association studies. Changes in the frequency of occurrence of genes known to be associated with thyroid carcinoma should be investigated. Currently these would include *RET, TRK, BRAF and PPAR* gamma rearrangements, and *BRAF* and *RAS* point mutations. Further genes could be added, either where newly discovered or where related to particular tumour types, including those linked to inherited syndromes e.g. *P53, PTEN, APC, BCatenin, GRIM19*. In addition mutation in genes involved in DNA repair, particularly double strand break repair, could be studied, e.g. *BRACA1* and *2*, *RAD 50* and *51, ATM, NBJ1* and *XRCC4*. Genome wide association studies could be used to confirm and extend recent observations linking certain polymorphisms to thyroid carcinoma and particularly to radiation induced thyroid carcinoma. The availability of a population with known doses would be a great advantage, because of the higher attributable fraction at high doses.

Feasibility

While data from study subjects are already collected, the main issue in addressing the feasibility of this study is whether it will be possible to collaborate with the Belarus, Ukraine and US investigators and agencies that have set-up and followed up these cohorts already.

Proposed approaches - Objective 2

What can we learn about the aetiology of radiation induced thyroid cancer?

Specific questions of interest are:

- Can we confirm the reported effects of iodine deficiency and supplementation?

- To determine the contribution of individual susceptibility (in particular genetic susceptibility and epigenetic effects) to the level of consequences observed after the Chernobyl accident. To identify mechanisms involved and, if possible individual genes.
- What other factors, if any (including host and environmental factors) may be modifying the risk of radiation induced thyroid cancer in these populations.

Further, careful, analytical epidemiological studies, planned in collaboration with all relevant specialties (including molecular pathology, genetics, biology of epigenetics and non-targeted effects, dosimetry) are needed.

Study Population

While such studies could in principle be built upon existing cohorts of screened children (BelAm and UkrAm), statistical power is likely to be low given the relatively small number of cases expected in these limited cohorts and population based case-control studies in the most contaminated areas are likely to be more informative (see, for example, the design of the previously published case-control study in Belarus and Russia (*11*)).

Study Design

Case-control studies are the common study design used in published studies involving susceptibility genes (*78*). Since thyroid cancer is a rare disease, a case-control study would likely to be the most efficient design to determine etiological factors contributing to radiation induced thyroid cancer. Cases can be collected all relevant treatment and diagnoses facilities in the participating regions (and completeness validated with the thyroid cancer registries). Population based controls could be recruited from the general population matched on sex, year of birth, and region as has been done in the previous population based case-control study in Belarus and Russia (*11*).

Doses

Within the previously conducted population based case-control study in Belarus and Russia, an approach was developed and validated to reconstruct individual doses and associated uncertainties. This approach can be applied to subjects in further population based studies of thyroid cancer.

Data collection

In a case-control study, a questionnaire can be used to collect individual level information on potential modifiers of risk and on potential risk factors for thyroid tumours.

Biological samples

Blood and tumour samples – either collected within the study or from the Thyroid Tissue Bank if it is active in the study region.

Molecular markers

Markers of individual sensitivity will need to be analysed. This includes variants in specific genes – for example involved in DNA damage recognition and repair – as well as markers of epigenetic effects. The field of markers of sensitivity is rapidly evolving and it will be important to ensure collection, processing and storage of adequate biological samples to ensure that markers identified in the future can be analysed.

Feasibility

A multi-disciplinary population-based case-control study of thyroid cancer has been conducted in the past in Belarus and Russia (*11*) thus demonstrating the feasibility of such an approach. That said, one must not underestimate logistic problem that can be encountered. These include continued access to sampling frames for selecting cases and controls and legal and logistic issues related to collection and analysis of biological material.

Proposed approaches - Objective 3

Studies aimed at determining optimum treatment for childhood thyroid cancer

To address Objective 3, the possible approaches include:

- The continuation of follow-up studies of all radiation associated childhood thyroid cancers to determine long-term morbidity and mortality, correlate treatment with outcome, document benefits and dis-benefits of treatment including radiation. If possible a lifespan cohort of treated children should be identified to ensure long-term studies.
- Studies linking tumour type and tumour oncogene changes with long term clinical behaviour and response to treatment. The oncogenes involved can be studied from frozen tissue from the original operation where this has been preserved, from the original paraffin blocks, and from recurrences.

Study Population

Cohort of children treated for thyroid cancer in Belarus, Ukraine and Russia.

Study Design

Prospective cohort study with active follow-up.

Data collection

During active follow-up, a consistent data set could be recorded at each regular visit, including a questionnaire concerning qualify of life and various risk factors.

Biological samples

Blood and thyroid tumours samples.

Feasibility

Feasible in principle. Such a cohort study is already underway in Belarus (Yu E and EP Demidchik in collaboration with Ch Reiners, personal communication)

Proposed approaches - Objective 4

Thyroid cancer risk in those exposed as adults

As most current studies of radiation effects indicate that risks from exposure in adults are likely to be somewhat less than from exposure in childhood, the most efficient approaches is likely to focus on cohorts of adults with the highest exposure levels and conduct nested case-control studies (which will allow more detailed dose reconstruction and collection of biological samples and information on other potential risk factors and modifiers)

Study Population

Cohorts of a priori importance for this are

- Cohorts of liquidators *(see section on Life-span cohorts)* – a recent study already suggests an increased risk of thyroid cancer in this population (*73*); prospective continuation of such nested case-control studies are feasible at relatively low cost and will be important to validate these results.
- Cohort of evacuees - more work is needed, however, to determine the feasibility of such studies and their likely statistical power *(see section on Life-span cohorts)*

Study Design

Because of the difficulties of following large populations and the need for collection of detailed information for dose-reconstruction and on risk factors, the most efficient design is likely to be nested case-control studies.

Doses

Within the previously conducted population based case-control study in Belarus and Russia, an approach was developed and validated to reconstruct individual doses and associated uncertainties for children. This approach can also be applied to adults.

Data collection

In a case-control study, a questionnaire can be used to collect individual level information on potential modifiers of risk and on potential risk factors for thyroid tumours.

Biological samples

Blood and tumour samples collected within the study. The Chernobyl Tissue Bank currently collects tissue only from those under 19 at exposure.

Molecular markers

Markers of individual sensitivity will need to be analysed. This includes variants in specific genes – for example involved in DNA damage recognition and repair – as well as markers of epigenetic effects. The field of markers of sensitivity is rapidly evolving and it will be important to ensure collection, processing and storage of adequate biological samples to ensure that markers identified in the future can be analysed.

Feasibility

A multi-disciplinary population-based case-control study of thyroid cancer has been conducted in the past in Belarus and Russia (*11*) thus demonstrating the feasibility of such a study in young people. There is no reason to think it would not be feasible in older adults though a more appropriate sampling frame will need to be found for controls (the previous study used the birth registry).

Proposed approaches - Objective 5

Effects of radiation exposure on the risk of other, non-cancer, thyroid diseases

While a number of epidemiology approaches could be used to clarify the role of radiation exposure from Chernobyl in these diseases, the most effective approach is likely to involve active screening of a cohort of children with a wide range of known thyroid doses who were exposed to Chernobyl fallout in the 3 most affected countries.

Study Population

Children recruited to the BelAm and UkrAm cohorts (see above and section on life-span cohorts) are likely to be the most suitable population to address this study objective, although children who were evacuated and received a high dose should also be studied if logistically possible.

Study Design

A prospective cohort with active screening such as the BelAM and UkrAm cohorts would be invaluable in addressing this objective needs without having to initiating a new study.

Doses

Individual thyroid doses from ^{131}I have been calculated, based on direct thyroid measurements, for all subjects in these cohorts and doses from intake of long-lived nuclides and from external radiation are being calculated. Thus all needed dosimetric information would be available for this study.

Biological samples

The most important information needed in this study would be derived from blood samples which should be available in further active follow-ups of these cohorts.
Analyses would include measurement of TSH, T_3 and T_4 and thyroid antibodies at, say, 5 year intervals over a 20 year period, and correlation with dose and TSH levels, together with assessment of iodine intake.

Data collection

Individual level information on potential modifiers of risk and on potential risk factors for thyroid tumours could be collected during the active screening if information is not already available.

Molecular markers

Various markers are known to be associated with susceptibility to autoimmune disease, but until an increased exposure related incidence has been demonstrated active study is probably not justified. Blood samples should be stored. While the same argument applies to hypothyroidism it is worth noting that because the mechanism of non-autoimmune radiation related hypothyroidism involves cell damage and death directly due to radiation it would be expected that subjects with an inherited susceptibility to radiation effects might show hypothyroidism at a dose less than the generally accepted threshold, and such cases should be investigated.

Feasibility

While data from study subjects are already collected, the main issue in addressing the feasibility of this study is whether it will be possible to collaborate with the Belarus, Ukraine and US investigators and agencies that have set-up and followed up these cohorts already.

Prioritisation

Objective	Feasibility	Priority
1. What will be the evolution of the Chernobyl thyroid cancer endemic?		
1.a The magnitude of the thyroid cancer burden due to radiation from the accident be in years to come.	Yes	High – short, medium and long term
1.b The molecular evolution of thyroid tumours occurring as a result of exposure to fallout from the Chernobyl accident and its correlation with changing patterns of clinical and morphological findings.	Yes	High
2. What can we learn about the aetiology of radiation induced thyroid cancer?	Yes	High – short term
3. To determine the optimum treatment of childhood thyroid cancer, and evaluate the long-term consequences of treatment on risk of cancer and other outcomes (including fertility and outcomes of pregnancy).	Yes	High – short and medium term
4. What can we learn about the effects of radiation exposure in adulthood?	Yes	Medium
5. What are the effects of radiation exposure on the risk of other, non-cancer, thyroid diseases?	Depends on collaboration with US, Bel and Ukraine	Low in short term?

LEUKAEMIA AND LYMPHOMA

The Chapter was prepared by A. Kesminiene and E. Ostroumova, IARC, Lyon, France

Background

General

Leukaemia is a haematological malignancy well-known to be associated with exposure to ionizing radiation based on evidence from atomic bombardments in Japan and medical radiation (*21*). The latency period for radiation-induced leukaemia is rather short, a with increases in disease rates that can be detected starting 2 years from initial exposure. Many forms of leukaemia are associated with specific genetic events often resulting in proto-oncogene activation (*79*). A particular cell's susceptibility is dependent on its lineage, as ionizing radiation seems to affect the various groups of marrow-derived cells in different ways. Chronic myeloid leukaemia, acute lymphoblastic leukaemia, and acute myeloid leukaemia have all been linked to ionizing radiation exposure and specific rearrangements (*80*).

Leukaemia was the first malignancy to be linked to radiation exposure among atomic bomb survivors (*81*) and has the highest radiation related relative risk of all cancers. It was estimated by Pierce and colleagues that 44% of all leukaemia deaths (78 out of 178) among survivors with dose above 0.005 Sv were due to ionizing radiation exposure (*82*). Preston and colleagues analyzed data from leukaemia registry, dose response relationships were found for acute lymphoblastic, acute and chronic myeloid leukaemia (*83*). Chronic lymphoid leukaemia (CLL) showed no excess but this cell type of leukaemia is infrequent in Japan. Results of the analyses of all types of leukaemia showed radiation risk dependencies on sex, age at exposure and time since exposure. Specifically, for those who were exposed early in life, risks decreased more rapidly than for those exposed later, and the decrease was less rapid for women than for men. Only two leukemic deaths were registered among exposed (dose at least 0.01 Sv) *in utero* who reached age of 15 - 46 years (*84*). The number was too small to allow a dose-response analysis.

In studies of medical exposures, the estimated ERR for leukaemia, excluding CLL, ranged from 0.88/ Gy in women treated for cervical cancer (average dose to the bone marrow of 7 Gy) to 12.4/Gy in subjects treated for ankylosing spondylitis (average dose 4.4 Gy) (*85*). Most of the studies focused on adults at the time of exposure, and only tinea capitis and haemangioma studies provide information about exposures in childhood.

The risk of leukaemia caused by low dose/low dose-rate exposures is not as firmly established. Studies of association between leukaemia risk and low-dose environmental exposure provide some evidence of an increased risk. Studies of leukaemia risk after the exposure to radioactive follow-up from the Nevada test site (*86*) and fallout from atmospheric nuclear weapons testing during the 1950s and 1960s (*87*) revealed little association between leukaemia and bone marrow dose at low (mean bone marrow dose due to the exposure from the Nevada test site was 3.2 mGy with maximum mean dose of 29 ± 5.6 mGy (*88*) and very low dose range (average estimated dose equivalent to the foetal bone marrow of about 140 μSv) (*87*). An ecological study in the territories around former nuclear testing site in Semipalatinsk revealed a significant risk of acute leukaemia in children with relative risk of 1.76 for those living closer to the air-testing site compared to those living more away (*89*). However, these findings need to be interpreted with precaution because of lack of control for potential confounders and absence of individual doses.

A case-control study of 22 leukaemia deaths (CLL deaths excluded) and 132 controls with individual dose estimates available (mean dose was 0.89 Sv raging from 0.01 to 5.71 Sv) found increased risk of leukaemia after the exposure from Semipalatinsk nuclear test site (*90*). Among

persons with dose above 2 Sv, the OR was 1.91 (95% CI: 0.38; 9.67) compared to those exposed to less than 0.5 Sv. The number of studied cases is too small to make firm conclusions.

Significantly increased risk of all types of leukaemia and non-CLL leukaemia was found in the Techa River Cohort numbering about 30,000 individuals exposed to protracted external and internal (mainly Cs-137 and Sr-90) radiation due to radioactive contamination of the Techa riverside area by Mayak production association (*91, 92*). The mean cumulated bone marrow dose in the study was 0.3 Gy with maximal dose of 2 Gy. Excess relative risk per Gray of bone marrow exposure for non-CLL leukaemia was 6.5 (95% CI: 1.8; 24.0) in a cohort mortality study and 4.6 (95% CI: 1.7; 12.3) in a case-control incidence study.

Increased risks of leukaemia were observed mainly in large groups of nuclear industry workers. In a 15-country study the estimated excess relative risk for leukaemia excluding CLL was 1.93 per 1 Sv but not statistically significant (95% confidence interval (CI): <0; 8.47) (*93*). Study of dose response for CLL in the same cohort found little evidence for an association between low doses of external ionizing radiation and CLL mortality with the relative risk (RR) of 0.84 (95% CI 0.39, 1.48) under the assumption of a 10-year exposure lag (*94*). Study in the Mayak workers revealed a significantly increased risk of leukaemia with ERR of 6.9 per Gy (90% CI: 2.9 – 15) for the period 3-5 years after exposure and 0.5 (90% CI: 0.1; 1.1) for the period 5 or more years after exposure (*95*). Most recently, leukaemia incidence and mortality were studied in the National Registry for Radiation Workers, UK, relative to earlier analyses, an enlarged cohort of 174,541 persons, with longer follow-up (to 2001) and, for the first time, cancer registration data (*96*). The estimated ERR per Sv from mortality from all leukaemia excluding CLL was 1.7 (95%CI 0.06, 4.3). Similar ERR per Sv was found in the incidence study - 1.8 (95%CI 0.2, 4.4).

Studies of leukaemia and lymphoma in the post-Chernobyl period

In utero exposure

An increase of infant leukaemia among those who were *in utero* at the time of Chernobyl accident was suggested by Petriodou et al. in Greece (*97*). The findings were based on very small numbers (12 exposed cases of infant leukaemia with no individual foetal dose estimates available and 31 unexposed cases). A comparison of the two groups of infants revealed 2.6 times higher leukaemia incidence in the exposed group (95% CI: 1.4 to 5.1; P ~ 0.003).

Michaelis et al. also reported an increased rate ratio (1.48, 95% CI: 1.02; 2.15) in Germany based on 35 cases of infant leukaemia in an exposed group compared to 143 cases in a control group, but no correlation between infant leukaemia incidence and ground contamination levels was found (*98*).

Steiner et al. observed an increased incidence of infant leukaemia in West Germany in a cohort of children born after the Chernobyl accident. Mean annual incidence rate per 10^6 newborns in those born between 1 July.1986 and 31 December 1987 was 37.7 *vs.* 23.0 among those born between 1 January 1980 and 31 December 1985. Further more detailed analyses, comparing areas with different contamination levels and dose-rate gradients over time after the accident, showed, however, no clear trend with regard to exposure to ionizing radiation from to the Chernobyl accident (*99*).

Study of infant leukaemia incidence in Belarus found non-statistically significant increase of leukaemia incidence in infants who were most likely exposed *in utero* compared to the rates in "unexposed" birth cohort with rate ratios 1.26 (95% CI: 0.76; 2.10) and 1.51 (95% CI: 0.63; 3.61) for entire Belarus and for Mogilev and Gomel oblasts, respectively (*100*). Results of an ecological study by Noshchenko et al. suggest an increased risk of leukaemia and acute lymphoblastic leukaemia in children born in 1986 in radioactively contaminated territories in Ukraine who were followed for 10 years after the accident (*101*).

Results of the infant leukaemia studies do not provide unequivocal evidence about increased risk of leukaemia in those exposed *in utero* due to the Chernobyl accident. Although several studies have

demonstrated a possible association, they did not show a clear trend with regard to radionuclide contamination levels. The major limitations of most of the studies are lack of individual dose estimates and very small number of cases included in the analyses.

Childhood exposure

Several ecological studies have examined the association between leukaemia risk and exposure to radiation from the Chernobyl accident in childhood, including the European Childhood Leukaemia-Lymphoma Study (ECLIS), the largest and most comprehensive study to date (*102, 103*). The ECLIS study found no evidence of a radiation-related increase in incidence of leukaemia in Europe in the first five years after the accident.

Most of these ecological studies followed the same design where childhood leukaemia rates before the Chernobyl accident were compared to post-accidental rates.

A study of Finnish children aged 0 – 14 years in 1976-1992 did not reveal an increase in childhood leukemia incidence rates resulted from the Chernobyl fallout (*104*). In Sweden, an investigation of risk of acute childhood leukaemia among children aged 0-15 years in 1980 – 1992 did not show a significant increase in acute childhood leukaemia in areas contaminated after the Chernobyl reactor accident (*105*).

A small study in northern Turkey showed that in one paediatric cancer treatment centre more patients with acute lymphocytic leukaemia were seen after the accident than before but no incidence rates were reported (*106*).

Studies in the most affected countries did not provide evidence for childhood leukaemia incidence increase in Belarus (*107, 108*) and in the Russian Federation (RF) (*109, 110*). None of these studies was sufficiently sensitive to detect small changes in the incidence of rare disease such as childhood leukaemia and all are the subject to methodological problems that may limit the interpretation of the findings.

Up to now two case-control studies of childhood leukaemia have been conducted in the most contaminated regions of Belarus, the Russian Federation and Ukraine (*111-113*). A significant association between leukaemia risk and radiation dose was reported by Noshchenko et al. but results are difficult to interpret due to problems in the selection and comparability of controls in Ukraine. A significant increase in leukaemia risk with increasing radiation dose to the bone marrow (median estimated radiation doses 10 mGy) was found in the study by Davis et al, which overlapped with the Noshchenko study This association was most evident in Ukraine, apparent (but not statistically significant) in Belarus, and not found in the Russian Federation (*112*). However, as stated by the authors themselves, the overall significant dose–response might be accounted for, at least in part, by an overestimate of risk in Ukraine possibly due to a disproportionate number of controls from less heavily contaminated rayons.

A study of leukaemia and lymphoma performed in a paediatric population of Kyiv city and 24 regions of Ukraine based on smears and samples of whole blood and bone marrow from patients with hematopoietic malignancies diagnosed between January 1993 and December 2004 did not reveal any significant differences as compared to corresponding data in Western Europe (*114*). The only distinctive feature in the patterns of leukaemia was found in a group of 227 diseased children born in 1986 and 1987. It concerned the ratios between the major forms of acute leukaemia (AL) with increasing number of acute myeloid leukaemia (AML) cases and T-cell variants of acute lymphoid leukaemia (ALL) characterized by more aggressive clinical course and unfavourable prognosis. No specific place of residence of this particular group of children was given.

Further epidemiological survey is necessary for elucidation of the question whether the association between the haematological malignancies in children and exposure to ionizing radiation following the Chernobyl accident exist.

Adulthood exposure

The results of studies of leukaemia risk among adults conducted in the most contaminated areas following the Chernobyl accident provided little evidence on an increase in risk. Ivanov et al. found no evidence of increase in leukaemia rates in the most contaminated rayons of Kaluga region of the Russian Federation 10 years after the Chernobyl accident (*115*). The analysis was based on 35 incident and 17 death cases of leukaemia reported by the cancer registry in Kaluga oblast established in 1994. SIR and SMR estimates based on comparison with the rates for the whole oblast over the period 1981-1995 were used as a measure of association between leukaemia risk and exposure resulting from the residence in the radioactively contaminated territories.

In an ecological study by Prisyazhniuk et al., adulthood leukaemia and lymphoma incidence trends in the most contaminated areas of Ukraine before and after the Chernobyl accident were examined (*116*). Incidence data were collected from all oncology hospitals in Zhytomir and Kiev oblasts. A steady increase was found for both men and women between 1980 and 1993, but there was no evidence of a more pronounced increase after the accident. Little evidence on increased leukaemia and lymphoma adulthood incidence rates in Kiev and Zhytomir oblasts of Ukraine with Cs-137 soil contamination $\geq$ 1-15 Ci/km^2 was found in a study by Bebeshko et al. (*117*). No differences were found both - in incidence rates and leukaemia's morphological structure in these populations before (1980-1985) and after (1992 – 1996) the Chernobyl accident.

All these studies of leukaemia risk in adult population residing in contaminated territories were ecological by their nature with no information on exposure levels and mainly compared disease rates before and after the accident. Lack of evidence of increased leukaemia risk in adults could be due to low power to detect (leukaemia is a relatively rare event) exposure effects as well as due to absence of exposure effect on leukaemia incidence in adults.

Clean-up workers

Small studies of Estonian, Latvian and Russian liquidators provided little information about leukaemia risk with very few leukaemia cases included in the analyses (*118-121*).

An initial analysis of all leukaemia types in a much bigger Russian RNMDR cohort after 9 years of follow-up found SIR of 1.13 (95% CI: 0.62 – 1.90) and 1.77 (95% CI: 1.22-2.47) for the follow-up period 1986 – 1989 and 1990-1993, respectively (*122*). ERR per 1 Gray was 4.30 (95% CI: 0.87; 7.75). Risk estimation in this study was based on comparison of observed incidence with the national incidence of leukaemia for males of the same age groups.

An apparent increase in leukaemia incidence in a large cohort of Ukrainian liquidators (*123*) was not related to dose while in a cohort of Russian liquidators an approximately two-fold increased risk was reported among those whose registered doses lied in a range between 150 and 300 mSv (*124*). Findings of these studies are questionable because of large uncertainties in officially recorded doses that are used in these studies and because of unknown case verification procedures.

Two case-control studies with detailed individual dose reconstruction – one conducted in Belarus, Russian Federation and the Baltic countries and coordinated by IARC (*14*), and the other in Ukraine, in collaboration with the National Cancer Institute (NCI) (*15*) reported similar estimates of leukaemia risk among Chernobyl liquidators: the ERRs for all leukaemia were 4.8/Gy (90% CI: n.d. – 33.1) and 3.44/Gy (95% CI: 0.47 - 9.78), in the IARC and NCI study, respectively. Interestingly, both studies found generally similar radiation effects for CLL and non-CLL with ERR/ Gy 4.7 (90% CI: n.d. – 76.1) and 5.0 (90% CI: <0 – 57.0 in the first study, and 4.09 (95% CI: <0 – 14.4) and 2.73 (95% CI: <0 – 13.5) in the later, respectively. Though the ERRs/Gy for CLL were not significantly different from 0, the fact that similar estimates were reported in two independent studies merits further investigation.

Objectives

1. The objectives of the leukaemia studies **among liquidators** following the Chernobyl accident are:

- to confirm the observed increase in leukaemia risk;
- to assess how the leukaemia risk pattern changes over time - if the observed increases in both non-CLL and CLL continue be elevated with increasing time since exposure;
- to study gene mutations and functional polymorphisms that influence the risk of radiation-induced leukaemia.

2. The objectives of the leukaemia studies **among those exposed *in utero* and in childhood** after the Chernobyl accident are:

- to investigate if there is an increase in leukaemia among those who were exposed early in life, in particular *in utero*
- if the increase is confirmed:
  - to assess if the increase is due to exposure from the Chernobyl accident;
  - to evaluate if leukemia radiation risk varies by time since exposure, age, gender;
  - to study gene mutations and functional polymorphisms that influence the risk of radiation-induced leukaemia.

Specific relevance (value-addedness) of Chernobyl populations

Continuation of studies of leukaemia risk in well-established cohorts of liquidators from Baltic countries, Belarus, the Russian Federation and Ukraine with relatively long follow-up, individual dose assessments can provide further insights about association of ionizing radiation and different cell types of leukaemia, identification of possible molecular markers of radiation-induced leukaemia. Findings from recent studies of the Chernobyl clean-up workers (*14, 15*), along with better understanding of the pathobiology of CLL, provide an opportunity to further elucidate possible association between CLL and exposure to ionizing radiation as previously it was believed that CLL is the type not sensitive to ionizing radiation.

Studies of leukaemia among individuals exposed *in utero* or in infancy as a result of the Chernobyl accident are very important for radiation protection purposes as they may provide evidence on the increased susceptibility in these particularly sensitive populations. Studies of childhood leukaemia incidence trends following the Chernobyl accident are of scientific and public health importance due to unique nature of population and exposure conditions, namely, low-dose rate protracted environmental exposure.

Proposed approach – Objective 1

Population and study design

An updated follow-up of the study in Ukraine (*15*) will ascertain cases for another 6 years (2001–2006). Extending the case-control study nested within the existing cohorts of liquidators from the Baltic States, Belarus and Russia (*14*) to include cases ascertained beyond 2000 can also be considered. Altogether, the two studies comprise approximately 110,000 liquidators from Ukraine (involved in the clean-up activities in 1986-1990) and 146,000 liquidators from the Baltic countries, Belarus and Russia (participants of clean-up in 1986-1987). The inclusion of recent cases would allow assessing if the observed increase in CLL risk declines which would indicate no association with the dose received during the clean up activities in Chernobyl. Both studies have similar design, International Panel of pathologists and haematologists verify diagnoses of the cases included in the studies. Pooling data from the two studies also can be considered to increase statistical power to detect radiation related risks.

Dosimetry

Doses to the bone marrow in the case-control studies of liquidators in the Baltic countries, Belarus, Russia and Ukraine are obtained using the same method RADRUE which was developed by a dosimetry committee in collaboration with epidemiologists. In the IARC study 78% of RADRUE doses were below 50 mGy and 14% were 100 mGy or more.

Biological samples

If there are improvements in sensitivity of existing biodosimetry methods (FISH, EPR), collecting of blood and tooth enamel could be considered for future biodosimetry.

Molecular markers

Blood samples for identifying molecular markers which may play an important role in radiation-induced leukemigenesis, e.g., BCR/ ABL translocations, should be collected within the case-control study of liquidators. Rearrangements that define the subtype of leukemia should be also studied. Collection of blood samples of genomic DNA would allow investigating possible inherited factors associated with an increased risk of radiation-induced leukaemia. Collection of leukemic blood from the patients would allow studying frequencies of different mutations in presumably radiation-associated and sporadic leukaemia.

Pathology

It is desirable to collect all bone marrow smears and/ or trepanobiopsies material from leukaemia patients and store them in the Chernobyl tissue bank (see a document on Chernobyl tissue bank).

Feasibility - Roadblocks that need to be overcome

Dosimetry (RADRUE) is a time-consuming and costly method that requires specific knowledge of conditions of work as a liquidator. The experts available to analyze questionnaire data are few and because of their age may be no longer available for the dose reconstruction. The method therefore requires documenting the procedure of analysing questionnaire data so that it can be replicated when needed.

Availability of slides, trepanobiopsy material, bone marrow smears (and their quality) for validating diagnoses of retrospective cases is achievable but requires many efforts and collaboration with hospitals.

Ethics requirements

Necessary permissions of national and international Ethical boards should be obtained depending on the specific requirements of each country.

Statistical power

Should be assessed before setting up a study.

Prioritisation

The priority for medium-term project is given to extension of the case-control studies of leukaemia among liquidators with pooling data from the two studies that allows getting further insights about association of ionizing radiation and different cell types of leukaemia, identification of possible molecular markers of radiation-induced leukaemia. Elucidation of possible association between CLL and exposure to ionizing radiation would have an important impact on public health as previously it was believed that CLL is the type not sensitive to ionizing radiation.

Proposed approach - Objective 2

Population and study design

Possible approach is monitoring of leukaemia incidence and mortality trends among those who could be exposed *in utero* or/ and in childhood through existing cancer registries. The study of infant leukaemia assessing possible effect of exposure *in utero* would collect additional data from European childhood cancer registries that participated in ECLIS study. The ECLIS study encountered problems to assess specifically the issue of exposure *in utero* because a number of cancer registries, particularly in Eastern European countries, collect year rather than exact date of birth.

If the increase in incidence of infant and childhood leukaemia is demonstrated - further analytical studies (case-control) could be applied in the most contaminated areas among those exposed *in utero* and/ or childhood. Possible case-control study should consider then including children born from female evacuees that were pregnant at the time of the accident.

Doses

Reconstruction of doses to the foetus (whole body and/ or red bone marrow) and to the child's bone marrow should be considered if an increase in leukaemia among those who were exposed *in utero* and early in life is confirmed.

Case-control approach would require dose assessment for each study subject with exact date of birth to be known.

Biological samples

Molecular markers

Blood samples for identifying molecular markers which may play an important role in radiation-induced leukemigenesis, e.g., *BCR/ ABL* translocations, should be collected within the case-control study of liquidators. Rearrangements that define the subtype of leukemia shall be also studied.

Pathology

In a framework of case-control studies it would be desirable to collect all bone marrow smears and/ or trepanobiopsies material from leukaemia patients and store them in the Chernobyl tissue bank (see a document on Chernobyl tissue bank).

Feasibility - Roadblocks that need to be overcome

Dose assessment for those who were possibly exposed *in utero* would require considerable efforts.

There is considerable interest in seeing the ECLIS analyses redone and completed, but this would require a sizeable effort to collect the needed information from hospitals and population registries, if ethics approvals can be obtained. The ECLIS study encountered problems in the past to assess specifically the issue of exposure *in utero* because a number of cancer registries, particularly in Eastern European countries, collect year rather than exact date of birth.

Ethics requirements

Necessary permissions of national and international Ethical boards should be obtained depending on the specific requirements of each country.

Prioritisation

Up to now the data on leukaemia risk following exposure in *utero* and in childhood due to the Chernobyl accident are not very conclusive and informative mainly, due to studies' methodological limitations. Higher susceptibility of foetus and child compared to adult to leukomogenic effect of ionizing radiation makes important to follow-up leukaemia risk in those exposed *in utero* and in

childhood. Results from the studies of childhood leukaemia incidence following the Chernobyl accident are considered by scientific and public communities to be of high public health importance. We are assigning a short-term urgent priority to the study of leukaemia risks following intrauterine and childhood exposure due to the Chernobyl accident. But all roadblocks related to the proposed study should be thoroughly considered, namely a continuation of the ECLIS study would require considerable efforts on collecting the needed information from hospitals and population registries, if ethics approvals can be obtained as well as obtaining of additional information on birth dates to possibly assess the effect of *in utero* exposure on infant leukaemia

OTHER TUMOURS THAN THYROID (BENIGN AND MALIGNANT)

The Chapter was prepared by A. Kesminiene and E. Ostroumova, IARC, Lyon, France

Background

General

Several reviews carried until now (*1*) (*2, 19*) concluded that apart from the large increase in thyroid cancer incidence in those exposed as children and young adults, there are at present no clearly demonstrated increase in incidence of other solid cancers related to the Chernobyl radiation. Increases in the incidence of cancers and other diseases have been reported in Belarus, the Russian Federation and Ukraine, but much of the increase appears to be due to other factors, including improvements in diagnosis, reporting and registration.

Cancer incidence rates in the most contaminated Gomel oblast of Belarus were compared to the corresponding rates in Vitebsk oblast with relatively low contamination. Overall cancer incidence rates, including colon, urinary bladder and thyroid, were significantly higher in Gomel than in Vitebsk oblast (*125*). In contrast, a study of cancer risks in Kaluga oblast of the Russian Federation 10 years after the accident did not show any significant effect of radiation on cancer incidence except for thyroid cancer in women (*115*). In Ukraine, an assessment of solid cancer incidence 20 years after the Chernobyl accident revealed a continuous increase for cancers of oropharyngeal cavity, rectum, female breast, prostate, urinary bladder, kidney and thyroid (*126*). All these studies were ecological with large variability in dose within the geographical study area and absence of control for important confounding factors. Their findings are therefore difficult to interpret.

Several important caveats should be borne in mind when considering evidence of increases in solid cancers among Chernobyl-exposed populations: studies are few and methodologically limited; doses to most organs (except thyroid) tend to be low; any expected increase is likely to be small compared to risk due to other causes ("baseline" rates); numbers of subjects are insufficient to allow conclusion; reliable individual (and even group) doses are generally not available; no information is available on other potentially much more important risk factors for the diseases (such as tobacco and alcohol); the typical minimal latency period for solid cancers seen in other studies is of the order of ten-fifteen years or more, no increase in risk for solid cancers would be therefore expected to manifest itself until the end-2000 (most of reviewed studies included earlier diagnosed cases). Thus studies, published to date provide little information about possible risk of neoplasms other than thyroid, breast and leukaemia from the Chernobyl accident. Given the very large number of people exposed, however, we might expect an elevated lifetime solid cancer risk that can be translated into a substantial number of radiation-related cancer cases in the future.

Existing resources such as national cancer registries in Belarus and recently in Ukraine allow following all sites and site-specific cancer incidence rates in the exposed populations (*125-127*).

Brain tumours including meningioma

The majority of tumours of the central nervous system are originated from glial cells, and glioblastoma is the most frequent and most aggressive form of malignant tumour (*128*). The most frequent benign nervous system tumours are meningiomas (originating from the brains covering) representing 13-26% of all intracranial tumours and schwannomas (arising from Schwann cells in the peripheral nerves). Meningioma is more frequent in females (*129*).

Several epidemiologic studies have reported increased risks of meningiomas and gliomas after radiotherapy, particularly in childhood (*130-138*).

Among A-bomb survivors, a statistically significant dose-related excess of nervous system tumours was found with excess relative risk (ERR) per Sievert (Sv) of 1.2 (95% CI: 0.6; 2.1) (*139, 140*).

The highest ERR/ Sv of 4.5 was seen for schwannoma (95% CI: 1.9; 9.2). When considered individually, a positive but non significant risk was found for gliomas, meningiomas, and other non-schwannoma nervous system tumours.

Studies where a significantly increased risk of radiation-induced meningiomas was observed include: the study of childhood exposure for tinea capitis (*130*); the study A-bomb survivors in Nagasaki (*141, 142*); studies of patients with dental radiographic examinations (*143-145*). Doses to the brain in those studies ranged from less than 0.005 Gy (40% of A-bomb survivors cohort) to 1.4 – 1.5 Gy (in the tinea capitis study). The risk was usually higher among those who were younger at the time of exposure and there appear to be differences depending on ethnic origin (*138*). The latency between exposure to low dose and meningioma diagnosis in different studies was about 30-36 years.

Radiation induced meningioma differs from spontaneous one in terms of patient's age at tumour occurrence, its multiplicity, aggressiveness and recurrence rate. Radiation related meningiomas may exhibit more aggressive behaviour with a high rate of recurrence after surgery and radiotherapy.

Studies of genetic predisposition for the development of radiation-induced meningioma suggest that DNA repair and cell-cycle control genes such as ATM gene could be plausible candidates for investigation (*146*). Findings by Sadetzki et al. suggest that *Ki-ras* and *ERCC2* SNPs are possible markers for meningioma formation whereas *cyclin D1* and *p16* SNPs may be markers of genes that have an inverse effect on the risk of developing meningioma in irradiated and nonirradiated individuals (*147*). A novel association between *rs*4968451 and meningioma risk was recently established (*148*). Since about 28% of the European populations are carriers of at-risk gene for *rs*4968451, the variant is likely to make a substantial contribution to meningioma development.

Chernobyl related studies

PubMed search made for key words "brain tumors" and "Chernobyl" provided very little results. There was only an abstract available from a study by Dumitrescu *et al.* where the authors discussed possible effects of Chernobyl accident exposure on the histological types of cerebral glial tumours diagnosed in 1981 – 1991 at the Department of Pathology of Neurosurgical hospital, Iasi, Romania (*149*). The authors reported a decrease of astrocytomas incidence and an increase of oligo-astrocytic tumours since 1986 but no information is provided on study subjects, contamination levels, and number of tumours under study.

An ecological study among Swedish children aged 0 – 19 residing in parts of Sweden with high ^{137}Cs contaminations as a result of the Chernobyl accident was published (*150*). The authors found a continuous increase of brain tumour incidence during the period of 1978 - 1992 but there was no clear relationship between the incidence rates and varying levels of Cs-137 contamination.

Descriptive analysis of childhood cancer rates over the period 1978 – 1994 based on the data from Belorussian childhood cancer registry showed a steady increase of brain tumour incidence with time that could be due to a better reporting in the later years, but a sharp increase in the rates in 1993 – 1994 warrants further investigations (*151*).

An increased incidence of brain cancers was found in a combined cohort of Latvian and Estonian liquidators (5,546 and 4,786 men, respectively) followed from 1986 to 1998 (*119*). SIR for brain cancers (based on 11 cases) was 2.14 with 95% CI: 1.07; 3.83 with lack of dose response. SIRs of brain cancer were significantly higher among those who were older than 30 when the follow-up started (SIR=2.69; 95% CI: 1.29; 9.96), who stayed in the Chernobyl area for more than 85 days (SIR=2.79; 95% CI: 1.12; 5.75), who had documented dose below 9.6 Gy (SIR=2.88; 95% CI: 1.06; 6.28), and after more than 10 years since return from Chernobyl area (SIR=5.16; 95%CI: 1.68: 12.06). Authors considered that study finding could be due by chance and further follow-up is required.

Parathyroid adenoma and other benign tumours

Anecdotal reports of parathyroid adenomas arising after radiation exposure have been published for many years, both for external radiation and from therapeutic ^{131}I. Experimentally, ^{131}I was shown to induce parathyroid adenomas in rats (*152, 153*). It should be noted that rodent parathyroids are almost invariably intrathyroid. The human parathyroid glands are anatomically close to the thyroid, particularly the upper glands which usually lie on its surface, may be within a surface depression but are rarely entirely within the thyroid so that the parathyroid dose from β- radiation from I-131 is difficult to estimate.

In humans, external radiation has been shown to be associated with the subsequent development of parathyroid adenomas in several studies (*154-157*).

From an epidemiological study of 23 parathyroid tumours detected in Hiroshima Prefecture among A-bomb survivors between 1974 and 1987, an elevated incidence of the tumours with increasing dose was shown ($p < 0.001$).

A significant effect of ionizing radiation on levels of calcium, parathyroid hormone (PTH) and calcitonin was found in 1,459 subjects in Hiroshima and Nagasaki even after patients with hyperthyroidism were excluded (*158*).

In the study of 27,925 individuals who were exposed in infancy for skin hemangioma the mean thyroid dose was 0.20 Gy (range from 0 to 28.5 Gy). 43 cases of adenomas were identified in the cohort through the Swedish Cancer Register in 1958 – 1977 (*154*). The estimate of excess relative risk (ERR) per 1 Gy was 3.84 (95% CI: 1.56; 8.99) for all cases and 1.56 (95% CI: 0.36; 4.45) with biased cases (when childhood radiation exposure at the time of diagnosis was known) excluded.

In the study by Schneider at el. in a cohort of 2,555 subjects with external beam radiotherapy to the head and neck area for benign conditions 36 confirmed cases of hyperparathyroidism were found with ERR per cGy of 0.11 (95% CI: 0.0 – 17.2).

In 53 patients with head and neck exposure for childhood malignancies five patients developed hyperparathyroidism with four conventional parathyroid adenomas and one parathyroid lypoadenoma (*155*). Four out of five patients with parathyroidadenoma were 1 – 2 years old at the time of radiotherapy when one patient of 16. Those exposed at early age developed parathyroid adenomas in less than 20 years after exposure. The number of cases was too small to allow meaningful dose-response analysis.

A study of 6,082 patients treated with radioactive iodine for thyrotoxicosis found no significantly increased risk for parathyroid adenoma (SIR=1.14; 95% CI: 0.57; 2.03), but the subjects were adults (mean age of 59 years) (*159*). Average latency period between thyrotoxicosis diagnosis and parathyroid adenoma in the study was 8 years within the range from less than 1 to 19 years.

There is a report on association between increased risk of developing parathyroid adenomas and x-ray treatment of benign diseases in the cervical spine in 27,415 adult patients (mean age of 53 years) (*160*). The calculated dose to parathyroid region was about 1 Gy. SIR was 1.83 (95% CI: 1.14; 2.76) compared to general population and 0.97 (95% CI: 0.62; 1.45) compared to the internal control group with no exposure of the parathyroid region. ERR estimate in the study was about 0.8 per 1 Gy with no information on confidence bounds provided.

Parathyroid adenomas have been recorded as occurring more frequently with thyroid carcinoma and radiation has been suggested as a possible reason for the association. However, the link could simply reflect the increased chance of finding one condition when being investigated biochemically or explored surgically for the other (*161*).

Objectives

Future studies of solid tumours in populations exposed to Chernobyl radiation should, whenever practical, examine cancers in specific organs. On the other hand, monitoring of trends of combined cancer types are perhaps of some interest for public health planning purposes. Regarding specific

cancer types, breast cancer (considered in a separate document), brain tumours, including meningioma, and parathyroid adenomas may be of particular interest.

The objectives of these studies would be:

1. To monitor trends of overall and site-specific tumour incidence and mortality following the Chernobyl accident.
2. In the longer-term, if increases in specific tumour incidences, such as brain tumours, including meningioma are detected, to evaluate if the increase is associated with ionizing radiation exposure from the Chernobyl accident and to assess the role of potential risk modifiers
3. To asses if there is an increase in parathyroid adenoma incidence following the exposure to high doses of I-131.

Specific relevance and proposed approaches are discussed separately according to each objective.

Specific relevance of Chernobyl populations

The widespread contamination of territories in Europe and elsewhere following the Chernobyl accident draws public attention to the possible cancer burden which may be attributable to Chernobyl radiation. Millions of people were exposed to and, in some cases, are still being exposed to radioactive contamination. These exposures are unique in their characteristics: protracted in time (from several days to decades), they resulted from a mixture of external and internal radiation. Monitoring tumour incidence and mortality trends in Chernobyl populations may fill in gaps in our knowledge about long-term radiation risks resulting from internally incorporated radionuclides and from mixtures of various radiation types.

The risk of meningioma following radiation exposure is one of the highest risks of radiation induced tumours after leukaemia, thyroid and breast cancer. The incidence of meningioma appears to be higher in Jewish populations and the observation of clustering of radiation induced meningioma suggests a genetic predisposition in some of the Jewish populations of Israel. If this is confirmed, the presence of a large proportion of Ashkenazi Jews in the contaminated areas of Belarus and Ukraine may provide an important opportunity to study this association in a different population.

The Chernobyl accident offers an unprecedented opportunity to study the risk of the development of parathyroid adenoma following exposure of large numbers of young children to significant doses of ^{131}I Given the variable anatomical relationship of the parathyroids to the thyroid a large study would be needed. Based on the knowledge that doses lower than those used for radiotherapy can cause parathyroid adenoma development with a relatively long latency, we might expect to detect an increased risk in the Chernobyl populations more than 20 years after the accident.

Proposed approach - Objective 1

Population and study design

Taking into account that a latency period to develop radiation-induced site-specific tumours is potentially long, as well as that radiation related risk of solid cancers remains elevated throughout life, it is important to ensure long-term monitoring of cancer incidence trends in the three most affected countries to be able to detect an increase in site-specific cancer rates if any, among populations exposed to low doses of ionizing radiation. The populations that should be monitored are:

- All population of Belarus and Ukraine
- All population of the four contaminated regions of the Russian Federation
- Chernobyl liquidators from the Baltic countries, Belarus, the Russian Federation and Ukraine

Monitoring tumour trends would include: obtaining site-specific cancer incidence/mortality data, monitoring of data quality and completeness, evaluating sex-, age-, birth cohort-, region- specific time trends in cancer incidence.

Studying cancer trends in the cohorts of the Chernobyl liquidators will require linkage of the Chernobyl registries or rosters in the Baltic countries with existing cancer registries.

Data on migration and vital status in all countries can be, in principle, obtained from the population registries/address bureaus/migration offices.

Doses

Simple monitoring of tumour trends in these populations does not require individual or group-specific doses. However, for the characterisation of the differences in trends between less and more contaminated regions, information on average deposition density can be used.

For more specific characterisation of the trends in relation to the Chernobyl radiation, average cumulative (for different time-period) doses per settlement are, in principle, available for Belarus, Ukraine and four contaminated regions of the Russian Federation. They can be used to calculate average doses at district (rayon) or region (oblast) level to evaluate relationship between radiation exposure and temporal oblast/rayon specific changes in tumour incidence, if such are observed.

Tumour trends in the cohorts of the Chernobyl liquidators can be compared with those of general population.

Roadblocks that need to be overcome

The absence of cancer registers in Ukraine and the contaminated regions of the Russian Federation at the time of the accident, recent changes in the longevity of the populations in the affected countries (both in contaminated and uncontaminated regions), improvements in cancer registration and cancer detection, and possible different reporting practices in most contaminated regions compared to less contaminated make it difficult to evaluate changes in cancer incidence trends in relation to radiation from Chernobyl.

Results of comparison of tumour trends in the cohorts of the Chernobyl liquidators with those in the general population also may be difficult to interpret because Chernobyl liquidators may be under increased medical surveillance compared to the rest of the population due to their personal interests or due to the existing law about regular health examinations.

However, continuing of monitoring of tumour incidence and mortality trends may provide basis for conducting further more specific well-designed analytical studies of carefully selected populations, if increases are observed.

Particular consideration should be given to evaluate feasibility of evaluating trends in meningioma incidence as this disease is not reported in most of the cancer registries. Registration and reporting on benign tumours in cancer registries in Belarus, Ukraine needs to be evaluated.

Ethics requirements

In some countries it is necessary to submit a proposal to the appropriate Institutional Ethics Board to get permission to use the data.

In the Baltic countries, ethics permission is necessary to obtain for linkage of the liquidators' rosters with population and cancer registries.

Proposed approach - Objective 2

Population and study design

It is expected that increase in radiation related brain tumours, particularly meningiomas, if any, may occur in the populations residing in the most contaminated districts of Belarus and Ukraine who potentially received the highest doses to the brain.

A case-control study would be the preferable approach to asses both the role of radiation from Chernobyl and genetic predisposition, if it exists.

A nested case-control study can also be conducted in the cohorts of the Chernobyl liquidators from the Baltic countries, Belarus, the Russian Federation and Ukraine, if the increase in brain tumours is confirmed in these cohorts.

Dosimetry

Individual doses to the brain need to be reconstructed.

For population residing in the most contaminated districts, models to calculate absorbed doses to the brain which take into account both external exposure and exposure from the internally incorporated radionuclides would need to be developed.

Official radiation doses received during their clean-up activities are available only for a part of the Chernobyl liquidators and are known to be inaccurate. Individual doses to the thyroid and bone marrow were reconstructed with the use of RADRUE method (*162*)for a limited number of liquidators who were included in the case-control studies conducted in the Baltic countries, Belarus, the Russian Federation and Ukraine. RADRUE can be also applied to calculate doses to the brain which for liquidators (except Belarusian) mainly have occurred from the external exposure while working in the area around the Chernobyl NPP or inside the buildings. For Belarusian counterparts, doses might have resulted from both – external exposure during participation in the clean-up around the Chernobyl NPP and in their places of residence in the contaminated villages and from the intake of contaminated food. The residential doses would require similar approach as for the population residing in the most contaminated districts.

Biological samples

Blood or saliva might be considered for screening of candidate genes identified to contribute to genetic susceptibility of radiation induced menigiomas.

Pathology

Fresh tumour tissue is desirable - for extracting RNA in order carry out gene expression array analysis.

Roadblocks that need to be overcome

Case ascertainment most likely is possible only through specialised neurosurgery departments. The completeness of the case ascertainment can be checked for malignant brain tumours using cancer registries but for meningeomas might be difficult to evaluate since benign tumours are not always reported in the cancer registries.

One of the major roadblocks for studying genetic predisposition due to specific mutation in the Ashkenazi Jew population could be considerable migration of this population outside Belarus and Ukraine that took place in the years following the Chernobyl accident.

Ethics requirements

Typical ethics requirements are necessary, the same as for other epidemiological studies.

Statistical power

This needs to be evaluated before setting up a case-control study, particularly for studying association between genetic predisposition to haemangioma and radiation dose to the brain.

Proposed approach - Objective 3

Population and study design

As parathyroid adenomas are almost always functional it would be possible to study those exposed as children, possibly based on existing screening cohorts with known thyroid ^{131}I doses (Bel-Am, Ukr-Am cohorts should be considered).

Doses

Individual thyroid doses from ^{131}I have been calculated, based on direct thyroid measurements, for all subjects in the existing cohorts are being calculated.

Biological samples

Blood samples (serum) - for biochemical measurements (parathyroid hormone, calcium and calcitonin levels) would be required to identify cases of hyperparathyroidism and investigate a possible dose response.

Roadblocks that need to be overcome

A major roadblock for this study is obtaining of information on parathyroid adenomas because it will not be reported and recorded in cancer registries. It would require regular screening approach.

There will be probably some uncertainties related to the variable anatomical relationship of the parathyroid to the thyroid gland that should be taken into account while assessing dose to the parathyroid.

Ethics requirements

Typical ethics requirements are necessary, the same as for other epidemiological studies.

Prioritisation

To monitor trends of overall and site-specific tumour incidence and mortality, including brain tumours

Monitoring of tumour incidence and mortality trends in the three most affected countries should be given a high priority in short, medium and long term. We do not recommend starting a new project but role of existing cancer registries should be acknowledged and their activities supported.

Wherever possible, medium priority should be given to analyze cancer incidence and mortality data using similar methods as it was done, for example, for breast cancer incidence trends (*9*).

Monitoring of tumour incidence and mortality trends in the cohorts of the Chernobyl liquidators should be also given a high priority in short, medium and long term.

If increase in brain tumours, including meningeomas is observed, to evaluate possible association with ionizing radiation exposure from the Chernobyl accident and to assess the role of potential risk modifiers

If an increased risk in the incidence of brain tumours, including meningioma is observed, analytical studies of brain tumours, including meningioma would be a high priority in the medium to long term (given the long latency for radiation induced brain tumours).

To asses if there is an increase in parathyroid adenoma incidence following the exposure to high doses of ^{131}I

This is not a short term priority, but it is important to monitor a possible increase in this disease in existing screened cohorts and to revise the necessity of setting up an analytical study in the future on the basis of these results.

RADIATION-INDUCED CATARACTS

The Chapter was prepared by A. Kesminiene and E. Ostroumova, IARC, Lyon, France

Background

General

A cataract is an ocular lens opacity associated with visual impairment and is one of the major causes of blindness worldwide (*163*). Depending on localization of the lens opacity, types of cataract can be classified as nuclear, cortical, posterior subcapsular and mixed. Based on a systematic review of ten population-based studies of the cataract prevalence in Europe, USA and Australia, when the cataract was defined as a lens opacity combined with a decreased visual acuity, the cataract prevalence ranged between 15% and 30% (*164*). Cataract prevalence increases with age when such factors as exposure to sunlight, smoking, alcohol consumption, diabetes and consumption of corticosteroids become of importance for cataractogenesis (*165*).

Lens of the eye is considered as a radiosensitive tissue with posterior subcapsular and cortical opacities to be associated with exposure to ionizing radiation (*21, 166*). A mechanism underlying radiation-induced cataract is a direct damage of lens epithelium dividing cells by ionizing radiation (*167, 168*). Further differentiation and migration of damaged cells to the lens posterior pole lead to opacity.

Experimental studies have shown that anatomic characteristics of the eye in rodents and primates are similar to human ones, so that many of the mechanistic models of cataract development are based on animal experiments. Radiation-induced lens opacity has been obtained experimentally after X or neutron irradiation (*169-171*).

Recent studies demonstrated that heterozygosity of the ATM gene was associated with higher radiosensitivity (*172*) and predisposition to cataract development (*173*) in mice. In humans, ATM gene heterozygosity in the Western population is estimated to occur in 0.5 – 1% (*174*). Also an effect of hyterozygosity of Rad9 on cataractogenesis was found in mice (*175*).

Results of recent epidemiological studies showing an increased cataract risk after low-dose dose exposure provoked considerable debate about the deterministic or stochastic nature of radiation-induced cataractogenesis. It was believed that cataract development is a deterministic effect which has a dose threshold of 0.5 – 2.0 Gy for acute exposure and of 5.0 Gy for protracted or fractionated exposure (*166*). This assumption was supported by the results of a cataract study 19 years after the atomic bombardment of Hiroshima where a threshold dose for radiation-induced cataract was found to be approximately 1.5 Gy (*176*). However, a later study (*177*) based on DS02 dosimetry system, found a statistically significant dose-response increase in the prevalence of cataracts with a threshold dose of 0.6 Sv (95% Confidence Interval (CI): <0;1.2). For postoperative cataracts, the threshold dose was estimated to be even lower - 0.1 Gy (95%CI: <0;0.8) (*178*). These results suggest a much lower threshold than that assumed by the radiation protection community and was statistically compatible with no threshold at all. The results also suggested a decrease of radiation effect with increasing age (*177*).

In the Beaver Dam Eye study of 4,926 subjects of both genders aged 43 to 84 years, a significantly increased risk of posterior subcapsular and nuclear sclerotic opacities was found to be related to self-reported CAT scans of the head: ORs were 1.45 (95% CI: 1.08; 1.95) and 1.28 (95% CI: 1.02; 1.61), respectively. An increased but non-significant association between posterior subcapsular and nuclear sclerotic opacities, and X-ray examinations of the head was also reported with ORs of 1.27 (95% CI: 0.98; 1.66) and 1.13 (95% CI: 1.02; 1.61), respectively. The study had a potential bias due to the fact that those who were referred for CAT scan of the head had underlying conditions (eye trauma, stokes or transient ischemic attacks) that may have led to the development of cataract.

A study showing an association between cataract and radiation for skin haemangioma was reported by Hall et al., 1999. The ophthalmological survey took place when the study subjects reached the age of 36-54 years. The prevalence of lens opacities was higher among those who underwent radiotherapy (37%) compared to non-exposed controls (20%) ($p < 0.001$). The OR per 1 Gy was 1.50 (95% CI: 1.15; 1.95) for posterior cortical cataracts and 1.49 (95% CI: 1.07; 2.08) for subcapsular cataracts (*179*). Age at examination was the strongest modifier of the risk.

Occupational studies have also provided some evidence that increase in risk of cataracts may occur at lower doses than it was believed before. In a study of 35,705 US radiologic technologists (median occupational radiation dose 28.1 mGy) odds ratio of developing cataract was 1.18 (95% CI: 0.99; 1.40) in the highest dose category (mean dose 60 mGy) compared to the lowest dose category (mean 5 mGy) (*180*). The study also found a statistically significant association between personal history of diagnostic X-rays and risk of cataracts: subjects who had 3 or more X-rays to the face or neck had 1.25 (95% CI: 1.06; 1.47) increase in risk compared to those with no X-ray procedures to the face or neck. The main limitation of the study is that cataract incident cases were identified through follow-up questionnaires without clinical confirmation. Possible outcome misclassification in the study can not be ruled out.

An increased cataract incidence was reported among astronauts with lens dose of 8 mSv and higher compared to those with dose less than 8 mSv (*181*). To investigate if employment as a commercial airline pilot and the resulting exposure to cosmic rays is associated with lens opacity, a population-based case-control study of 445 men was set up by Rafnsson et al. The odds ratio for nuclear cataract was 3.02 (95% CI: 1.44; 6.35) among pilots compared to non-pilots after adjustment for age, smoking and sunbathing habits (*182*).

A study of lens opacities following protracted low-dose-rate γ exposure was performed in Taiwan in the 1980s (*183*). In this study, the exposure doses ranged from 0.001 up to about 1,5 Sv. A significant dose-dependent increase in the numbers of focal lens defects among those less than 20 years old at the time of examination was demonstrated. These findings, however, are based only on 114 subjects with minor lenticular changes.

Chernobyl

Among the examined 77 survivors of the Acute Radiation Syndrome (ARS) with doses from 2.6 to 8.7 Gy, 11 cases of radiation cataract were found. The time of occurrence and dose dependence of the cataracts did not reveal any new features (*184*).

Risk and threshold dose of radiation-induced cataract development among Chernobyl clean-up workers was assessed in a prospective cohort of 8,607 subjects at 12 and 14 years after exposure (*16*). The dose reconstruction modeled the gamma doses by validating the official recorded exposures against dose estimates obtained by EPR analyses of teeth from a sample of workers, including uncertainty estimates (*31*). Beta doses to the lens of the eye were further reconstructed using information about beta exposure levels at various work locations within the Chernobyl area by time period. The doses to the lens of the eye were nearly all low to moderate: 94% of study subjects received lens doses lower than 400 mGy. The median estimated lens dose was 0.12 Gy. More than three quarters of the liquidators were below 50 at the time of examination. A statistically significant odds ratios (OR) per 1 Gy of lens dose were found for stage 1 superficial posterior cortical changes (OR=1.51; 95% CI: 1.09; 2.10), for early posterior subcapsular changes (OR=1.89; 95% CI; 1.25; 2.84) and for stage 1 posterior subcapsular changes (OR=1.42; 95% CI: 1.01; 2.00). All of these types of cataracts may be radiation related, Analyses for dose thresholds for stage 1-5, stage 1, stage 1 non-nuclear, stage 1 superficial cortical, stage 1 posterior subcapsular cataracts showed that the data are compatible with thresholds of moderate dose level within the range of 0.3 – 0.5 Gy (the upper bound ranged from 0.5 to almost 0.7 Gy). A small number of stage 2-5 cataracts in the study cohort did not allow for meaningful analysis of dose threshold in these higher grade categories.

Study of prevalence of lens changes in 5 – 17 year old 1,787 Ukrainian children living in the permanent control zone around Chernobyl nuclear reactor found a small (3.6%) but statistically significant excess (p=0.0005) of posterior subcapsular changes (*185*). The weaknesses of the study were related to the fact that individual doses were unavailable and that examiners were not 'blinded' as to the examinee's exposure status (which was defined by the geographical location of the examination). There were no further studies performed on children to confirm or challenge the findings by Day et al.

Objective(s)

1. The main questions to be answered about cataractogenesis following the exposure due to Chernobyl accident among the Chernobyl liquidators are:
    - to confirm the observed risk in developing radiogenic cataracts;
    - to estimate the value for dose threshold, in particular for higher grade cataracts;
    - to clarify if some fraction of radiation-associated Grade I opacities progress to become more severe, vision-disabling.
2. The main questions for those exposed as children are:
    - to asses if the early reports on excess in lenticular changes among children residing in the contaminated territories can be confirmed to provide more evidence on catarogenic sensitivity in young ages;
    - if the increase in risk is confirmed, then to quantify it, to estimate value for dose threshold and to characterize dose-response shape.

Specific relevance of Chernobyl population

As a number of studies on cataracts conducted at the low-to-medium-dose range is limited and a controversy exists about deterministic or stochastic nature of the radiation-induced cataracts, it makes it important to continue investigation of radiation-induced cataracts following the Chernobyl accident. Results of the previously performed studies indicated that cataracts arising in the population of Chernobyl liquidators, corrected for the most important confounding factors, were related to the dose received. For the most part, the doses were less than 0.5 Gy of low-LET radiation acquired in a protracted/fractionated manner. A key finding was that the data were not compatible with a dose-effect threshold of more than 0.7 Gy, although this needs to be tempered by consideration of the uncertainties in the dosimetry.

Studies of health consequences among Chernobyl liquidators therefore provide a unique opportunity to assess the risk of the radiation induced cataracts following exposure to low doses, to find out about magnitude of such risk, about rate of progression from early lens lesion to more advanced visual-impairing lesions, to evaluate possible cataractogenesis threshold following the exposure at a wide range of doses, with dose to the lens to be within low-to-medium range. The duration of the follow-up of 23 years is sufficient for detecting a time since exposure effect on cataract occurrence.

A role of genetic predisposition for cataractogenesis can be also studied in this unique population, if biological samples can be obtained.

Study of cataracts in those exposed as children can also provide an opportunity to evaluate cataract risk after low-dose-rate protracted exposure at young ages. However, there are some considerations that should be taken into account and these are discussed below.

Proposed approach – Objective 1

Study population

The prospective Ukrainian/American Chernobyl Ocular Study (UACOS) was set up in the 90's (*173*). The cohort consists of 8,607 liquidators who have undergone two rounds of

ophthalmological examinations. The existing structure can be used to continue following this population for further development of lenticular changes.

Study design

As the detection of early lens changes requires regular ophthalmological examinations, a prospective cohort study design is most suitable.

Cohort members will be examined at regular intervals for manifestations of possible radiation effects on the lens. Ophthalmological examinations will be performed by trained staff using the same technique and uniform evaluation and staging criteria. The results of ophthalmologic examination of the lens should be documented (photographed) to allow a panel of international experts to verify cataract diagnoses.

Individual questionnaire will be distributed to obtain information on other risk factors for cataracts.

Dosimetry

Considerable efforts have been made to assess doses to the lens within the UACOS study. The median estimated lens dose for the cohort was 123 mGy, while 4.4% of the cohort members received dose >500 mGy. Although bias corrections and uncertainty estimates based on available information were applied in this study and for each individual the uncertainty distributions were randomly sampled to estimate his/her summed γ-ray and β-particle dose, the dose estimation is still a work in progress. There may have been some underestimation of uncertainties, and correlated uncertainties were not modeled as such. Obtaining detailed information on work locations for the workers should also increase the validity of the dose estimates.

Biological samples

If assessment of genetic predisposition is desirable then blood or saliva samples shall be collected for each consenting cohort member.

Molecular markers

Based on results of experimental studies, *ATM, rad9* gene heterozygosity could be considered as a potential marker of genetic predisposition to cataract development.

Feasibility

Roadblocks that need to be overcome:

- Continuation of ophthalmological screening requires substantial funding;
- Can be tempered by consideration of the uncertainties in the dosimetry estimation;
- If funding cannot be obtained rapidly, validation of retrospective cataract diagnosis becomes problematic.

Statistical power

The study on liquidators, as shown in the previous work by Worgul et al, has sufficient power to detect risks of radiogenic cataracts in the cohort of nearly 9,000 liquidators.

Ethic requirements

Typical ethics requirements are necessary, same as for other epidemiological studies. A signed consent form will be obtained for all study participants.

Proposed approach – Objective 2

Similarly to the study of liquidators, a prospective cohort study design is most suitable.

Study population

To identify population of children suitable for studying risk of radiation induced cataracts following exposure in childhood is more difficult as such population with known doses to the lens does not exist. One possibility is to use the existing two screened BelAm and UkrAm cohorts (more detailed information provided in the Internationally supported Life-Span cohorts proposal) as it includes individuals under 18 years at the time of the accident. Another option, although less feasible, is to trace those children who were involved in the study of Day et al. The principle investigators should be contacted and asked if these subjects can be identified and traced.

Dosimetry

For the cohort study of children at the time of the Chernobyl accident, an assessment of dose to the lens will require major efforts and will be a critical point to evaluate feasibility of this study. Hopefully, information on places of residence (and contamination density) since the accident has been already collected in the UkrAm and BelAm cohorts and can be used as the starting point for evaluating doses to the lens from external exposure in the place of residence. Replication of Day study would require even more investment in reconstruction of doses to the lens.

Biological samples

The same as for the study of liquidators

Molecular markers

Studies of *ATM, rad9* gene heterozygosity could be also considered.

Feasibility

Roadblocks that need to be overcome:

- Identifying the appropriate cohort for the study;
- Setting up an ophthalmological screening in the study group;
- Assessment of lens doses and their uncertainties.

Ethic requirements

Typical ethics requirements are necessary, same as for other epidemiological studies. A signed consent form will be obtained for all study participants.

Statistical power

For studying cataracts in those exposed as children, power to be determined based on the population size, dose range in the study and various assumptions on effect magnitude.

Prioritisation

In the UACOS, staff are well-trained and experienced, development of the dose reconstruction method is well advanced, two rounds of the ophthalmological examinations have been completed and there is potential risk that delay in conducting the next screening round will cause problems in loss of the staff and in retrospective validation of ophthalmological findings, thus the study in **liquidators** is considered as **potentially high priority** for short term project.

The study in those exposed as **children is not necessarily urgent**, can be set up as a part of the life-span cohort study later.

CARDIOVASCULAR AND CEREBROVASCULAR DISEASES

The Chapter was prepared by E. Cardis and M.T.Do, CREAL, Barcelona, Spain

Background

General

The significant burden from diseases relating to the circulatory system represents a major population health concern and a challenge for public health risk management in many countries world wide. Substantial epidemiologic evidence to date has implicated cigarette smoking, obesity, low density lipoprotein cholesterol, genetic dispositions, and high fat diet as independent risk factors for developing cardiovascular diseases (*186*). High (radiotherapy) doses of radiation have also been shown consistently to increase the risk of cardiovascular and cerebrovascular diseases (*187-189*) .

In recent years, evidence has emerged suggesting moderate doses of ionizing radiation can also contribute to excess cardiovascular and cerebrovascular disease risks. Among atomic bomb survivors, significant excess relative risks have been observed for heart disease (ERR=0.17, 90% CI; 0.08-0.26) and stroke (ERR=0.12, 90% CI; 0.02-0.22) below 2 Gy (*7*). The data, however, are consistent both with a linear no-threshold dose response and with a threshold around 0.5 Gy. In the absence of a proper understanding of the biological mechanism(s) that would lead to a radiation-related risk at low doses, it is therefore not possible at present to draw conclusions about implications for the risk (if any) at low doses.

The risk of cardiovascular and cerebrovascular diseases related to lower doses of radiation has become a topic of substantial concern in radiation protection as the existence of a risk at low doses would challenge the current radiation protection system. The risk of these diseases has therefore been studied in a number of populations with lower doses recently (*190-192*) but results have been inconsistent. McGeoghegan and colleagues analyzed data from 64,937 males workers from the British Nuclear Fuels facility and found significant excess in mortality from circulatory system disease (ERR=0.65, 90%CI 0.36-0.98 per Sv) (*193*). Similar results were observed for a large Canadian cohort of nuclear workers (*194*). In contrast, Vrijheid and colleagues assessed mortality experience from diseases other than cancer following lower doses of ionizing radiation using data of nuclear workers from 15 countries and found non-significant excess relative risk for circulatory diseases (ERR=0.09, 95%CI; -0.43-0.70 per Sv) (*195*). Among a cohort of German uranium miners, no excess in cardiovascular diseases risks were observed (*196*). Studies of radiologists reviewed by McGale and Darby (*192*) did not show significant excess in cardiovascular risks.

Chernobyl

Evidence for the risk for cardiovascular diseases associated with exposure from radiation emitted from the Chernobyl accident is limited. Recently, an analysis of non-cancer incidence and mortality rates in various groups registered in the Ukrainian State Chernobyl Registry (USCR), including workers, evacuees and residents of contaminated areas, was published by Buzunov and colleagues (*197*). The authors made an attempt to evaluate total and disease-specific incidence and mortality rates from 1988 to 2004. The most surprising finding was a decrease in the incidence of non-cancer diseases among recovery operations workers since 2000. However, this article lacks any presentation of the methodology used in estimating the rates. Recently, Ivanov and collaborators studied cardiovascular and cerebrovascular incidence among the cohort of Russian recovery operations workers. They followed 61,017 men from 1986 to 2000 (*198*). A statistically significant dose-related increased risk of ischemic heart disease (ERR= 0.41, 95% CI: 0.05-0.78 per Gy) and cerebrovascular diseases (ERR= 0.45, 95% CI: 0.11- 0.80 per Gy) was found (*198*) Results should, at present, be interpreted with caution as the study lacks information on other risk factors for these diseases (including smoking and other lifestyle factors). These early results from Chernobyl,

combined with inconsistent results from the literature based on other occupational cohorts provide impetus for more comprehensive examination of the effects of radiation on cardiovascular diseases. In particular, the effects of low and moderate doses of radiation experienced by the liquidators can shed some light on the current discussion regarding the effects of low and moderate doses on cardiovascular and cerebrovascular diseases.

Objectives

Cardiovascular and cerebrovascular diseases are an important group of circulatory diseases with complex etiology and a high prevalence in the general population. Radiation protection standards and recommendations to date do not consider a possible effect of low to moderate doses on the risk of these diseases. Should such a risk exist, however, estimates from atomic bomb survivors studies suggest that the detriment could be similar to that due to radiation induced cancer, thus challenging the current basis for radiation protection. Because of this, the High Level Expert Group on European Low Dose Risk Research has identified research into low dose effects on cardio- and cerebro-vascular diseases as a priority (*199*), thus providing motivation for contributing to this area of research. The overreaching objective is therefore:

- To determine whether morbidity and mortality from cardiovascular and cerebrovascular diseases are associated with low to moderate doses of ionizing radiation.

Specific relevance (value-addedness) of Chernobyl populations

Currently, there is great uncertainty regarding the effects of low to moderate doses on cardiovascular and cerebrovascular diseases. The Chernobyl exposed populations – particularly the liquidators and possibly the evacuees – are particularly suitable to address this knowledge gap because of their large size and relatively higher dose levels (hence higher statistical power) than other low dose populations. Knowledge to be gained from analyses of data from Chernobyl will be invaluable for radiation protection.

Proposed approaches

There are many epidemiological approaches that could be used to examine the effects of radiation from the Chernobyl accident and cardiovascular and cerebrovascular risks among exposed populations. A well conducted case-control or case-cohort study on selected population is likely to be the most efficient way to obtain information on the effects of radiation doses on these diseases, taking into account effects of other risk factors. However, a coordinated approach will be necessary for any study to be successful.

Study Population

Ideally, a prospective cohort of all affected persons (liquidators, inhabitants, and evacuees) from the areas most affected (Belarus, Russia, and Ukraine) by the Chernobyl accident would provide a 'gold standard' and would be most desirable to determine the effects of exposure to radiation on cardiovascular disease risks. This type of study has been instrumental in understanding cardiovascular diseases in other populations (e.g., Framingham Heart Study). However, given the size of the affected population, it would not be feasible or cost-effective to follow everyone prospectively. Instead, it would be more feasible and efficient to focus the research on a specific sub-population.

The most informative population would likely be the Chernobyl liquidators included in the Chernobyl registries of the Russian Federation, Belarus, Baltic countries and Ukraine who participated in the clean-up activities in 1986-87 in the 30-km zone (see section on "life-span" cohort for a description of the population). This is one of the best defined populations of exposed persons, with the highest average whole body dose levels. The large size of this population allows for identification of large numbers of cases and hence ensures statistical power. Given that a cohort of liquidators has been enumerated, focusing on this population would be most fruitful.

Study Design

Research questions into radiation effects on cardiovascular and cerebrovascular diseases could be addressed efficiently using either a nested case-control or a case-cohort study design, based on the roster of liquidators included in the Chernobyl registries of the most affected countries. In both of these designs, data collection is restricted to only a sub-sample of the entire cohort. For large follow-up studies with relatively infrequent disease occurrence, limiting data collection to only a subset of the cohort provides significant cost savings over time, particularly in countries such as Belarus, Russia and Ukraine where passive follow-up of large cohorts is likely to be difficult (*200*). The nested case-control study design has already been used successfully to study the risk of leukaemia, lymphoma and thyroid cancer among Chernobyl liquidators (*14*) and is particularly well suited for the study of relatively rare cancer and non-cancer outcomes. The possibility of conducting a case sub-cohort approach (with the sub-cohort serving also as comparison group for other diseases) is also well worth investigating.

Dosimetry

Dose-reconstruction to a number of specific organs (red bone marrow and thyroid) from external radiation and from intake of long-lived radionuclides has been successfully conducted in nested case-control studies of liquidators using Realistic Analytical Dose Reconstruction with Uncertainty Estimation (RADRUE) (*162*). In these studies, individual bone marrow dose estimates were found to range from less than one Gy to 3,300 mGy, with an arithmetic mean of 71 mGy. RADRUE can also be used to estimate doses to other organs from these radiation types. Although, due to the detailed information needed and to the labor intensive process, RADRUE could not be used to estimate individual doses to very large cohorts, it is feasible to use it in a nested case-control or case-cohort study. As RADRUE involves the participation of dosimetrists knowledgeable in the organization of work, dosimetry control and monitoring at the time of Chernobyl, and as these dosimetrists are few and aging, RADRUE may no longer be feasible in some years and any study requiring this resource should be done in the near future.

Data and biological sample collection

One of the major limitations of current studies of Chernobyl populations is the lack of information on other factors that could contribute to cardiovascular risks (*198*). A case-control or case-cohort approach would allow the collection of all necessary information, including smoking, anthropometric indicators (e.g., body mass index), diet (e.g., alcohol and fat consumption) and others.

Biological samples, in particular blood, could also be collected to examine markers of disease.

Detailed confirmation of diagnoses and consistent diagnostic criteria will be needed for all diseases included in the study.

Feasibility

Although the cohort of liquidators have been enumerated, method of identifying the cases in a complete and comprehensive way may be a challenge as, like in most other countries, no population-based registry of these diseases exists, registration in the Chernobyl Registry (see section on “Life-span studies”) may be incomplete and exact diagnoses may be inaccurate.

A successful study would need to involve, presumably, periodic checks of all relevant hospital departments in the study regions, linking with the roster of liquidators, and validating a much wider range of possible diagnoses in order to find those of interest. The number of hospitals in the coverage areas can be large; therefore, appropriate amount of time would need to be allotted to ensure appropriate contacts with all hospitals in the catchment area. Furthermore, medical records may be in paper format. Adequate amount of time would need to be allotted for searching and reviewing of medical records.

Selecting controls could be based on random sampling of potential controls from the roster of liquidators, as was done in previous case control studies (*14*), matching on age, sex and region of residence. While selection from the roster is relatively straightforward, a challenge would be to be trace the potential controls as the information on current address in the Chernobyl Registries may not be up to date. Liquidators who moved out of the catchment area are considered lost to follow-up. Whether the mobility of certain groups within the liquidator population will have resulted in selection bias would need to be determined to ensure appropriate interpretation of study results.

Ethical Considerations

As all other epidemiological studies, all appropriate ethics permissions would need to be obtained prior to study implementation.

Study Power

The study cohort is in principle very large population consisting of approximately 66,000 Belarus, 65,000 Russian Federation, 15,000 Baltic country and 110,645 Ukrainian liquidators who took part in the clean-up activities and were included in the Chernobyl Registry in their countries.

If there truly is an effect at low doses of the order reported among a-bomb survivors (an order similar to that seen for cancer risk), then the statistical power, if careful studies can be conducted within the large cohorts in Belarus, Russia, Ukraine and possibly Baltic countries, should be sufficient to detect an increased risk.

Prioritization

The Chernobyl experience could be invaluable in shedding some light into the current debate regarding the potential effect of low to moderate doses to cardio and cerebro-vascular diseases risks. Currently cohorts of liquidators have already been enumerated. This project would be very important to the understanding of the effects of low and moderate dose radiation exposure and cardiovascular risks. Doses for these workers can be calculated. A case-cohort or nested case-control study of Chernobyl liquidators can be rapidly implemented to derive cardiovascular risks for this population. Given that epidemiological resources are currently available and that given the importance for radiation protection of resolving the question of the burden of cardiovascular and cerebrovascular diseases at low to moderate doses, a high urgent priority should be considered for this area of research.

IMMUNOLOGICAL EFFECTS

The Chapter was prepared by A. Kesminiene and E. Ostroumova, IARC, Lyon, France

Background

General

The immune system consists of cells and tissues spread widely in the human body and plays major role in providing protection against infection and cancer. Bone marrow and thymus are the primary lymphoid tissues where maturation of lymphocytes takes place. There are two types of the immune response, namely: innate, when monocytes, macrophages, polymorphonuclear leucocytes, dendritic cells, natural killer cells and mast cells are involved, and acquired, when B- and T-lymphocytes are predominant players.

Whole body exposure to ionizing radiation at medium and high doses leads to immunosuppression as well as does localized radiotherapy (*201*). In contrast, reports on the effects of the low-dose exposure lack consistency.

Data from experimental studies at low-doses and low-dose rates show activation of immune function through enhancement of the proliferative response of splenic and thymic lymphocytes to mitogens, enhancement of natural killer (NK) cell activity and increased secretion of cytokines with a regulatory effect on immune cells promoting their activation (*201*). In addition, animal experiments demonstrated suppressive effects of low-dose radiation on tumour growth, metastasis and carcinogenesis (*202, 203*). However, data are not entirely consistent and the observed effects were highly dependent on the range of dose and dose-rate, and upon the animal studied (*201*).

Results of continuous follow-up of the atomic bomb survivors allowed short-term and long-term immunological effects of the acute exposure to be assessed. In various follow-up periods, effects on incidence of autoimmune diseases (1958-87), systemic bacterial infections (1954-67) and granulocyte functions (1947-79) (*204*) were reported. A study of 139 survivors showed a significant decrease in mixed lymphocyte culture response (evidenced by impaired thymic function) with increasing dose, especially pronounced among those who were under 15 years at the time of bombardments (*205*). The most remarkable late effects on immunity were functional and quantitative abnormalities of T- and B-cells in survivors exposed to doses above 1 Gy (*206*). Studies of immunoglobulin levels among 2,061 a-bomb survivors with the dose ranging from 0 to 5.6 Gy found a significant increase in immunoglobulin (Ig) A levels in females, a significant increase in Ig M levels in both sexes and no changes in the prevalence of antinuclear antibodies, antithyroglobulin antibodies, antithyroid microsomal antibodies and in levels of Ig G and Ig E (*207, 208*). Imaizumi reported that there is little evidence on statistically significant dose-response in relation to the prevalence of increased antithyroid autoantibodies or clinical hypothyroidism with increased autoantibodies among atomic bomb survivors (*209*). Among other immunological effects observed among a-bomb survivors, were: increased frequencies of somatic mutations and chromosome aberrations (*210, 211*); quantitative and qualitative changes suggesting a radiation–induced acceleration of the normal process of immunological aging (*212*); radiation-induced chronic inflammatory responses (*213*); decreased proportion of CD4+ cells with increased dose and history of myocardial infraction, and a higher prevalence of myocardial infraction among those survivors who had a lower proportion of CD4+ cells (*214, 215*).

As stated before, the effects of the chronic low dose exposures on the human immune system are less studied. A dose-dependent decrease of cellular immunity, mainly evaluated by CD4+/CD8+ ratio and HLAMHC-DR+ activated T-cells was reported in residents of buildings constructed using 60Co contaminated materials (*216*). The dose estimates used in this study are, however, subject to uncertainty and exposure protraction varies from 2 to13 years. On the contrary, no significant changes were observed in the same parameters of workers occupationally exposed to external low-

LET radiation in nuclear facilities (*217, 218*). It should be noted however that most of these studies included very small numbers of workers. The results of studies concerning the impact on the immune system of living in high natural radiation areas (HLNRAs) were controversial and their significance remains unclear (*201*).

Chernobyl observations

Liquidators

Early reports related to immune response have yielded conflicting results. A decrease in lymphocytes in recovery workers has been reported, however, this lasted only about a year (*219*). Helicopter pilots who received higher doses did not show this effect (*220*), nor did Chelyabinsk recovery workers (*221*).

More recent studies of emergency and clean-up workers have focused on levels and function of T cells and natural killer (NK) cells. Early studies reported a decrease in T cell counts and immunoglobulins (*222-224*). In the period from 1 to 5 years since exposure, there was variable recovery of cellular and humoral immunity. There were also variable responses of B cell counts. Thirteen years post exposure, none of the patients had developed symptoms of the classic autoimmune disease.

- An initial decrease in CD3+ and CD8+ cells was reported by (*225*);
- a decrease in both CD4+ and CD8+ cells was found by (*226*);
- Kurjane et al. observed that doses between 0.01Gy and 0.5 Gy reduced CD3+, CD4+ and CD8+ T-cells (*227*). Kuzmenok et al. did not find any changes in the levels these cell populations 11–14 years after the accident (*228*).

The same study reported a possible increase in response of CD25+ cells to the cytokine interleukin-2, which was not proportional to dose. Kurjane et al., 2001, reported a decrease in NK cells. In addition, some authors have reported a confounding toxic effect of lead (which was dumped on the reactor) on CD4+ and CD16+ cells (*229*). Also, elevated blood levels of lead, zinc and iron were found in Latvian and other clean-up workers (*227, 230*). Chumak et al. reported an increase in CD4+ cells and a decrease in CD8+ cells in heavily irradiated workers (*223*). Another study (*231*) of 730 emergency and 1212 recovery workers also reported decreases in CD3+ and CD4+ counts, but unexplained substantial, although smaller, decreases for the control groups .

Studies in children

Results of the studies of children were also conflicting. In a study of children evacuated from Pripyat (*232*) no significant differences in immunological parameters compared to control group were found. Children from Mogilev and Gomel examined two years after the accident did not show abnormalities in levels of T-lymphocytes, but showed a minor increase in B-lymphocytes (*233*).

The immune status of children residing around Chernobyl has also been studied. Titov et al. reported a variety of findings, including a decrease in B-cells and IgM but only for 30–45 days after the accident (*234*). A decrease in IgG was reported for 90 days, which later returned to normal and then increased. A study (*232*) of 1,118 children performed 5 years after the accident reported decreased CD3+ and CD4+ levels in children living in contaminated territories compared with children of the same age living in “clean” villages. A study by Chernyshov et al. showed lower levels of CD3+CD4+ cells in children with doses >1 mSv with respiratory disease compared to control children in uncontaminated areas (*235*). However, no decrease was seen in healthy children living in the contaminated zone. Koike et al. reported that children in Gomel had abnormalities in NK cell activity, but this was not correlated with the level of 137Cs contamination (*236*).

Autoimmune thyroid disorders in children have also been studied. In many of these studies, the number of subjects is small, the method of study population selection is unclear, and absorbed doses were not estimated (*61*).

Study of autoimmune thyroiditis (AIT) among those who were exposed to 131I under age of 18 in Ukraine, found little evidence of an association between AIT prevalence and thyroid dose (*237*). At the same time a modest significant association was shown between 131I dose and prevalence of elevated ATPO (antibodies to thyroid peroxidase).

A study of group of 1,251 children residing in the territory of Narodichesky district of Zhitomir region in the period of 1993 – 1998 showed a significant decrease in a count of red and white blood cells with increasing of 137Cs soil contamination (*238*). However, this study has serious methodological limitations: no explanation how the study sample was drawn is given; study subjects were not followed on a regular basis, only individuals with available measurements were included in the analyses; reasons for exclusions are not presented; it is not clear if an adjustment for medical history and medical conditions that may affect results of the blood tests was made.

The above reported immunological effects of radiation exposure from the Chernobyl accident appear to be related mostly to changes in the number or function of peripheral lymphocytes and serum immunoglobulin levels. These effects have been detectable up to the present time. Some of these effects may be due to confounding factors other than direct radiation such as stress, chronic infections, diet and chemicals. As a result, it is difficult to interpret the results.

Current gaps in knowledge also include the clinical significance of abnormal immune function on the increased risk of cancer and non-cancer morbidity.

Objectives

The main objectives are:

1. to evaluate late health effects of the suppressed immune system following acute radiation syndrome (ARS) among the ARS survivors;
2. to assess if there are any delayed immunological effects among those who were exposed as children in the contaminated territories around Chernobyl.

Specific relevance of Chernobyl population(s)

The ARS survivors are an informative population to look at the late health effects following the immunosuppression after the high dose radiation. Studies of the Chernobyl liquidators are unlikely to yield significant information. Studies of populations with neoplastic and non-cancer diseases in relation to the status of the immune system after exposure in childhood may be of value.

Proposed approach

Population

- ARS survivors - for evaluating late health effects of the suppressed immune system following ARS;
- BelAm and UkrAm cohorts - for assessing if there are any delayed immunological effects among those who were exposed as children.

Study design

Active follow-up of the ARS survivors should be continued in the clinical centres of Moscow and Kiev.

Cross-sectional study could be conducted on a basis of existing BelAm and UkrAm cohorts. This would require performing standard blood tests and clinical examinations at regular intervals and also follow-up for neoplastic and non-cancer diseases that potentially could be related to the effect of radiation on the immune system.

Dosimetry

Individual doses are necessary to evaluate effects of external and internally incorporated radio nuclides (^{137}Cs, ^{90}Sr) on the immune system.

ARS survivors' doses – see for details the chapter on ARS.

BelAm and UkrAm cohorts: whole body dose (dose to the bone marrow) and dose to the thyroid needs to be reconstructed (see for details the chapter on Life Span cohort).

Biological samples

Whole blood and isolated lymphocytes (frozen at -70^0 C) are needed for looking at the biomarkers of the studied endpoints.

Other - not applicable

Feasibility

Roadblocks that need to be overcome:

1. Unification and standardization of methods and reference ranges of the immunological assays in different labs;
2. Necessity to obtain (collect) detailed information on health status, current and previous diseases that could affect immunological parameters;
3. Individual bone marrow dose reconstruction for those who were exposed as children;

Ethics requirements

Ethical clearance should be obtained in the same way as in any other study.

Statistical power

Power calculations should be conducted before setting up these studies.

Prioritisation

That it is extremely difficult type of the study with many roadblocks to overcome. We consider the priority of this study as low.

ACUTE RADIATION SYNDROME SURVIVORS

The Chapter was prepared by A. Kesminiene and E. Ostroumova, IARC, Lyon, France

Background

The Chernobyl accident can be considered as a large industrial accident with severe acute health consequences where about a quarter of the people situated at the accident site in the nuclear power plant (586 individuals) developed acute health effects including acute radiation sickness (ARS) (*239*). Initially the diagnosis of ARS was applied to 237 persons based on symptoms of nausea, vomiting and diarrhoea. Of this group, 115 patients were transported to the clinic No 6 of the Russian State Research Centre of the former Institute of Biophysics (SRC-IBPh), Moscow. Within several days, ARS was diagnosed in 104 of those persons (*240*). Later on, ARS was retrospectively verified in 30 persons. Overall, ARS diagnosis was confirmed for 134 persons with bone marrow depression observed in all ARS patients (*241*). Their whole-body (or bone-marrow) doses due to external gamma radiation ranged from 0.8 to 16 Gy (Table). Doses due to external exposure were evaluated using chromosomal analysis of peripheral blood lymphocytes. During the acute period (first four months of the accident), 28 fatalities were recorded; underlying bone marrow failure was the main contributor to all deaths during the first 2 months (*242*).

Degree of ARS	Dose range, Gy	Number of patients	Number of short term deaths	Number of survivors
Mild (I)	0.8 – 2.1	41	0	41
Moderate (II)	2.2 – 4.1	50	1	49
Severe (III)	4.2 – 6.4	22	7	15
Very severe (IV)	6.5 – 16.0	21	20	1
Total	0.8 – 16.0	134	28	106

Table: Doses, number and outcome among 134 patients with ARS (Metler, Jr., Guskova AK, & Gusev IA 2007)

Within the first five years after the accident all the ARS survivors were followed in the same medical centre – Clinic No 6 of the IBPh (*240*). By 1996, the number of patients being followed-up at the SRC-IBPh clinic had reduced to ten persons. Since 1990, a large group of ARS survivors, as well as a group of people initially suspected of having ARS, but later confirmed as not having ARS, continued medical surveillance at the Research Center of Radiation Medicine (RCRM), Kyev, Ukraine (*243*). By 2008, the number of ARS survivors alive and followed by RCRM, was 62 persons, including 28 patients with 1st degree of ARS, 27 with 2nd degree and 7 with 3rd degree of ARS severity (Dimitry Bazyka, personal communication, 2009). The numbers of patients seen in RCRM, as reported in different publications, vary; this is because of different time periods of observation and because some have been lost from the follow-up. No residents from Belarus were diagnosed and followed for ARS.

In both clinics in Russia and Ukraine the patients undergo annual inpatient examinations. The follow-up data are difficult to analyze, compare and use because the data of the two clinics have been presented in different formats, have used different diagnostic criteria and overlap in time. The majority of publications refer to the ARS survivors' follow-up results from the SRC-IBPh clinic and lately from RCRM.

The data refer to the following specific health outcomes:

- transient peripheral cytopenia,

- immune status,
- cataracts,
- thyroid disorders,
- local skin injuries,
- neuropsychological disorders,
- sexual behavior and fertility,
- cancer incidence and mortality,
- all non-oncological diseases,
- overall mortality.

The follow-up of ARS survivors indicates that the initial haemotological depression has decreased substantially in many patients.

Studies of immune status revealed abnormalities in T-cell immunity for those survivors who received high doses of radiation. But these abnormalities were not associated clearly with clinically manifested immunodeficiency (*240*).

The major consequence is severe local skin radiation injuries in 8 patients and various degrees of cutaneous injuries in 20 survivors who were treated in Germany. Most of the patients with cutaneous fibrosis have been treated with low dose of interferon. Results of this treatment are mixed. Results of follow-up of 15 patients with local radiation injuries in the period between 1991 and 2000 revealed two cases of basal cell carcinomas on the nape of the neck and the right lower eyelid, areas that received lower exposures (*244*).

For ARS survivors seen at the SRC-IBPh the prevalence of eye disease was 15% versus 6% in the group with unconfirmed ARS. This was due to a rise in the incidence of radiation cataracts (*245*). At least 17 of the survivors have developed radiation cataracts. The cataracts formed 3 – 8 years after exposure (*246*). Cataract patients were operated for artificial lens implantation. According to the SRC-IBPh findings the threshold for the development of radiation cataracts due to beta and gamma irradiation is 3.2 Gy. For the group of persons followed-up at RCRM, numerous cases of radiation (subcapsular posterior) cataract were reported, i.e. 23 cases among the ARS survivors and 3 cases among persons with unconfirmed ARS (*247*) . The researchers at RCRM indicate that radiation cataracts have been found at absorbed doses of less than 1 Gy (*248*).

Among the patients monitored at the SRC-IBPh during the period 2001–2007, only one of ten patients was hypothyroid (*245*). The RCRM researchers have reported that three cases of moderately pronounced hypothyroidism were observed in the recent years (*247*).

During 1986 – 1991 follow-up period, 74 cases of arterial hypertension and 22 of coronary heart disease were diagnosed among 91 ARS survivors at the RCRM, but with no correlation between cardiovascular disease risk and radiation dose (*249*). Up to 2005, there were 38 patients suffering from the consequences of local radiation injuries under observation (5 at SRC-IBPh and 33 at the RCRM). There was a strong dependence of the frequency and intensity of long-term effects on the grade of ARS and on the grade of local skin injury in the acute period (*245, 248*).

Sexual function and fertility among ARS survivors were investigated up to 1996. Fourteen normal children were born to ARS survivor families within the first years after the accident (in one family, the first newborn died from sepsis, but a second healthy child was born subsequently) (*21*). Among 15 patients, the follow-up results reported (*244*), in 2000, an aspremia was diagnosed in one patient with evidences of high local exposure of his scrotum. In 3 out 12 patients azoospermia was diagnosed. Seven of 15 survivors had an elevated level of follicle-stimulating hormone.

Among 15 ARS survivors, 5 patients have developed hepatitis C. In one patient a microadenoma of the pituitary gland (prolactinoma) was detected.

The incidence of oncohaematological diseases was elevated at 4.6 % and included 4 cases of solid cancer among confirmed ARS survivors (a kidney cancer, a colon cancer and 2 cases of thyroid cancer) with an average latent period of about 11.5 years. There also were 3 cases of myelodysplastic syndrome, one case of acute myelomonoblastic leukaemia and one case of chronic

myeloid leukaemia. The average latent period was 11.8 years. No malignant thyroid neoplasms were detected in the 10 patients under observation in recent years at SRC-IBPh (*245*).

Among 13 solid cancer cases diagnosed in patients seen at the RCRM, four were diagnosed in ARS survivors and nine in persons with unconfirmed ARS (*247*). The mean latent period after exposure was 14 years for both groups. No statistically significant dependence of the disease frequency or severity either on the grade of ARS or respectively on the dose, has been observed.

By the end of 2001 14 ARS survivors with grade I – III died from different causes, including 1 case of acute myelomonoblastic acute leukemia, 2 case of myelodysplastic syndrome, 2 cases of liver cirrhosis, 3 cases of ischemic heart disease, 3 cases of coronary heart disease, 1 case of lung gangrene, 1 case of lung tuberculosis, 1 case of fatty embolism. In some cases of death an autopsy was not performed (*241*).

During 20 years (1987–2006), 19 ARS survivors and 14 persons with unconfirmed ARS have died for various reasons (*245, 247*). Among the ARS survivors, the most notable cause of death (4 cases out of 19) was haematological malignancy with a latent period of 9 years (*245*).

Objectives

The potential objectives for research on the ARS survivors are the following:

- To evaluate late health consequences of acute radiation syndrome;
- To assess relevance of the experience gained from the rehabilitation and follow-up of the ARS patients after the Chernobyl accident for other acute high dose radiation situations.

Specific relevance (value-addedness) of Chernobyl population(s)

The Chernobyl accident can be considered as a large industrial accident with severe health consequences and has produced the world's largest group of the ARS survivors. Data on the ARS survivors of the Chernobyl accident should be analysed with reference to ARS consequences in other accidental situations.

The exposure of the ARS patients has a unique pattern with severe local exposures that occurred in a part of the patients. The wide spectrum of late sequelae among ARS survivors reported by 2001 justifies a multidisciplinary, possibly, life-long follow-up of these patients.

Potential approaches

As it reported by Ilyin et al., information about 134 ARS cases was entered in the Registry of radiation accidents and incidents of the State Research Centre – Institute of Biophysics (SRC-IBPh, currently Burnasyan Federal Medical Biophysical Centre), Moscow (*250*). A database on 75 ARS survivors was established and maintained at the RCRM (*251*).

A continuation of cohort active follow-up is strongly recommended.

The active follow-up of the subjects who had ARS should be maintained, especially because more than twenty years have passed since the accident and late carcinogenic effects may only now be manifesting.

To fully evaluate the late health effects, it would be desirable that the two centres (one in the Russian Federation and another in Ukraine) following these patients use the same methodology and criteria for diagnosis and that these would be carefully reported in publications. This would make the results of the two clinics compatible. It is also recommended to continue the follow-up of the subjects with non-confirmed ARS.

Special attention should be given to the haematological proliferative diseases, tumours of the endocrine system and skin cancers. A detailed case review of all tumours that arose among ARS survivors is of importance to see if the tumours are different in structure.

Population

The study population would be the cohort of ARS survivors (134- including deceased), also inclusion of the initially suspected but later non-confirmed cases of ARS (referred as to Grade 0 ARS (103)) should be considered, if they can be traced.

There is an issue of identification of an appropriate comparison group for long-term effect assessment among the ARS survivors. The ARS Grade 0 patients are sometimes used as a comparison group; however, they clearly do not represent a true unexposed control group and appear to have been exposed to doses in the range of about 0.1 to about 1.0 Gy. A comparison with rates among liquidators probably is more appropriate.

Study design

Active long-term, if not life-long, follow-up of the ARS survivors should be continued for late health outcomes.

Dosimetry

The dominant exposures were external irradiation of the whole body at high dose rates and beta irradiation of the skin. Internal contamination was of relatively minor importance, neutron exposure was insignificant.

The 134 ARS patients received whole-body (or bone-marrow) doses due to external gamma radiation ranging from 0.8 to 16 Gy; skin doses exceeded bone-marrow doses by a factor of 10 to 30 for some individuals; and among these, some received skin doses estimated to be in the range of 400 to 500 Gy.

Doses mainly were estimated using biodosimetry, i.e. on the basis of cytogenetic parameters of lymphocytes and/or blood formula.

Biological samples

Molecular markers

DNA repair genes should be studied as a possible explanation for different susceptibility to deterministic effects.

Pathology

If the outcome is a tumour, it is recommended that tumour tissue samples be collected and deposited in the Chernobyl tissue bank to check if the tumours occurring among those exposed to high doses have different pathological structure. Blood sample should be collected and store for future study of different genetic susceptibility. .

Feasibility

Roadblocks that need to be overcome:

It is important to reach a consensus between the two clinics in charge of the ARS survivors to develop a core protocol and procedures for following and reporting outcomes.

Ethics requirements

The same as in other epidemiological studies.

Statistical power

Not applicable.

Prioritisation

The follow-up of this group is ongoing. It is important that it is continued using harmonised diagnostic criteria, the same approach for examining and reporting outcomes.

NONTARGETED RADIATION EFFECTS IN CHERNOBYL POPULATIONS INCLUDING PRECONCEPTIONAL IRRADIATION

The Chapter was prepared by K. Baverstock, University of Eastern Finland, Kuopio, Finland and A. Karotki, IARC, Lyon, France

Background

Various in vitro and in vivo studies show induction of the non-targeted effects, especially genomic instability in the progeny of irradiated cells as well as the bystander effects (effects in the nonirradiated cells in direct proximity to the irradiated cells), which may influence risk estimation in radiation epidemiology. The mechanisms for these effects are not fully understood, although they have been shown to have a nontargeted nature (*252-255*). The evidence for the non-targeted effects in radiation-exposed populations in general, and in those exposed to the radiation from the Chernobyl accident is significant.

Two major lines of non-targeted effects are shown as a result of Chernobyl radiation exposure. First is the induction of the parental germ line mutations revealing themselves in the offspring families (*17, 18, 256, 257*). Second is the genomic instability and clastogenic factors in the blood of the clean-up workers persisting in some cases for more than 10 years after the accident (*258-261*). This proposal aims to improve our knowledge on non-targeted effects in Chernobyl populations and, what is more important, to understand their mechanisms.

Somatic effects

Persisting chromosomal damage in the lymphocytes of the populations living in the proximity of the Semipalatinsk nuclear test site and who had mean effective doses (including internal and external irradiation) form 1.6 Gy to 4.47 Gy, according to different estimates, was evident in comet assays, which detect the amounts of single and double strand breakage in DNA, as well as its alkaline-labile lesions, 30 years after the last weapons test in the area (*262*). However, the studies made in the nuclear test site area must always take into account chronic doses, internal and external, received due to persisting contamination with ^{137}Cs and Pu isotopes (*263*).

Evidence of possible genomic instability in hematopoetic cells comes from occupational exposure studies. *In vitro* studies of the uranium miners' lymphocytes revealed a dose-dependent decreasing ratio of micronuclei with centromeres compared to those without compared to healthy donors, indicating possible ongoing chromomal instability and predisposition to cancers decades after the exposure. The changes were greatest in the miners with a lung cancer history (*264*). In contrast, analysis of the chromosomal instability in radiation workers of the Sellafield British Nuclear Fuels facility having more than 20% of the maximum permissible body burden of plutonium and receiving doses to the red bone marrow ranging up to 1.8 Sv showed constant decrease in the chromosomal aberrations both after 10 and 20 years post exposure and, accordingly, no evidence for chromosomal instability (*265*).

Cultured peripheral blood lymphocytes collected on average 41 years after the exposure from 19 A-bomb survivors, did not reveal any increase in clonal chromosomal translocations with FISH analysis compared to controls (*266*).

Further controversial evidence comes from medical exposure studies. For example, patients in remission from Hodgkin's lymphoma and treated locally with 22-40 Gy of ^{60}Co γ-rays showed elevated frequencies of dicentrics, rings and chromosomal fragments in their lymphocytes 6-24 years after the treatment. The anticancer drug bleomycin was tested and was excluded as a possible source of chromosomal aberrations in this study, however, the fact that Hodgkin's lymphoma itself is associated with fragile chromosomes could be a strong confounding factor (*267*). In the other work 18 cancer patients, who received 35-80 Gy of fractionated radiotherapy as a treatment of various types of malignancy, did not show any evidence of genomic instability in the blood lymphocytes (*268*). Similarly, 25 adult survivors of the childhood cancer and their offspring did not

have any signs of persisting genomic instability in peripheral blood lymphocytes, thus also providing negative evidence for the transgenerational chromosomal instability (*269*).

However, in addition to chromosomal instability, several studies of the radiation-induced cancers raise the possibility of the radiation-induced microsatellite instability in somatic cells. The frequency of microsatellite instability in Thorotrast-induced intrahepatic cholangiocarcinoma (ICC) patients was 62.5% compared to 22.7% in non-Thorotrast ICC for 29 patients injected with Thorotrast intravascularly 39-51 years ago compared to 22 cases ICC patients not administered Throtrast. Hypermethylation of the *hMLH1* mismatch repair gene was strongly associated with the microsatellite instability in Thorotrast patients, with no association in non-Thorotrast patients (*270*). There is an indication of microsatellite instability also in the A-bomb survivors developing myelocytic leukaemia. Analysis of the leukaemic cells for microsatellite variability in 10 loci by a fluorescent analysis of the PCR-amplified target fragments revealed significantly ($p < 0.001$) higher frequency of the multiple microsatellite changes in the exposed patients compared with unexposed ones.

Hereditary effects (in the germ line)

The mechanisms by which parental irradiation can affect the health of offspring are far from clear but there is some sketchy evidence that there are effects in humans. There are indications from animal experiments that associated health damage, including increased liability to cancer, may occur in offspring as a result of preconceptional exposure. These effects appear to be mediated by a non-genetic, i.e. an epigenetic, process that is not well understood but for which there is significant evidence (see radiobiology report).

An increase in tumours in the offspring of male mice, which had been subject to x-rays and other mutagens prior to mating, was reported, especially where the offspring were exposed to the tumour promoting agent urethane (*271, 272*). Moreover, when the tumour bearing males were subsequently mated to their litter mates the offspring also exhibited an excess of tumours, the effect being observed up to F34 (*273*). However, extensive efforts to reproduce the results in at least one other laboratory have failed, probably due to the strain dependency of the effects observed (*274*). Some other mouse studies aiming to overcome the criticisms of Nomura's results showed that paternal preconceptional exposure of mice to ^{239}Pu alpha particles in one study and Auger electrons from ^{55}Fe in another, significantly increased the incidence of lymphohaematopoietic malignancies after the exposure of offspring to secondary carcinogens, i.e., nitrosourea and X-rays (*275-277*). The preconceptional doses used in these experiments were too low to be responsible for specific locus mutations, e.g. 65 and 130 mGy from ^{239}Pu exposure and few mGy in the case of the ^{55}Fe irradiation. Recently, the analysis of the expanded simple tandem repeat (ESTR) frequencies showed increased mutation rates in the F1 generation of the *in utero* radiation exposed fathers (*278*). This instability was general and observed in all the tissues of the experimental animals.

Induced sensitivity of the progeny chromosomes to the genotoxic agent cyclophosphamide or secondary irradiation, manifesting in high total numbers of bridges and fragments in anaphase/early telophase hepatocytes, bone marrow cells and fibroblasts, was observed in the F1 progeny of the male rats irradiated with 4.5 Gy X-rays (*279*). Moreover, in a similar study 4.2 Gy given to the male parents increased the average amount of lung adenoma nodes in their F1 progeny (*280*).

Studies of the children of the Atomic Bomb Survivors in Japan showed no increase in frequencies of sex chromosome aneuploidies, mutations of *hprt* and glycophorine A genes, stillbirths, major congenital defects, death during the first postnatal week, and death in live-born children, (*281*). In accordance with the previous studies the observations of cancer and noncancer mortality among the offspring of the Japanese A-Bomb Survivors (*282*) after 45 years of follow-up and the studies of congenital abnormalities and cancers in children whose parents were occupationally exposed to ionising radiation (*283, 284*) did not indicate any significant increase in risk. However, a reanalysis of the data is currently in progress at Radiation Effects Research Foundation, Japan (private communication Dr Roy Shore).

Epidemiological evidence in humans supporting a statistically significant increased rate of leukaemia and lymphoma in children whose fathers were working at the Sellafield nuclear power plant and received relatively high doses has been reported (*285*). However, this study has been heavily criticised due to the methodological problems (*286*). Another cohort study of cancer in children of nuclear industry employees showed, based on a very limited number of cases, 5.8 times increased rates of leukaemia in children whose fathers received a dose of more than 100 mSv (*287*). It remains unresolved as to whether the small excess found by Gardner is attributable to the preconceptional exposure or some other cause (*274, 288, 289*). The Tri State Study in the USA suggested that a sub-group of apparently susceptible children were prone to develop leukaemia after antenatal exposure to low doses of x-rays (*290*). Susceptibility was defined as occurrence of illness more than 6 months prior to diagnosis for leukaemia. A highly significant relative risk was demonstrated for preconceptional exposure in this group (*291*) compared to the risk for all children where a radiation history of the mother was absent, with a latency of between 4 and 7 years. It is notable that there is no increase in leukaemia unless the susceptible child is exposed to antenatal irradiation, thus the effect could be an increased sensitivity to a carcinogen rather than a risk of cancer *per se*. In subsequent papers (*292, 293*) a model was derived based on preconceptional irradiation of either parent being the cause of the susceptibility, with a relative risk of 5, and a highly increased risk of leukaemia. However, this study has been criticised for its failure to employ any dose estimates.

In another study the diagnostic X-ray examination of 55,908 mothers, as a part of the Collaborative Perinatal Project of the National Institute of Neurological and Communicative Disorders and Stroke (USA), found that the preconceptional exposure of the mothers to X-rays was associated with a relative risk of 2.2 (90% CI: 1.3-5.8) for malignancies among their children (*294*). Other studies have cast doubt on the link between preconceptional irradiation and an increase in childhood malignancy. Acute lymphoblastic leukaemia (ALL), the commonest childhood malignancy, was not found to be increased either after maternal (OR, 0.9; 95% CI, 0.8-1.2) nor paternal (OR, 1.1; 95% CI, 0.8-1.4) preconceptional lower abdominal X-ray exposure (*295*). Moreover, a large cohort study of US radiological technologists showed that leukaemia and solid tumours in offspring (105,950 offspring in total) were non-significantly elevated for maternal exposure with hazard ratios (HRs) for lymphoma of 2.3, 1.8, and 2.7 if estimated doses were <0.2, 0.2-1.0, and >1.0 mGy, respectively. Paternal preconceptional exposure to estimated cumulative doses above the 95th percentile (82 mGy, n=6) revealed a non-significantly increased risk of childhood cancer of 1.8 (95% CI 0.7-4.6) (*296*). In both these more recent studies there were greater effects associated with paternal rather than maternal irradiation. These results make a strong case and urge for a careful study of the children of parents exposed to radiation from Chernobyl.

Dubrova reported an increased frequency of minisatellite mutations in the children of fathers exposed as a result of the fallout from the Chernobyl accident, from the exposures from the Techa river, and the nuclear tests in Kazakhstan (*17, 297, 298*). It is not clear whether this increased mutational frequency is an indicator of health effects and evidence for the effects in other radiation exposed populations is inconsistent (*reviewed in 299, reviewed in 300*).

Chromosomal instability was shown to contribute significantly in increased frequencies of non-clonal aberrations in the progeny of irradiated cells both *in vivo* and *in vitro* (*253, 301-303*). As this phenomenon is also found to be associated with the tumorogenesis (*303*) the studies of its prevalence in the preconceptionally exposed children of Chernobyl clean-up workers and evacuees is required.

Despite the collected evidence of the effects of preconceptional exposure the mechanism underlying these effects is not clear. Basically, there are two ways to explain the predisposition to disease or increased cancer incidence in the offspring of irradiated parents, genetic and epigenetic. The first is based on the inheritance of the specific mutations in germ cells as a result of the exposure. In contrast to the situation in humans the transmission of radiation induced genetic effects in mice is well established as a result of large experimental programmes at Harwell, UK and Oak Ridge, USA

(*304*). These studies form the basis for radiological protection standards today. It is therefore conceivable that a genetic predisposition to cancer resulting from preconceptional irradiation could be inherited. A number of genetic disorders, summarized in the National Research Council report, are known to enhance the carcinogenic response to radiation (*305*).

The second possibility is an epigenetically inherited deregulation of the cellular processes leading to genomic instability (GI). Ionising radiation has been shown to trigger several types of GI including minisatellite instability, chromosomal instability, multiple point mutations and various epigenetic effects, like the CpG island methylator phenotype (*reviewed in 306, 306, 306, 307, 307, 308*). The induction of GI is dependent on radiation quality or linear energy transfer (LET), dose, and the genetic background of the exposed animals. Low doses of high and low LET radiation produce GI, for low LET a low-dose saturation is observed, beyond which no additional genomic instability is induced (*307*). Recent models of carcinogenesis consider extracellular signals (*309*) and epigenetic changes (*310*) as very important in triggering malignant transformation. Therefore, studies of GI occurrence and inheritance in the offspring of Chernobyl population are an important issue even in the current absence of the established health effects. The experimental evidence on the mechanisms of radiation-induced transgenerational GI including the dysregulation of DNA methylation and other epigenetic processes indicates its non-mendelian mode of inheritance (*311*). Today there is no consensus as to what underlies genomic instability.

In humans microsatellite (tandem DNA arrays of <10 bp length units) instability is often associated with inflammatory conditions, like ulcerative colitis (*312, 313*), and carcinogenesis, for example development of colorectal, endometrial, gastric and other cancers (*314*). Despite the fact that different mechanisms can be involved in the instability and hypervariability of the microsatellite and minisatellite repeats (*315, 316*), both can be associated with human diseases, although the experimental evidence of the association of minisatellites (tandem DNA arrays of 10>length<100 bp units) with human diseases is less evident. However mutations in some minisatellites are shown to interfere with gene transcription and generate fragile sites on chromosomes (*reviewed in 299*). The minisatellites are the most unstable loci in the human genome and their mutation rates in the paternal human germlines were shown to be on average 4 times higher than in the maternal (*299*). Recently, transgenerational inheritance of tandem-repeat instability by the F1 generation was demonstrated in the murine model (*278*). Increased mutation rates in germline minisatellite sequences have been observed in families exposed to both high and low doses of ionising radiation after the Chernobyl accident (*257*).

Bouffler et al. address an important feature of the transgenerational inheritance of minisatellite mutations (*299*), noting that mutations may originate both in the developing parental germ cells and in the cells of the offspring (with no evidence of the same mutational damage in the parental germ cells). Therefore, mutations can be regarded as transgenerational if they are observed either in F1 or F2 generations. If the radiation-induced tandem-repeat mutations are an expression of GI and have at least a partial epigenetic origin the above definition of "transgenerational" is incomplete, as the genomically unstable cells may appear normal in terms of their molecular content, and the mutations in the next generations may develop with delay. Therefore, the definition of transgenerational inheritance should be any inherited effect derived from a parental germ cell that has undergone at least one division since irradiation. This is clearly demonstrated in the transgenerational transmission of the pink-eyed mutation, p^{un} (*317*).

Evidence of non-targeted effects in Chernobyl populations

Both stable and unstable chromosomal aberrations have been unexpectedly observed in Chernobyl clean-up workers even after 13 years after the last exposure. Even those with the average whole-body doses of less than 20 cGy showed persistently increased statistically significant ($p \leq 0.05$) level of dicentrics and rings. In addition, acentrics and chromatid translocations were increased in the lymphocytes of those exposed to the whole range of doses from 20 to 100 cGy during the cleanup process. However, the increase in the last two types of chromosomal aberrations should be analysed with caution due to the possible confounding from smoking and age (*258, 259*). A significant

increase in the aberrant kariotype frequencies, TCR-mutations and increased levels of $CD95^+$ apoptosis predictor in the peripheral blood lymphocytes both in the irradiated clean-up workers and their children born after the Chernobyl accident or exposed preconceptionally has been noted (*256, 260*). The repair of genomic DNA in lymphocytes after secondary γ- and UV-radiation treatment was reduced (*318*). Taking into account a well-established association of chromosomal aberrations with tumours, the studies of the chromosomal instability in the children of irradiated parents can be of great importance.

Human thyroid carcinoma cells were analysed for genomic instability. The study was conducted on 17 paediatric post-Chernobyl papillary thyroid carcinomas at 27 microsatellite loci and 3 minisatellite loci by PCR-amplification. No evidence of minisatellite instability was found in 20 controls of sporadic thyroid carcinoma, but 18% of the post-Chernobyl tumours revealed minisatellite instability in 1 or more loci. However, only 1 post-Chernobyl tumour had a microsatellite mutation in 1 locus, with no differences between sporadic and radiation-induced cases in the other tumours analysed (*319*). Although, the controls were not age matched in this study, there are clearly no indications of microsatellite instability in post-Chernobyl papillary thyroid carcinomas. However, with the increasing evidence for the changes in molecular evolution of thyroid carcinomas and increase in incidence of the follicular subtype, it may be useful to analyse the microsatellite and minisatellite instabilities in the tissue from the late thyroid cancer cases, as well as the expression and methylation profiles, especially for the MMR genes. It is important to investigate possible precancerous genomic instability in the post-Chernobyl thyroid tissues, as more evidence suggests that RET amplifications and rearrangements could be the consequence of genomic instability (*320*).

The UNSCEAR and UN Chernobyl Forum reports (*2, 21*) concluded that no significant health effects had occurred in the offspring of parents exposed to Chernobyl radiation. However, an elevated minisatellite mutation rate detected in the germline of irradiated Chernobyl families was evident in the offspring of people living at the heavily contaminated after Chernobyl accident rural areas (*18*). Using short random-sequence PCR primers to amplify DNA segments for fingerprinting Weinberg reported in a strongly criticised study a large increase in prevalence of DNA mutations in preconceptionally exposed children of clean-up workers (*321, 322*). It should be noted that no increase in minisatellite mutations was detected in the Estonian clean-up workers of Chernobyl accident (*323*) or in the Japanese atomic bomb survivors (*324*). Moreover, efforts to find an increase in minisatellite mutation rates in preconceptionally exposed children of Ukranian clean-up workers were unsuccessful (*325*), and no change in microsatellite mutation rates was shown in the children of Chernobyl clean-up workers (*326*).

Justification of the methods to be applied in the study

The non-Mendelean inheritance of the minisatellite instability in Chernobyl families as well as the chromosomal instability manifested in the persisting levels of the chromosomal aberrations in the peripheral blood of the clean-up workers could be potentially investigated in a study on Chernobyl families. Although initially the project should focus on establishing the association of these effects in the F1 generation of the exposed humans with Chernobyl radiation exposures, a second stage could focus on investigating the possible mechanisms of these effects or at least evaluating their molecular markers, based on somatic mutation quantification, epigenetic perturbations in the cells and several biochemical biomarkers.

Testing the possible inherited methylation patterns and methylation stability in the F1, would require a tissue-specific global methylation analysis, and methylated DNA immunoprecipitation (MeDIP) as a first step. In case of distinct methylation patterns in the DNA of nonirradiated F1 progeny this analysis should be followed by the specific checks of the methylation network elements, like DNA methyltransferases DNMT1, DNMT3a, DNMT3b or methyl-CpG–binding protein MeCP2 genes transcription (*327*). At the same time the methylation of several major

downstream genes previously shown to be involved in radiation-induced bystander effects and genomic instability, for example COX-2 or iNOS could be examined.

Several recent studies suggest the aberrant regulation of the DNA repair pathways as an important mechanism of GI. The methylation and transcription checks of the genes involved in the SSB or DSB repair processes can be also performed. Here, BRCA2, XRCC2 and XRCC3, and DNA-PKcs would be the most promising candidate proteins for transcription analysis as well as their interaction network partners (*328-330*). Less promising is the analysis of the methylation status of the mismatch repair genes, *hMLH1* and *h MLH2*, which can predict possible microsatellite instability (*331*) as microsatellite instability was not previously found in the F1 generations of the Chernobyl clean-up workers (*326*). The analysis of the DNA repair machinery in the identified cases of GI in lymphocytes can be recommended if a prior application of the γ-H2AX fluorescence assay and comet assay to reveal the level of DSB (*332*), or alkaline Comet assays to reveal SSB (*333, 334, 334*) show significant increases. However, no point mutations or other DNA sequence changes will be seen by these assays. The latter will be analysed by *hprt* clonal assay in the cultured lymphocytes, as well as by the glycophorine A gene loss assay (GPA) in the erythrocytes.

The methods for analysis of the minisatellite mutations are well described for the peripheral blood lymphocytes (*17*) and the thyroid tissues (*319*) and do not require further detalisation here.

Application of microarray techniques for analysis of the entire cellular transcriptome by multivariate analytic methodologies, for example, hierarchical clustering analysis can narrow the search for the mechanisms involved in the Chernobyl-related GI, to several major protein interaction networks, related, for example, to oxidative stress. Moreover, as the changes in the miRNAome, which represents approximately 1-2 % of the total RNA of the cell, may be important for GI occurrence and inheritance (and more generally to predict the cancer pathology). miRNA analysis utilizing miRNA chips should be carried out within the families studied.

Also, as the development of the relatively cheap approaches for massively parallel sequencing is in progress, application of this methodology to the analysis of mutation rates in somatic cells of irradiated parents and their offspring in Chernobyl populations is another potential approach to assess the increase in mutation frequencies in the next generations. A cheaper method to obtain substantial information on mutations could be the SNP analysis utilizing the DNA chips. Recently the levels of the heritable individual-specific and allele-specific variation in gene expression within and between parent-child trios of the different ethnic origin were estimated as a function of chromatin structure and transcription factor binding (*335*). This analysis allows the estimation of the relative impact of genetic and epigenetic (including environmental) factors in this kind of heritable regulation of the genome. Despite the expense of the experimental setup such work, first on mice and then on human lymphocytes would answer a number of mechanistic questions concerning radiation-induced transgenerational genomic instability. The need for this work is emphasised by recent reports on human intergenerational mutation rates and Mendelian disease inheritance (*336*).

Kits for the fluorescent detection ROS/RNS and specific cytokines can be used for microscopic detection of oxidative bursts in the leukocytes of the clean-up workers and their offspring where transgenerational effects can be separated from the other lifetime factors.

Objectives

a. To investigate persisting genomic instability in the peripheral blood lymphocytes of Chernobyl clean-up workers and evacuees.
b. To estimate the level of radiation-induced transgenerational genomic instability in the preconceptionally exposed F1 (and possibly F2) offspring of the Chernobyl evacuees and clean-up workers using the whole range of endpoints. To explore possible association of these biological effects with detectable health effects.
c. To search for any mechanistic hallmarks of transgenerational effects of ionising radiation in addition to chromosomal and minisatellite.

d. Test Chernobyl-related thyroid cancer cell samples and irradiated normal thyroid tissue samples (if available) for the genomic instability, and evidence of major changes in cellular genome and proteome of thyroid tissues associated with radiation exposure and carcinogenesis.
e. To assess the extent to which chromosomal instability impairs the dosimetric use of chromosomal aberrations in Chernobyl studies

Populations and approaches

A. Studies of genomic instability in the blood of the clean-up workers can be carried out within the study of the transgenerational effects of radiation, using unexposed family members of the clean-up workers and families where there was no parental Chernobyl exposure as a control group.

B. Scoring of minisatellite germline mutation rates and chromosomal aberrations in the families of the most exposed clean-up and emergency workers with two or more children fulfilling all the following conditions:

- One or both parents exposed to ionising radiation during the Chernobyl accident as an evacuee or a clean-up worker.
- At least one child should has been conceived before the Chernobyl accident

- At least one child should have been conceived after the accident

- The family should be resident in clean territory at the time of the birth of post-Chernobyl children and afterwards

According to data from Belarusian registries, the number of children born to liquidators is 12,262 and the number of children born to evacuees is 2,402. In the Ukrainian Chernobyl State registry the total number of children born to parents exposed to Chernobyl fallout is 373,846. Of interest are children from Kyiv city living in clean territories, including 1,616 born to liquidators, 7,687 born to evacuees and 38 born to acute radiation syndrome survivors. Some external control families with no Chernobyl exposure history, matched by ethnicity, parental age and smoking habits should be chosen from the clean regions of Ukraine, Russia, or Belarus.

The collection of health status data on these offspring would assess, firstly, an overall morbidity of the unexposed offspring in comparison to exposed children from the same families and to children from control families and secondly, the rates of the malignancies in the same children as appropriate and available.

Tissue and blood samples should be collected and stored in the country of origin following agreed protocols and with quality control assurances. Tumour tissue samples should be classified according to standard histological criteria, invasiveness, latency time and other clinical criteria. The possibility of releasing samples for study by collaborators in other countries should be explored, however, the possibility of conducting the analysis on Russian, Belarussian or Ukrainian soils would be highly preferable.

Blood samples should be collected from the members of at least 100 families and lymphocytes cultured. Scoring of chromatid and chromosome aberrations, e.g., dicentrics, centric rings, acentrics, chromosomal fragments etc. in the peripheral blood lymphocytes should be carried out in the first division metaphases using Giemsa staining and differential FISH. Anaphase spreads can be used for quantification of chromosomal bridges. Further assessment of SSB and DSB in lymphocytes can be performed by alkaline comet assay and γ-H2AX fluorescence analysis, respectively. Tests for the timescale of DNA repair should be applied by exposing the cultured lymphocytes to secondary radiation sources and scoring the induced SSB and DSB decreases as a function of time after exposure.

Genomic DNA should be extracted by the phenol/chloroform method and used for further analysis without intermediate freezing steps. Southern blots after PCR amplification can be used to score minisatellite germline mutations in hypervariable single-locus and/or multilocus minisatellite

probes. DNA can also be analyzed for global methylation chromatographically and, if needed, by methylation-specific PCR at the specific loci.

C. Depending on the results of the first objective several further laboratory techniques can be applied to the samples where signs of GI are detected, depending on the nature of the expression of GI, for the mechanistic analysis. The blood cells from families showing increases in minisatellite mutation rates or chromosomal aberrations should be further used for subsequent chromosomal aberration and transcriptome analysis, *hprt* and glycophorine A assay, SSB/DSB analysis, including tests for DNA repair, and miRNA/siRNA analysis.

D. Comparative analysis of GI in Chernobyl-associated thyroid adenocarcinoma cells, non-cancerous thyroid cells extracted from the same patient and the cells from the thyroid adenocarcinomas found in people with no or minimal history of radiation exposure should be compared. The control tissues would be mostly thyroid cancer tissues from the existing European or American tissue banks. It will be crucial, although difficult, to obtain the control tissues from the thyroid cancer patients with no or minimal history of radiation exposure. The success of such studies will be dependent on availability of tissue and DNA samples from the Chernobyl tissue banks and morphological samples (parafinised formalin-fixed samples) from the hospitals involved in the treatment of the patients with Chernobyl-related thyroid cancers. The study population will be the children and adults, who received a range of thyroid doses and developed PTC and follicular thyroid carcinomas over the last 20 years, especially those with tumours with an atypical morphology.

RNA should be isolated from the snap-frozen samples and immediately used for the mRNA and miRNA profiling by the array techniques, followed by multivariate analysis. Such RNA extraction might prove to be a very difficult task due to the high sensitivity of RNA to some manipulations. Around 20-80 mg of tissue per sample will be needed for a co-purification and further RNA and miRNA differential profiling for comparison with the healthy but irradiated thyroid tissues and the thyroid tissues from the patients with no history of Chernobyl radiation exposure. The same DNA and RNA isolated from thyroid tissues can be used also for the analysis of mutations in "Thyroid cancer evolution" proposal after PCR amplification of the loci of interest or RT-PCR with the specific primers for gene rearrangements.

At the same time, using the extracted DNA, instability analysis can be carried out on the extracted DNA, using several single-locus and multi-locus minisatellite loci as well as microsatellite loci (*319*). If the microsatellite instability is found, methylation patterns in *hMLH1* and *h MLH2* genes should be analysed. This study can be combined with the study of molecular evolution of thyroid cancers from the "Thyroid cancer evolution" proposal, using the same samples and infrastructure. DNA and RNA isolated from thyroid tissues will be than used for the analysis of mutations after PCR amplification of the loci of interest or RT-PCR with the specific primers for gene rearrangements.

Dosimetry

For the study three types of dose information will be collected. Firstly, the accumulated total body doses for clean-up workers and evacuees are required for the chromosomal instability studies. Secondly, accumulated dose to the father's and mother's gonads before the child's conception should be reconstructed for the transgenerational effects study. For dose reconstruction purpose information contained in Chernobyl State registries of the affected countries could be used but may require additional dosimentric assessment from,, for example occupation history for liquidators (function performed, dates of service, officially recorded doses), residential history for evacuees (time and place of residence in the radioactively contaminated territories, date of evacuation).

Dose estimates are expected to be in a low-to-medium dose range. For example, for the majority of evacuees from Pripyat town (98.6% of evacuated residents) and from 30 km zone (86.2% of evacuated individuals) doses due to external γ-irradiation do not exceed 50 mSv, and dose due to

internal exposure to cesium-137 does not exceed 20 mSv (personal communication with Dr. Bazyka).

Third type of dose information will be the information from the medical records about doses received by thyroid cancer patients, whose tissues will be used for the studies.

Ethical issues of the proposal

Ethical considerations will be a major issue for project approval, especially the handling of the nonpaternity cases. The preliminary discussion of the issues with the EC (Dr Andre Jouve) has suggested a rather straightforward procedure for project submission to be followed by an assessment of the ethical issues by the responsible authorities in the countries from where the tissues originate and where the experimental work will be carried out. .

Roadblocks to be overcome

- Reconstruction of the doses to gonads
- Possibly small transgenerational effects
- Possible confounding factors, i.e. medical exposures of parents and children, smoking and age strongly modifying the chromosomal aberration frequencies in the families, high sensitivity of the array techniques to the homeostatic changes in the individual organism.
- Ascertainment of suitable study subjects 24 years after the accident
- Absence of a centralized body in Russia or Belarus mandating the organizing/supervising of the sample collection and analysis. In Ukraine this responsibility is mandated to the RCRM.
- Identifying controls for this study from the subjects born at the “clean” territories will be difficult
- Large quantities of cell material (blood) may be needed for a stepwise mechanistic analysis (more than 20 ml, depending on the methods applied).
- Blood and DNA banks in the countries conducting the project need to be established
- Expensive set-up and equipment in both studies
- Difficult access to the extracted thyroid tissues

Prioritisation

This project is very important to the understanding of the non-targeted effects of low-dose radiation exposure. Further work, including the verification of the animal studies and the resolution of the inconsistencies therein, has a high priority. It is a long-term project.

MENTAL RETARDATION FOLLOWING IN UTERO EXPOSURE AS A RESULT OF THE CHERNOBYL ACCIDENT

The Chapter was prepared by A. Kesminiene and E. Ostroumova, IARC, Lyon, France

Background

General

Among various health effects of prenatal exposure to ionizing radiation observed in the atomic bomb survivors, impaired cognitive function, including cases of mental retardation, is not so common phenomena when compared to the incidence of cancer, but nevertheless, it has been well-documented (*337-339*). It has been shown that the critical time of exposure, when the most significant damage occurred, was the period of 8-15 week of gestation, with less damage effect at 16-25 weeks. The interval from 8 to 15 weeks after fertilization is characterized by a fast development of the cerebral cortex when a rapid increase in the number of neurons occurs. These immature neurons lose their capacity to divide and migrate from the proliferative zones to the cerebral cortex. At 16th week after fertilization cellular differentiation accelerates, synaptogenesis increases and the definite cytoarchitecture of the brain unfolds.

Studies of behavioral changes and structural defects in rats irradiated in utero with whole body doses in a range of 0.25 – 1.25 Gy demonstrated that a spectrum of functional and morphological changes can be produced even by low-dose in utero irradiation (*340*). For most behavioral endpoints a dose-dependent association was found with the biggest changes being determined by the specific day of gestation on which irradiation took place. Dose-dependent changes in postnatal behavior and decrease in the cerebral cortex thickness following in utero exposure in rats at two doses of 0.5 Gy given in 6 hours interval were also reported by Vidal-Pergola et al. (*341*). Extrapolation of the experimental results to the human should always be performed with a caution, but those studies provide an additional evidence of high sensitivity of the fetus especially at the early stages of organogenesis.

Studies of those *in utero* exposed due to the atomic bombardments of Hiroshima and Nagasaki are main source of information on mental health effects following fetal exposure. In a group of about 1,600 in utero exposed a-bomb survivors, 30 cases of severe mental retardation were diagnosed, whereas one could expect 9 or 10 spontaneous cases only (*338*). Normal (spontaneous) incidence of mental retardation in liveborn children is 5 cases per 1000 (*342*). The severity of mental retardation was assessed by the following criteria: inability to make simple conversation, to perform simple calculations, to care for him/ herself or if he or she was completely unmanageable or had been institutionalized. A linear dose-response association between severe mental retardation and DS86 uterine dose was observed with 50-fold increase at 1 Gy (*337*). When two probable non-radiation related cases of Down's syndrome were excluded from the 18 cases of severe mental retardation, a statistically significant threshold was found that ranged from 0.46 Gy (95% CI lower bound 0.06 Gy) to 0.56 Gy (95% CI lower bound 0.31 Gy) if exposed 8-15 weeks after ovulation (*338, 343*). For the group of in utero exposed in 16-25 weeks after ovulation, the lower 95% limit of the estimated threshold was 0.28 Gy (*338, 343*).

As for the other measures of brain cognitive functions, a linear decrease of intelligence quotient (IQ) score was demonstrated with dose increase in the children exposed 8-15 weeks after ovulation with estimated decrease in IQ score to be 29 points per 1 Gy of DS86 uterine absorbed dose (95% CI ± 8.2 points) when mentally retarded cases were included in the analyses and 25 points (95% CI ± 9.8 points) when they were excluded. No evidence of radiation effect on inelegance was found in children exposed prior to week 8 or at 26 or more weeks after ovulation (*338*).

A decline in average school performance was observed in the groups exposed at 8-15 and 16-25 weeks, with a linear dose-response association between school performance results and absorbed

uterine dose among those exposed at 8-15 weeks after ovulation with or without individuals with severe mental retardation (2).

When testing about possible dose threshold for IQ and school performance scores, there was no statistically significant excess risk at dose below or equal 0.10 Gy (*338*).

However, there are some limitations of the studies of brain damage following *in utero* exposure due to the bombardments of Hiroshima and Nagasaki. First of all, the data are limited and the number of heavily exposed is small. There are other factors, except radiation, that can damage the central nervous system of the embryo and fetus, such as genetic variation, bacterial and viral infections during pregnancy, nutritional deprivations, etc. But it is unlikely that these concomitants, if they exist, would have dose-dependent effects.

On the whole, it is still uncertain if a threshold in radiation-related fetal brain damage exists and it is unclear what molecular mechanisms are involved in radiation-related damage to the brain. Thus, further systematic studies, including animal experiments, are essential for better understanding of the effects of radiation on the developing central nervous system especially at the low dose range.

Chernobyl-related studies

First assessment of mental health in children prenatally exposed due to the Chernobyl accident by each affected country was performed in the framework of the International Programme on the Health Effect of the Chernobyl Accident (IPHECA) as a "Brain damage *in utero*" project (*344*). Cohorts of children exposed *in utero* included all individuals (alive or dead) born between 26 April 1986 and 26 February 1987 and resided in the "strictly controlled" zones (rayons with radioactivity level above 555 kBq/ m^2) or "clean areas" (rayons or oblasts with contamination level less than 37 kBq/m^2). Children resided in the "clean" areas considered as a control group. The total number of children initially identified as *in utero* exposed in the "strictly controlled" zones of Belarus, Russia and Ukraine was 1,400; 1,200 and 1,400, respectively. About 55% of all eligible children were included in the study (2,189 out of 4,000 identified as *in utero* exposed according to the study protocol). The reasons of exclusions from the study were: mother's non-residence on the radioactively contaminated territories during pregnancy; study subjects' migration, parents' refusal to participate in the study.

Based on the Ratter's scale test completed with parents participation, children exposed *in utero* from the "strictly controlled" zones of Belarus expressed two times higher frequency of emotional, behavioral and non-differentiated disorders compared to the children of control group (42.69% and 25.56%, respectively) Scientific report: International Programme on the Health Effects of the Chernobyl Accident (IPHECA) (*344*). In the Russian Federation, *in utero* exposed children from the "strictly controlled" zone showed significantly lower scores for non-verbal ($P<0.05$) and verbal intellect ($P<0.01$) as compared to the results in the control group as well as statistically higher frequency of emotional and behavioral disorders based both on parent's and teacher's evaluations ($P<0.05$). Findings in Ukraine were even more striking, where a slight degree of mental retardation was detected twice more frequent in the group of *in utero* exposed from the "strict controlled" zone compared to the controls (4.1% in the exposed group vs. 2.1% in the control group). Emotional and behavioral disorders based on parental and teacher's evaluation also were more frequent in the exposed group as compared to the controls.

Significant loss at follow-up of the study subjects, lack of individual dose estimates and test results based on parental evaluation which could be affected by higher levels of anxiety and stress among parents resided in the "strictly controlled" zones, are the major limitations of the findings reported.

More recent study conducted in Ukraine also showed significantly higher frequency of mental retardation as well as of emotional and behavioral disorders and lower IQ scores in children exposed *in utero* (*345*). The study results rest upon examinations of only 50 *in utero* exposed children and 50 age-and-gender matched unexposed children.

Assessment of intellectual and physiological development of *in utero* exposed in Belarus (*346, 347*) found significantly lower full-scaled IQ score among 250 prenatally exposed children aged 6-7 and 10-12 years compared to the control group.

Study of neurobehavioral and cognitive performance among 1,629 children up to 4 years at the time of the accident, including 270 who were *in utero*, did not reveal any association between attention-deficit/ hyperactivity disorder index and exposure level (*348*). The study population included children emigrated to Israel from the Gomel region (considered as a highly exposed group) and a sample of immigrant children from Mogilev and Kiev regions (mildly exposed) and from the cities of Moscow and St-Petersburg (nonexposed).

Objectives

To assess prevalence (incidence) of mental retardation following radiation exposure *in utero* due to the Chernobyl accident.

Specific relevance (value-addedness) of Chernobyl population(s)

Currently the presence of a threshold for mental retardation in children exposed prenatally has been questioned. The population affected *in utero* after Chernobyl is greater than that after Hiroshima, confirming the value of the proposed study, if the set up of the cohort of children exposed *in utero* appears to be feasible.

Proposed approaches

Study Population

Study population would include children born between 26 April 1986 and 26 February 1987 in regions of Ukraine, Belarus and, possibly, Russia contaminated as a result of the Chernobyl accident. A feasibility study should be performed assessing possibility to obtain information on exact date and place of birth as well as information on parents (parental birth year to define parental age). Table shows number of children exposed *in utero* identified in the framework of "Brain damage *in utero*" project of the IPHECA (*344*).

Table 1. Number of *in utero* exposed children identified for the "Brain damage *in utero*" project of IPHECA by country and level of territory radioactive contamination

Country/area	Number of *in utero* exposed subjects
Contamination level > 555 kBq/m^2	
Belarus	
Braginsky, Hoyniksky, Narovlyansky, Vetkovsky rayons of Gomel oblast and Kostyukovichsky and Cherikovski rayons of Mogilev oblast	906 (1,400)*
Russia	
Bryansk oblast with the towns of Novozybkov and Klincy	725 (1,200)
Ukraine	
Evacuated from the towns of Pripyat and Chernobyl, Narodichesky, Ovruchsky, Korostenski raoyns of Zhytomir oblast, Polesski and Ivankovsky rayons of Kiev oblast	558 (1,400)
Subtotal	**2,189 (4,000)**
Contamination level < 37 kBq/ m^2	
Belarus	
Slavgorodsky rayon of Mogilev oblast, Volkovysski rayon of Grodno	962 (1,100)

Country/area	Number of *in utero* exposed subjects
oblast, Zhabinsky rayon of Brest oblast	
Russia	
Kaluga oblast with the towns of Obninsk and Borovsk	300 (1,400)
Ukraine	759 (1,400)
the city of Kharkiv and Kharkiv oblast	
Subtotal	**2,021 (3,900)**

* number of all eligible subjects indicated in parentheses

Study design

Both a cohort and a case-control study approach could be applied.

Doses

Preliminary analysis can be done using level of soil radioactive contamination as a dose surrogate to find out if there is an association between mental retardation frequency and exposure levels. If mental retardation appears to be related to the contamination level, then more thorough analysis using individual dose estimates is required. For that purpose, individual doses need to be reconstructed. It is not clear though which organ dose would be the more appropriate for the risk analysis. The absorbed dose to the uterus due to external exposure and also from internally incorporated Cs-137 could be used.

In cases of significant exposure to I-131, it is necessary to consider dose to the fetal thyroid. Previously, reconstruction of individual dose to the fetal thyroid and absorbed uterine dose due to external exposure has been performed for 250 *in utero* exposed children in Belarus by V. Drozdovitch (*349*). Maximal dose to the fetal thyroid was 4.1 Gy (mean and medium dose 0.39 and 0.23 Gy). Maximal absorbed uterine dose as a measure of antenatal exposure was 110 mGy with mean and median of 9.6 mGy and 6.2 mGy, respectively (*349*).

Biological samples

Biological samples, in particular buccal imprints and blood could also be collected.

Feasibility

Feasibility stage is required to investigate possibility of cohort identification and collection of information on study outcome, i.e. mental retardation diagnosis or intelligence performance indicators.

Roadblocks

1. Identification of study population members and tracing eligible study subjects might be difficult.
2. Collection of information on their mental status (IQ score, school performance, education level, etc.) is problematic and potential sources need to be identified.
3. Setting up well defined mental retardation diagnostic criteria to be used in the study. It would assure data quality and compatibility of data from all participating countries. Panel of international experts could advice on harmonization of diagnostic criteria as well as verify the diagnoses.
4. Collection of information on non-radiation risk factors is also important since there are other factors, in addition to ionizing radiation, which can affect normal development of the brain/ central nervous system of the fetus. It is therefore important to obtain information on potential confounders, such as parental age, family history of mental disorders and inherited

metabolic disorders, pregnancy and labor course, mother's infectious during pregnancy, mother's malnutrition, parental alcohol consumption, occupational hazards, etc.

Statistical power

A spontaneous frequency of mental retardation is about 1 case per 200 births. We could therefore expect about 20 cases of spontaneous mental retardation in a cohort of about 4,000 *in utero* exposed. A radiation-related excess would be possible to predict when doses of fetal exposure in the study population are available. A study would have more power if some other intelligence performance indicators (such as school performance, IQ score, education level etc.) are considered for dose-response analysis, suggesting a sufficient dose range, or for comparison with unexposed controls.

Ethical requirements

Typical ethics requirement are necessary, same as for other epidemiological studies.

Prioritisation

Assessment of the feasibility of setting up the cohort of children exposed *in utero* is considered of high importance medium-term priority.

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IARC, 150 Cours Albert Thomas, 69372 Lyon CEDEX 08, France - Tel: +33 (0)4 72 73 84 85 - Fax: +33 (0)4 72 73 85 75
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The Radiobiology Report

Introduction

From the outset of the ARCH project the need for the evaluation of the effects of the CA to contribute to an "*improved understanding of radiation effects and help with future radiation protection measures"* was recognized. In 1986 the phenomena of genomic instability (GI) and bystander effect (BE) were unknown and there was considerable confidence that microdosimetry, based on target theory provided a sound theoretical basis for radiological protection. Target theory had directly connected the number of cell hits and the molecular damage with the detrimental effects on the tissue and organism. A biological underpinning of the effects of radiation is vital in radiological protection because causality and effect predictions cannot be inferred from epidemiology alone. Thus, a "partnership" between underlying mechanism studies and epidemiology is essential.

The first clear evidence on radiation-induced genomic instability, GI, and bystander effect, BE, in 1992 led to a long series of radiobiological discoveries that could not be assimilated into the then current theoretical framework based on target theory: they thus represented a serious diminution in the certainty of radiation risk estimation. Therefore, this was one of the challenges for ARCH when it commenced in 2008.

With these considerations in mind the objective of the radiobiology report was focused as follows:
To review the literature related to the non-targeted effects of radiation and the possible consequences for understanding the health effects of the low dose protracted exposures experienced as a result of the Chernobyl accident.

Executive Summary

The report consists of three Chapters and an Annex to Chapter 3. Also included is a paper prepared during the ARCH project and now published in Mutation Research.

Chapter 1 briefly and selectively reviews the status of radiation risk assessment from the time of the Chernobyl accident to the present date emphasizing the scientific issues that would need to be addressed in a comprehensive theoretical underpinning of the biological basis of radiation effects, which is what radiobiology should provide. Paramount among those scientific issues is the understanding of the nature and causal relationships within and between the genomic instability and the bystander effect.

Chapter 2 reviews comprehensively (up to June 2010) the literature on proposed mechanisms to account for GI and BE. Genomic instability is the greater challenge as the BE is well established to be a consequence of well known (but not necessarily well understood) communication between cells through expressed cellular factors. Six proposed mechanisms for GI were identified and evaluated against a set of criteria drawn from the extensive empirical data on the effect.

Chapter 3 explores the theoretical basis for the qualifying proposal, which represents a distinct departure from the traditional approach to radiobiology. This traditional approach has focused on how radiation damages the material of the cell, most notably the genomic DNA, in terms of causing mutations which have implications for the phenotype. The qualifying proposal is *process* rather than *materially* oriented envisaging the irradiated cell as a dynamic system rather than a simple set of targets. This approach is based on thermodynamic openness and dynamical irreversibility; a more appropriate basis for biology in physics than provided by the traditional radiobiological dogma.

The report concludes that a number of health consequences of radiation exposure, including cancer and non-cancer conditions, arise not as a result of mutational damage *per se* but through an irreversible dynamical perturbation of the normal operation of the cells damage processing capacity.

Implications for the Commission

Descriptive epidemiology, in the absence of an understanding of the processes underlying the generation of the health effect being studied is a less than ideal basis for the risk assessment in radiological protection. For this reason the hybrid subject “molecular epidemiology” has been advocated. However, in terms of the effects of radiation very little in the way of justification for any specific procedure has been advanced. The findings of the radiobiology report, which specifically relate to ionizing radiation, offer some guidance.

It is axiomatic that the principle cause of a radiation health effects lay in what radiation does to the cell in which the energy deposition occurs (leaving aside the BE). The survivors of the atomic bombings in Japan provide the greater part of the quantitative evidence of the carcinogenic effects of radiation on human health. In this case (instantaneous exposure) the physical and chemical sequelae of exposure are complete within the lifetime of the cell in which the energy is deposited and thus play no physical and chemical role in the progeny of that cell. Consistent with this situation extensive efforts were devoted to finding DNA mutations caused by radiation in that initial cell and replicated in all subsequent generations causing subsequent carcinogenesis. This has not been realized despite the opportunity provided by the radiation-induced thyroid cancer.

In the view of the basis for carcinogenesis advocated in the radiobiology report the observation of molecular damage associated with the initiation of the cancer would not be expected, but rather evidence of modifications of gene expression and regulation would be expected and are observed in diagnosed cases of thyroid cancer.

This is not to say that molecular damage is irrelevant to carcinogenesis. The presence of mutations caused in the initiated cell or which might arise as a result of the process underlying carcinogenesis, may influence the behavior of a cancer through influencing the proliferative and adaptive capability of the cell. The activity of certain genes are essential to regulate cell growth and thus if lost due to mutations may accelerate the development of the cancer. It may then be possible to regulate the development of the cancer by restoring, in some way, those functions.

What mutations and other chromosomal aberrations observed in the development of a cancer will not do is reliably indicate either the origin of a cancer (e.g., radiation) or whether cells bearing specific molecular markers in exposed persons will become malignant. Molecular markers are therefore of very limited application in these aspects of so called “molecular epidemiology”.

The upside of this situation is that “process markers” such as proteomics, metabolomics and transcriptomics are more likely candidates to be used in conjunction with epidemiology. However, before this form of molecular epidemiology can be useful there is a need for further collaborative research between those interested in underlying mechanisms/processes and epidemiologists equipped to understand the basic science.

As authors of the radiobiology report we have no wish to force our ideas on the Commission and would welcome peer review of the radiobiology report, indeed we recommend that this is done and are willing to address any issues raised as a result.

Andrei V Karotki PhD
Keith Baverstock PhD

Chapter 1

The state of the modern radiobiology and its main problems

1.1 Brief and selective overview of radiation biology 1986 - 2010

It can be argued that the origin of modern radiobiology lies in the comment made by Crowther in 1924 (Crowther 1924) suggesting, based on some crude dose response results on irradiated cells entering mitosis, that biological effects may depend exponentially on dose and if so it might "represent the probability that a given structure in the cell would actually be affected by the incident radiation" implying that an effect due to the x-rays, rather than the biology, was being observed. This was the birth of target theory[1] which, in one form or another, has dominated radiation biology up until 1992. Well before the discovery of the identity of the heritable material, DNA, target theory had been used to estimate the size of loci (now known to be genes) mutated in the offspring of the fruit fly when exposed to x-rays (Timoféeff-Ressovsky, Zimmer et al. 1935) and mated. Schrödinger, in his celebrated lecture entitled "What is Life?" in Dublin in 1943 (Schrödinger 1944), cited Delbrück's estimate of ~1000 atoms.

The idea carried over into the 1960s with the theory of microdosimetry (Rossi 1991; Rossi and Zaider 1991) which sought to rationalise the effects of radiation quality in biology in term of volumes within which radiation-induced sub-lesions could interact. In the 1980s with increased computing power microdosimetry evolved into track structure theory (Paretzke 1987; Nikjoo and Uehara 2004; Nikjoo, Uehara et al. 2006) which sought to rationalise biology in terms of the spatial distributions of the ionising events within the cell. The theme throughout was to find a theoretical underpinning framework for the relationship between biological effect and some critical target volume within the cell.

This radiation biological theme fitted well with the mainstream developments in biology from 1953 onwards with the discovery of DNA as the heritable material and subsequently the base sequence as the information that defined phenotype. The Central Dogma, namely that information flowed uni-directionally from the DNA sequence to define the biologically functional proteins enabled the heritable effects of radiation to be understood in terms of sequence mutations in the DNA. Considerable advances in radiation genetics were made in the 1950s onwards in large scale mouse experiments in the UK and USA and led to a

[1] The precursor to target theory was hit theory which did not imply that what was hit by a radiation event was a biologically sensitive target. Rather effect was related to the number of hits necessary to induce the biological effect. Thus, target theory attempts to link the radiation events in the cell to relevant structures within the cell.

relatively secure knowledge of genetic risk from radiation (Sankaranarayanan and Chakraborty 2000).

Less progress was made in respect of understanding the somatic effects of radiation which, with the realisation in the mid 1970s of an excess of solid cancer in the survivors of the Japanese bombings, took on a greater importance. Nevertheless the somatic mutation theory (SMT) of cancer (Weinberg 1998; Hanahan and Weinberg 2000) seemed mechanistically the most reasonable in view of the ability of radiation to cause mutations. It had been clear for a long time that radiation caused mutations and chromosomal damage similar to that found in malignant cells.

What was clear from experimental work between 1960 and 1990 with short range x-rays and Auger electrons among other radiation sources, was that DNA was the target for the somatic effects of radiation and thus a dogma that entailed damage to the DNA that on replication if it was not repaired was transmitted to all progeny, emerged as the basis for radiobiology (reviewed in (Baverstock and Belyakov 2005)).

However, in 1992 two new phenomena emerged in *in vitro* experiments, namely genomic instability (Kadhim, Macdonald et al. 1992) (delayed appearance, sometimes by several cell generations, of cellular and molecular damage) and the bystander effect (Nagasawa and Little 1992) (where an unirradiated cell neighbouring the target or irradiated cell exhibits the effect) that could not be rationalised with that dogma, in the first case because it was caused by doses so low that target theory denied the possibility that for a given end point a specific target was affected (Baverstock 2000), for example a gene and in the second case the DNA of the cell exhibiting the effects was certainly not damaged by the radiation. Significant research effort has been expended in an attempt to understand the mechanistic bases for these two effects, for example in the FP6 non-targeted effects (NOTE) project. This project seeks to find a new paradigm that embraces both the classical radiobiology and the non-targeted effects (Salomaa, Wright et al. 2010).

In addition to genomic instability, GI and bystander effect, BE, a number of other phenomena caused by radiation have been revealed, for example adaptive response, clastogenic effects, low dose hypersensitivity and various abscopal effects. None of these fits easily into the classical dogma. To date they have not been as extensively studied or found to be as reproducible across a large number of biological systems as have GI and BE. They have been briefly reviewed (Baverstock and Belyakov 2010) but they are not considered further here.

In effect the target size for GI has been determined to be of the order of the whole nucleus or whole cell. For the BE the target concept is not useful. In the case of GI it can then be argued

that *any* damage in the cell has a finite probability of leading to the effect and in the case of BE that some form of signalling from the irradiated cell to its neighbours must be implicated. Furthermore, in the case of GI the fact that molecular damage may not appear for several generations after exposure can be taken to be indicative that the mechanism does not involve any direct relationship between whatever is the initial molecular damage by the radiation and what eventually emerges as damage in distant progeny. It was therefore correctly argued from early in the 2000s that these effects must involve a new paradigm (Baverstock 2000; Barcellos-Hoff, Park et al. 2005) for the effects of radiation on biological entities. This will be addressed in Chapter 3.

GI has mainly been observed *in vitro* (Morgan 2003) but there are instances in *in vivo* studies (Morgan 2003), not least in germ cells (Barber, Plumb et al. 2002; Barber, Hickenbotham et al. 2006; Barber, Hardwick et al. 2009). In mice spermatogonial irradiation leads to extended sequence tandem repeat (ESTR) mutations in the offspring (Barber, Plumb et al. 2002; Barber, Hickenbotham et al. 2006; Barber, Hardwick et al. 2009) and mini-satellite mutations in humans (Dubrova, Plumb et al. 2000; Dubrova, Bersimbaev et al. 2002; Dubrova, Grant et al. 2002; Dubrova, Ploshchanskaya et al. 2006). However, such observations are not universal as is reviewed by (Bouffler, Bridges et al. 2006). The phenomenon is much more difficult to observe *in vivo* in somatic cells but at least one notable experiment (Lorimore, McIlrath et al. 2005) is convincing. It demonstrates both GI and BE which had already been observed *in vivo* (Watson, Lorimore et al. 2000) in mouse bone marrow.

In addition, in the offspring of irradiated rats radiation and other mutagens more easily induce chromosome damage (Vorobtsova 2000). More recently an increased sensitivity to radiation has been reported in the children of fathers exposed to Chernobyl fallout (Aghajanyan and Suskov 2009).

The mouse studies on GI transgenerational inheritance are of particular importance partly because of their comprehensiveness and partly because they appear to “mirror” an equivalent effect in humans in terms of the minisatelite mutations referred to above. This raises an important question as to whether the ESTR and minisatellite mutations are the same phenomenon as the GI observed in studies with somatic cells. Important evidence in support of this contention comes from the observation that the somatic cells of affected F_1 mice show evidence of GI in terms of an increased rate of mutation at the *hprt* locus and in terms of strand breakage as measured by the comet assay and gamma-H2AX fluorescence assay (Barber, Hickenbotham et al. 2006). A second line of supportive evidence comes from the observation that paternal exposure to the chemical mutagen ethylnitrosourea (ENU) induces ESTR mutations in the offspring while inducing quite a different type of damage in the genomic DNA of the parent (Dubrova, Hickenbotham et al. 2008).

These studies supplement a quite extensive body of evidence indicating that irradiation of males at the spermatogonial stage leads to a number of effects in the offspring which cannot be accounted for by sequence mutations. These include the reversion of the pink eyed unstable mutation, p^{un}, as observed in terms of coat colour (Carls and Schiestl 1999) and eye markings (Bishop, Kosaras et al. 2000), the increased sensitivity of offspring to malignancy induced by mutagens (Lord, Woolford et al. 1998; Lord, Woolford et al. 1998; Lord 1999), the appearance of dominant lethal mutations in the offspring of F_1 male mice with a paternal radiation history but no exposure to radiation themselves (Luning, Frolen et al. 1976) and the reduced cell proliferative capacity in chimeras of offspring from irradiated and un-irradiated fathers (Wiley, Van Beek et al. 1994; Wiley, Baulch et al. 1997; Vance, Baulch et al. 2002).

From these results we can conclude that GI and BE are effects of radiation (and other agents such as heavy metals (Coen, Mothersill et al. 2001) and ENU (Dubrova, Hickenbotham et al. 2008)) that have important implications for biology.

As a consequence of the earlier dogma based on cell survival curves and microdosimetry it was believed that radiation risk was dependent on dose-rate as well as on dose. This is indeed true for inherited sequence mutations in mice as shown in the figure 1.1, which compares the locus specific mutation rate per unit dose based on extensive studies carried out at the Medical Research Council in the UK and at Oak Ridge in the USA (from (Baverstock 1991). There is a factor about 3.6 between the highest dose rate (~3Gy/min or 300R/min) and the region of dose-rate independence (<10mGy/min or 1 R/min).

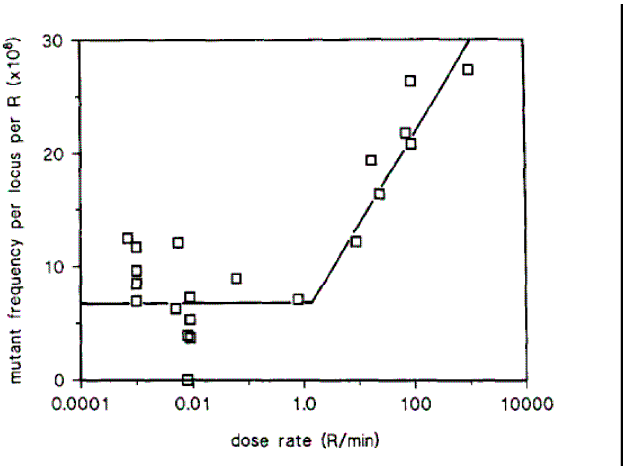

Figure 1.1.

The influence of dose-rate on the induction of seven recessive mutations in mice. (1R/min is approximately equal to 10mGy/min)

However, the question of whether this dose-rate reduction applies to cancer has been a matter of debate for many decades. Based on evidence from the induction of chromosome

anomalies by x-rays, the ICRP has adopted a dose and dose-rate effectiveness factor (DDREF) equal to 2, in effect assuming that at low doses (<100mGy) and low dose-rates the risk per unit dose will be half that at high doses and dose-rates. There is no evidence to support this in terms of cancer induction and the curvilinear dose response data upon which it is based are not confirmed if later, more sophisticated, methods of measuring chromosomal aberrations, such as FISH, are used (D T Goodhead: presentation at ICRR 1998); in this case the response is much more linear, suggesting a DDREF of unity.

Based on the fact that biological organisms are composed of cells and therefore, compared to the initial dimensions of ionising events (nanometres), “grainy”, there is a strong physical argument for any biological response to have a linearly increasing response at low doses, that is, where the effect is determined by the number of cells in the organism that are hit. Above some 5 to 10mGy increasing the dose involves increasing the number of times a cell is hit and under these conditions the dose response may be more complex. This argument effectively rules out a dose threshold below which there is no effect and has become known as the “linear no-threshold” hypothesis (LNT). It does not, however, necessarily entail a linear interpolation from doses at which effects can be observed back to zero dose although that would be consistent with LNT (Brenner and Sachs 2006; Brenner 2009). A threshold above about 6mGy is ruled out on evidence from the irradiation of pregnant women and the incidence of childhood leukaemia and solid cancers in the irradiated *in utero* child (Doll and Wakeford 1997).

However, over the past decade the evidence for a linear dose response extrapolated to zero dose from doses of the order of a few Gy is strengthening. See for example (Brenner, Doll et al. 2003).

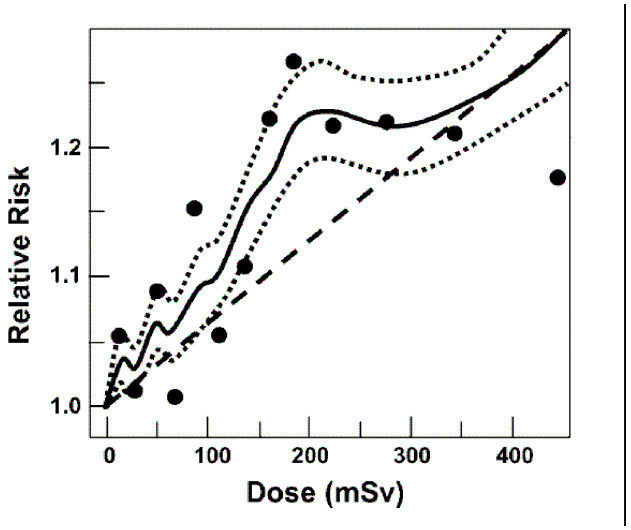

Figure 1.2

The figure is taken from (Brenner, Doll et al. 2003) 2003 in which carefully re-analysed data from the survivors of the atomic bombings in Japan were reviewed. The dashed line is the linear dose response

curve computed from all the data between zero and 2 Gy. The majority of the points fall above this line, thus arguing in favour of a DDREF <1.

Other evidence is also supportive of risks based on a DDREF of 1 or even less and includes evidence from radiation worker studies (Cardis, Vrijheid et al. 2007) and from the analysis of the Techa River data (Krestinina, Preston et al. 2005; Eidemuller, Ostroumova et al. 2008; Eidemuller, Ostroumova et al. 2010; Krestinina, Preston et al. 2010). A study made in Sweden compared the cancer incidence in a region of the country where rainfall increased the precipitation of ^{137}Cs after the Chernobyl accident with that seen in populations in uncontaminated areas (Tondel, Hjalmarsson et al. 2004; Tondel, Lindgren et al. 2006). The doses received by the population of the contaminated area were of the order of a few mSv at the most and yet some increases were observed in the succeeding decade. This study is currently being repeated in Finland.

As far as cancer is concerned a rather simplistic picture of radiation as an initiator has dominated thinking on radiation carcinogenesis, largely based on the survivors of the atomic bombings in Japan, and has to be replaced by a more complex picture where radiation may not only initiate but also promote cancer. For example, post-mortem examination of the tissues of trauma victims provides evidence of latent pre-cancerous lesions in many tissues of adults (Folkman and Kalluri 2004). In addition a proportion of the population will be carrying developing cancers which may take several years to become evident. Were the latent lesions to progress to full scale tumours or the developing tumours be accelerated, the age specific tumour incidence rates would be increased. Enderling (Enderling, Anderson et al. 2009) has proposed that the latent lesions are surrounded by very slowly dividing cells that constrain their further development and that disturbance of these lesions (by radiation) could provoke their proliferation in tumours. Thus, there are ways in which radiation could act to increase cancer incidence quite independently of its ability to initiate cancer(but see Appendix A3; Chapter 3).

Until comparatively recently the late stochastic effects of radiation at low doses comprised only hereditary disease and cancer. In 1992 certain non-cancer diseases were found to be related to dose in the survivors of the Japanese bombings (Shimizu, Kato et al. 1992; Shimizu, Pierce et al. 1999). Circulatory disease was an established consequence of radiotherapy but thought to be relevant only at high doses. More recently as reviewed by Schultz-Hector (Schultz-Hector and Trott 2007) and Little (Little, Tawn et al. 2009) it seems to be also associated with low dose exposures.

Thus, since 1986, the year of the Chernobyl accident, there have been substantial changes in the basic understanding of the effects of ionising radiation in the direction of increasing the credibility of the effects of low doses but also in increasing the uncertainty about risks. Had

the Chernobyl accident occurred in 2006 rather than 1986 the initial risk assessment might have been rather different.

1.2 Problems in modern radiobiology

The principle factors inhibiting the advancement of radiobiology at the present time stem from biology and physics, the subjects that underpin radiobiology. These will therefore be discussed first.

2.2.1 Problems of biology

Until 2001 biology was firmly based on a deterministic interpretation of the Central Dogma (CD) which stipulated that information coded into the genomic DNA flowed uni-directionally to determine the properties of the functional proteins. The assumption that this would be a deterministic process was central to the human genome sequencing enterprise commenced in 1991and it was the dominant interpretation of the CD at least from then to 2001. This was despite the fact that the protein folding problem was unsolved, as it still is, and thus, even if determinism did apply, biological function could not be predicted from sequence.

Under the CD, phenotype would be easily derived from the genotype once the code had been broken and the functions of the resultant proteins known. Thus, the derivation of phenotype was widely believed to an intellectually trivial problem; one for sequencing technology to solve.

With the completion of the sequencing of the human genome in 2001, when it was realised that there were only some 21,000 gene coding sequences for the more than 100,000 known protein products (Carninci 2008), the determinism was seen to have been an unwarranted assumption[2]. While how it is possible to get an average of 4 to 5 products from a single sequence is well understood, how the cell "chooses" which of those products is needed at any given time is not understood; this is in essence a question of how the cell is regulated to produce a phenotype from a genotype.

In respect specifically of cancer the so called somatic mutational theory (SMT) based on the CD, has also been questioned on the basis of genome wide sequencing, which has shown that rather than a few driver mutations many cancer types carry numerous mutations suggesting, at the very least, several independent pathways leading to malignancy

[2] In an interview with Der Spiegel Dr Craig Venter (http://www.spiegel.de/international/zeitgeist/0,1518,709345,00.html), the leader of one of the two major and competing human genome sequencing projects says "nothing has been learned from genome sequencing". In that study it was Venter's own DNA that was sequenced and he notes that it not possible to determine the colour of his eyes from the sequence.

(Greenman, Stephens et al. 2007; Sjoblom 2008). Furthermore, once considered as primarily a phenomenon of cells (witness Weinberg's book of 1998 (Weinberg 1998) entitled "One renegade cell") a much wider involvement of many cells and signalling between them is now recognised (Barcellos-Hoff and Brooks 2001). Cancer is now increasingly seen as an emergent property of tissue.

This last statement raises a more fundamental issue, namely whether biology is, as it has since the time of Descartes been assumed to be, "complicated" or whether it is "complex". The distinction here is that things that are *complicated* have *dependencies* between their component parts, in this case the genes or their products, or the cells within a tissue, or the tissues within an organism, and if they are *complex* they have *interactions* at these levels. Emergence is a property of complex systems. If indeed biology is complex the ramifications are not trivial, particularly in the context of understanding the processes that go on (Feinendegen, Hahnfeldt et al. 2008). There is no half-way house; a given aspect of biology will be either complex or complicated (see Appendix A3; Chapter 3) and if "emergence" is invoked in a particular respect (e.g. in (Barcellos-Hoff 2008)) then the full implications of complexity should follow (Feinendegen, Hahnfeldt et al. 2008). It would not be an exaggeration to say that complex systems operate under a different paradigm from complicated systems.

Complex systems raise important problems in the physics that underpins biology and, therefore, radiobiology so they will now be addressed.

1.2.2 Problems with physics

If biology is not to attract the label of "vitalism", i.e., being based on some mysterious vital force, it must be based securely on physical foundations, yet we know that the traditional physics must be inadequate. Traditionally the relevant physics is Newtonian dynamics, the thermodynamics of closed systems and the kinetics of small molecules. Newtonian dynamics is time reversible but life is not; biological systems are not closed but open; the molecules responsible for biological function are rarely small but usually large polymers. It might be argued that considerable progress has been made without a more relevant physics but as in many other areas past progress is no guarantee of future progress.

It is not yet possible to conceive of a comprehensively revised physical basis for biology since each of these aspects present significant problems. Dynamical irreversibility has been a major problem for physics for decades, as has thermodynamic openness (Prigogine and Stengers 1997). Proteins fall into the spatial category termed the mesosphere by Laughlin (Laughlin 2005), that is, the size range larger than single atoms and smaller than cells, a neglected area of physics. Therefore, at the moment, the best that can be done is to recognise that the

physics underpinning biology may be seriously inadequate to handle the processes that characterise living systems.

One neglected “physical” approach to biology is “relational biology” pioneered by Rashevsky in Chicago in the 1950s and developed further by one of his students, Robert Rosen (Rosen 1991; Rosen 2000). The *relations* referred to are the relations between component parts of the system (cell, tissue or organism) which are explored rather than their material properties. This approach, despite its early beginnings, resonates well with the physics of complex or non-linear systems (Scott 2007).

1.2.3 Problems in radiobiology

Thus, the feature that underpinned classical radiobiology from its earliest days to 1992, the concept that biological effects were primarily a matter for physics and not biology and that the whole issue was really about what happened at the molecular level in discrete volumes in the irradiated cell, is seen to be only a part of radiobiology. In addition there are processes that are not readily captured in terms of molecular changes induced by radiation in DNA although there is no doubt that DNA is still very much involved. The term “non-targeted” is perhaps mis-leading (Baverstock and Belyakov 2010) and may usefully be replaced by “epigenetic”, that is, “over and above” genetics and without any implications regarding chromatin marking. At root the problem is how the cell is regulated to produce its phenotype from the genotype and how interfering in some way with the genotype modulates the effect on phenotype. Until this is understood radiobiology, which in the past has relied so heavily on an underpinning by a theoretical framework, cannot be complete.

In Chapter 2 the proposals for mechanisms to explain GI and BE are comprehensively reviewed. Using the well established features of GI and BE six candidate mechanisms are evaluated.

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Chapter 2

The biological bases for the epigenetic effects of radiation.

2.0 Introduction

As is clear from Chapter 1 the challenge is to understand the origin of the non-targeted, or epigenetic, effects of radiation. Classical radiobiology provides a sound basis for the deterministic and some inherited effects of radiation based on chromosomal damage and sequence mutations in the genomic DNA. Moreover, this formulation has been extended to radiation-induced carcinogenesis in the form of the somatic mutation theory (SMT), in which it is assumed that an initial hit causing a mutation in a single cell is required to trigger the cancer programme depending on further mutations, resulting in a clonal cancer expansion. However, as we have seen much evidence does not support this theory and proposals to introduce an epigenetic element into the understanding of carcinogenesis have been made (Haslberger, Varga et al. 2006; Herceg 2007; Sonnenschein and Soto 2008; Sharma, Kelly et al. 2010). Classical radiobiology does not account for the non-Mendelian inheritance mechanisms (Barber and Dubrova 2006), the induction of non-cancer disease by radiation (Schultz-Hector and Trott 2007) and some of the experimental evidence relating to the apparently increased minisatelite mutation frequency of persons with a parental radiation history (Dubrova, Nesterov et al. 1996; Dubrova, Nesterov et al. 1997) all of which have been observed in Chernobyl exposed populations. This Chapter is therefore exclusively devoted to understanding the origin and inheritance of the non-targeted or epigenetic effects of radiation.

2.1.0 The phenomenology of non-targeted epigenetic effects

2.1.1. What is included?

As noted above the best established effects that fall into this non-targeted, and therefore epigenetic, category are GI and the BE. These effects are known to be epigenetic not because DNA is not involved but because target theory tells us that the target for the effect is much larger than any single genetic entity, such as a gene or even a chromosome. While it can be argued that cell transformation (Kennedy, Fox et al. 1980) is possibly closely related to GI it is not discussed in detail here. Other effects mentioned in Chapter 1, for example adaptive response, clastogenic effects etc. are relatively too poorly understood to contribute to this argument (Baverstock 2008).

2.1.2. Definitions of GI and BE

GI is exemplified by a progressive irreversible accumulation during replication of non-clonal DNA damage, that is, damage that is not being faithfully inherited (at cell division and transgenerationally) in the genomic DNA. Indeed, there may be no evidence of molecular damage in the early generations after irradiation so absence of a molecular marker is not evidence of the absence of GI. This is well illustrated by experiments with the pink eyed unstable mutation, p^{un}, in mice. This mutation causes black coated mice to have a white/grey

coat. The p^{un} mutation is relatively unstable after radiation exposure, but at doses well below those that target theory would dictate for a direct hit on the gene coding sequence. Male mice with the mutation were irradiated six weeks prior to mating with unirradiated females. Most offspring were white coated (indicating the inheritance of the mutant gene) but some developed black patches on the coat due to the reversion of the mutation during embryogenesis (Schiestl et al PNAS 1997) The spectrum of endpoints that are indicative of GI is broad, including increases in chromosome aberrations, apoptosis, frequency of micronuclei, spontaneous mutational frequency (at the *hprt* locus in somatic cells, for example), mutational frequency of repeat sequences (minisatellites in humans and extended tandem repeat sequences (ESTR) in mice, increased and persistent level of reactive oxygen species (ROS), etc. (Morgan 2003). Early evidence showed that the *hprt* mutations induced by genomic instability (Chang and Little (Mut Res 1992)) were predominantly point mutations whereas those directly caused by interaction of the locus with radiation events were predominantly deletions (Little et al 1997: Radiat Res: Grosovsky et al 1996 Mol Cell Bio). This result confirms the sporadic nature of GI induced mutations and the mutator phenotype of the GI cell. To some extent the endpoints might be correlated with cell type and the efficiency with which GI can be induced and sustained is variable among cell types. For example, Paquette and Little (1992) observed ESTR mutations in *in vitro* irradiated mouse C3H embryo-derived fibroblast line cells some of which were cultured for 25 passages *in vitro* while others were subcutaneously injected into mouse skin to form tumours, i.e., they were cultured *in vivo*. The latter showed higher levels of ESTR mutations than the former. It should be noted that the mouse “tumours” did not involve the tissue of the host mouse. Mouse bone marrow cells irradiated *in vitro* with either neutrons or gamma-rays and transplanted into mice with oblated bone marrows were scored for chromosomal aberrations for up to 24 months. Chromosomal instability was observed in 3 to 6% of bone marrow cells (Watson, Pocock et al. 2001) . Where ESTR mutations are inherited transgenerationally they appear in the somatic as well as the germ cells of the offspring. Furthermore, somatic cells show increased levels of DNA damage (according to the “comet assay”) and increased levels of mutation at the *hprt* locus (Barber, Hickenbotham et al. 2006).

The case that GI:
is a universal radiation induced effect, which maybe present in the absence of molecular damage;
is heritable both at somatic cell division and transgenerationally;
is a mutator phenotype and thus generator of molecular damage including mutation and
is contingent on properties of the affected cell, is overwhelmingly supported by evidence.

The BE clearly indicates the importance of cell-to-cell communication. Irradiated cells secrete some agent, either through gap junctions or into the extra cellular medium which acts as a signal to neighbouring unirradiated cells causing them to adopt a phenotype similar to that induced in directly irradiated cells, including the features of GI cells (Hei, Zhou et al. 2008). There has been considerable debate as to whether BE is protective or detrimental, in that it can induce effects like apoptosis as well as increased mutational frequency. Although most commonly observed *in vitro* the BE has also been observed *in vivo* (Lorimore, McIlrath et al. 2005).

2.2 Mechanisms/processes proposed to explain GI and BE

2.2.0 Introduction

To understand the possible impact of the non-targeted or epigenetic effects on the health of the Chernobyl populations over several generations one needs to understand the mechanisms or processes underlying these effects and their origins. It is especially important in this respect to distinguish between *causal* and *consequential* events. Epigenetics is about regulation and process and as such "markers" of events of significance in terms of causality are less obvious with current research technologies than those for genetic events, namely mutations. For example, chromatin marking is widely regarded as causal in terms of the epigenetic phenomenon, imprinting. An extension to this is the proposal that such marking underlies cell regulation (Jaenisch and Bird 2003). However, unlike proposals for regulation by genetic regulatory networks (GRNs), where the genomic DNA sequence provides the information for regulation (albeit in a potentially flawed way as described below) no proposals for the origin of the information that dictates marking, other than the genetic code, have been put forward. Without that "origin" it is impossible to say whether marking is causal or consequential, i.e., a product of some other unidentified regulatory process. The above are points to be borne in mind in what follows.

2.2.1 GI in somatic cells

Extra cellular signalling between the cells through the microenvironment is hypothesized to be the cause of and support for the persistence of, both GI and BE (Barcellos-Hoff and Brooks 2001) and ultimately the appearance of cancer in the affected tissues. This hypothesis gives a major regulatory role to the extra-cellular matrix (ECM) (Bissell 1981; Bissell and Barcellos-Hoff 1987). In accordance with the stem cell niche theory and experimental data, the disruption of communication between cells in the tissue or with the ECM, removes the limiting factors for cell proliferation and malignant transformation (Gordon, Dowding et al. 1987; Lochter, Galosy et al. 1997; Whetton and Graham 1999). Cells in tissues tend to be more quiescent than single cells or those cultured *in vitro* (Soto and Sonnenschein 2004) and this is attributed to inhibition of proliferation by the tissue. Thus, alterations in stroma signalling

resulting from the presence of GI cells and the BE can be expected to have major effects on cancer progression in stromal tissues (Wiseman and Werb 2002; Prehn 2005). In this context radiation can then be seen as a non-specific stress factor altering the signalling between cells. For example, *in vivo* studies showed changes in ECM of irradiated murine mammary gland as well as the activation of the transforming growth factor β (TGF β) (Barcellos-Hoff 1993). This signalling molecule regulates cell differentiation, apoptosis, cell growth, and chemotaxis (Roberts, Flanders et al. 1988; Massague, Blain et al. 2000). TGF β is postulated to be an important molecule for mediating the effects of radiation exposure in the ECM (Ehrhart, Segarini et al. 1997), and is suggested as a central mediator of abnormal extra-cellular signalling resulting from radiation-damage. The authors recognise that tissue function is greater than the sum of its parts and they envisage radiation-induced GI closely related to carcinogenesis seen as a "two-compartment" problem, encompassing genomic sequence damage and damage to cellular communication (Barcellos-Hoff and Nguyen 2009). They assume that cellular phenotype is *dictated* and *regulated* by tissue microenvironment (Spencer, Xu et al. 2010)

In the context of the above hypothesis a leading role for centrosome aberrations in the triggering and inheritance of chromosomal instability is proposed based on *in vitro* studies on normal human mammary epithelial cells (Maxwell, Fleisch et al. 2008). The centrosome is an organelle hypothesised to be responsible for organising the microtubule cytoskeleton of animal cells (Bornens 2007). However, it is not essential for this purpose as microtubules self-organise (Karsenti 2008). In this work the induction of GI, manifested as aberrant karyotypes, had a threshold > 10 cGy (Maxwell, Fleisch et al. 2008). The centrosome amplification is associated with aneuploidy and cell death in many cancers due to spindle multi-polarity. Such spindles cause unequal distribution of chromosomes to daughter cells (Sluder and Nordberg 2004). Centrosome deregulation can be silent in the first generation of radiation-treated cells, but have detrimental effects in cell progeny by unidentified mechanisms. Such un-repaired centrosome aberrations, if they do not trigger the TGFβ-mediated cell death, are posited to cause persisting GI over generations (Maxwell, Fleisch et al. 2008).

Another theoretical approach to understanding the origins of the radiation-induced GI is on the borderline between targeted and non-targeted effects and is based on telomere damage as a causal event. Telomeres, are the end-capping DNA-protein complexes on chromosomes, protecting them from degradation and fusion (Raynaud, Sabatier et al. 2008). Damaged telomeres are problematic regions for repair by non-homologous end joining or homologous recombination pathways and their maintenance by telomerases is limited to normal germ cells, embryonic cells and abnormal somatic cells as a stage in carcinogenesis (Murnane 2006) and in stem cells (Ju and Rudolph 2006). Telomere loss causes a series of the breakage/fusion/bridge (bfb) cycles resulting in DNA amplification and large deletions (Murnane 2006). It is proposed that such radiation-induced damage of telomeres can be the causal event for the unmasking of primary recessive genomic damage accumulated in previous cell generations and the cell's own history, through chromosome imbalance or loss of heterozygosity (Ayouaz, Raynaud et al. 2008). In addition, radiation-damaged or mutant repair and maintenance pathways will indirectly contribute to the telomere shortening and the effects described and reviewed in (Ayouaz, Raynaud et al. 2008). The short term effects of

telomere region damage can be proliferative cell death, and the long-term effect can be positive pressure for clonal selection of cells which bypass the telomere-based mechanism of cell proliferation control, due to telomerase reactivation or other mechanisms, and immortalization of the cells, as a part of oncogenic transformation. In addition to telomere-telomere fusions, telomere-DSB fusions may contribute to the mechanism described above (reviewed in (Bailey, Williams et al. 2007)), generating also free unfused DSBs which can propagate and damage multiple chromosomes very much in the way the free radical reactions propagate the chain damage through the unpaired electron transfer.

An association between free-radical mediated processes and GI has been proposed and extensively studied (reviewed in Lorimore, Coates et al. 2003; Barcellos-Hoff, Park et al. 2005). Triggered by ionising radiation, oxidative stress and inflammation in tissues are tightly bound to the production of the reactive oxygen (ROS) and nitrogen (NS) species by the affected cells. Moreover, persistent inflammation is associated with an increase in DNA mutations and even induction of cancer (Coussens and Werb 2002; Lorimore, Coates et al. 2003). *In vitro* studies of haematopoietic and CHO cells showed that DNA damage, cell membrane damage and apoptosis or necrosis were due to the increase of ROS in the cell cultures (Clutton, Townsend et al. 1996; Limoli, Hartmann et al. 1998; Limoli, Kaplan et al. 2001; Limoli, Giedzinski et al. 2003). Mitochondria, which produce high concentrations of ROS as a part of normal metabolic processes are proposed as the origin of chronic oxidative stress also associated with unbalanced respiration processes, reduced activity of superoxide dismutase, or mutations in succinate dehydrogenase genes (Samper, Nicholls et al. 2003; Kim, Chandrasekaran et al. 2006; Kim, Fiskum et al. 2006; Wright 2010).

Modification of DNA and chromatin through methylation and acetylation may serve in the long-term to modify directly or indirectly transcription leading to changes in the cellular phenotype. GI has been associated with global DNA hypo-methylation in irradiated somatic tissues (Pogribny, Raiche et al. 2004; Raiche, Rodriguez-Juarez et al. 2004). This is proposed to be linked to activation of specific transposons, chromosomal aberrations, and an increase in mutation rates (Kovalchuk and Baulch 2008). Moreover, alterations in DNA methylation influencing protein synthesis patterns are characteristic of cancer cells (Baylin 2005; Baylin and Ohm 2006). Widely used as a marker of DNA double strand breaks, phosphorylated histone H2AX, is an example of histone modification mediating changes in transcription. Acetylation, methylation and ubiquitination are other histone modifications relevant to transcriptional regulation (Jenuwein and Allis 2001).

A recent extensive review (Aypar, Morgan et al. 2010) of the potential role that chromatin marking, chromatin reorganisation and miRNAs, which all have been found to be influenced by exposure to radiation, supports the idea that these epigenetic mechanisms may all be involved in genomic instability although the mechanisms “remain unclear”.

2.2.2 BE in somatic cells

In terms of the BE ROS- and RNS-mediated oxidative metabolism in irradiated cells, leading to inflammation in the surrounding tissues and cytokine signalling as a result of radiation damage, promotes the generation of secondary ROS in cells affected by bystander signalling. This is observed both in medium-transfer experiments and in tissue studies *in vitro* (Mothersill and Seymour 1998; Mothersill and Seymour 1998; Lyng, Seymour et al. 2000). These effects are transmitted by soluble humoral factors excreted by the irradiated cell possibly through low molecular weight signalling molecules via gap junction proteins in the tissues (Azzam, de Toledo et al. 1998; Narayanan, LaRue et al. 1999; Iyer, Lehnert et al. 2000; Iyer and Lehnert 2002; Lehnert and Iyer 2002; Shao, Furusawa et al. 2003). The search for the proteins or other factors inducing oxidative metabolism and subsequent GI in the progeny of the irradiated cells and in distant or surrounding tissues is in progress. This factor(s) must have low molecular weight (1000–10,000 Da) and its/their production involves lipid peroxidation and oxidative stress pathways (Emerit 1994; Mothersill and Seymour 2001).

Based on numerous studies a unifying model of radiation-induced BE based on cytokine and ROS mediators is proposed by Hei et al.(Figure 1) (Hei, Zhou et al. 2008). Very recently the authors of the model updated it to include the IL-33 – regulated blockage of NF-kB pathways and IGF-1-receptor kinase into this picture of bystander pathways (Ivanov, Zhou et al. 2010).

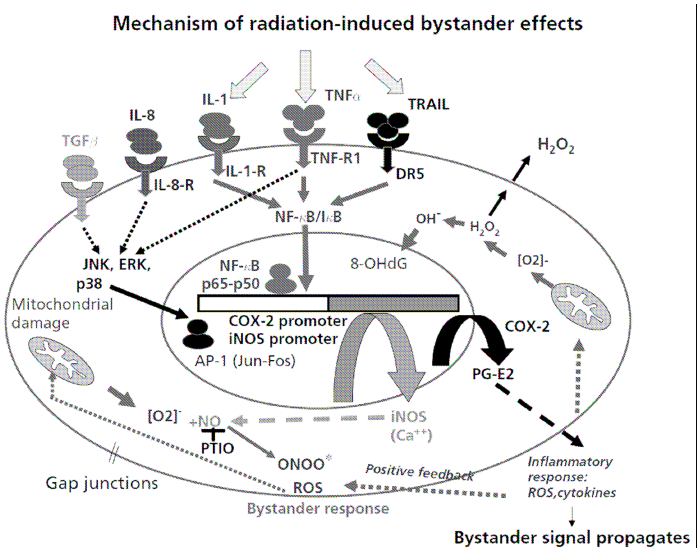

Figure 1.

The mechanism of radiation-induced bystander effects as proposed by Hei et al. Expression/secretion of the inflammatory cytokines strongly increased after exposure to ionizing radiation or oxidants. Secreted or membrane-associated forms of cytokines such as tumour necrosis factor (TNF)*a* activate I*k*B kinase (IKK)-mediated phosphorylation of I*k*B, which releases nuclear factor (NF)-*k*B. NF-*k*B enters the nucleus and acts as a transcription factor for cyclooxygense-2 (*COX-2*) and inducible nitric oxide (NO) synthase (*iNOS*) genes. TNF*a* also activates mitogenactivated protein kinase (MAPK) pathways (extracellular signal-related kinase (ERK), c-Jun N-terminal kinase (JNK) and p38) that, via the activation protein

(AP)-1 transcription factor, additionally up-regulate expression of *COX-2* (Zhou, Ivanov et al. 2005) and *iNOS,* which stimulates production of NO. Mitochondrial damage facilities the production of hydrogen peroxide, which migrates freely across plasma membranes and is subjected to antioxidant removal. Activation of COX-2 provides a continuous supply of reactive radicals and cytokines for the propagation of bystander signals through either gap junctions or medium. H2O2, hydrogen peroxide; IL, interleukin; $OH^{\cdot}$, hydroxyl radicals; $ONOO^{\cdot}$, peroxynitrite anions; PTIO, 2-4-(-carboxyphenyl-4,4,5,5 tetramethylimidazoline-1-oxyl-3-oxide (an NO scavenger); -R, receptor; PG-E2, prostaglandin E2; ROS, reactive oxygen species; TGF, transforming growth factor, TNF, tumour necrosis factor; TRAIL, TNF-related apoptosis-inducing ligand (original figure from (Hei, Zhou et al. 2008)).

Other studies confirm that primary radiation-induced ROS and as well as TGF-β1 and interleukins were shown to be involved in perpetuating bystander effects (Iyer, Lehnert et al. 2000). BE may reveal itself not only in endpoints typical for GI, e.g. apoptotic death, chromosomal aberrations, but also in such effects as premature cell differentiation (Belyakov, Folkard et al. 2002; Belyakov, Folkard et al. 2006) or even radioresistence through NO-signalling (Matsumoto, Hayashi et al. 2001). There is discussion as to whether bystander effects have a protective or a harmful role in the cell colonies or tissues neighbouring the initially radiation-damaged cell (Belyakov, Folkard et al. 2002; Mothersill, Moriarty et al. 2005).

The possibility that miRNA is involved in the BE is raised by indications of alterations in miRNAome in bystander rat spleen. These show that the expression of miR-194, which targets both DNA methyltransferase-3a and MeCP2, was altered in the bystander spleen cells (Koturbash, Boyko et al. 2007). However, no other experiments have been conducted to measure perturbations in the inherited miRNA profile of genomically unstable cells to our knowledge.

Compared to GI there is relatively good agreement on the processes underlying the BE albeit that the chemical identity of all the agents involved remains to be determined. However, the question arises as to whether BE is a response to the induction of GI in a neighbouring cell. Arguments have been made that the two effects are related (Lorimore, Coates et al. 2003; Morgan 2003; Morgan and Sowa 2007). Wright notes that the bystander response from an irradiated cell seems to act both directly and in a delayed manner (similar to GI) on a neighbouring cell and that GI cells can also initiate the BE. (Lorimore and Wright 2003)

2.2.3 GI in germ cells

Dubrova et al. argue that much evidence points to the non-Mendelian inheritance of mini-satellite mutations in humans and ESTR mutations in mice. The small size of the repeat sequences that are mutated, the frequency of mutation observed and the persistence of these effects are taken to indicate an epigenetic nature and identity with the phenomenon of GI (reviewed in Dubrova 2006). The clearest illustration of such epigenetic effects are the experimentally determined mutation rates of ESTR in mice (Barber, Plumb et al. 2002; Barber, Hickenbotham et al. 2006; Barber, Hardwick et al. 2009). However, very similar results have been seen in the mutation of mini-satellite sequences in humans (Dubrova, Nesterov et al. 1996; Dubrova, Nesterov et al. 1997; Dubrova, Bersimbaev et al. 2002; Dubrova, Grant et al. 2002) which are increased in the germline of the irradiated males. Furthermore, paternal exposure of mice to ethylnitrosourea (ENU), a mutagen that acts through base damage rather than strand breakage, results in ESTR mutation in the offspring (Dubrova, Hickenbotham et al. 2008). Similar results are obtained for cyclophosphamide that

forms adducts with guanine leading crosslinks and base substitutions (presented at NOTE Workshop on 14 June 2010). The ESTR mutations are thought to be triggered by, for example, replication slippage in repair processes (Bouffler, Bridges et al. 2006). Dubrova is categorical on the point that the "memory" of irradiated cells (organisms) is epigenetically transmitted to their offspring. He rejects the ideas that this memory is carried in the sperm by free radicals or RNA species. He believes the most plausible mechanism involves chromatin marking by methylation (Dubrova 2006).

Several authors have drawn attention to the level of expression of several genes, for example Tpr53 or protein kinases which have been shown to be altered in the progeny of irradiated cells (Baulch, Raabe et al. 2001; Baulch and Raabe 2005). The inherited epigenetic signals that can mediate such effects are RNA silencing, histone modifications and DNA methylation (Jaenisch and Bird 2003). *S*tudies of the progeny of irradiated mice (paternal and combined paternal/maternal exposure) show a methylation loss in several organs of the offspring as well as the changes in DNA methyltransferase levels, and methyl-binding protein MeCP2 (Koturbash, Rugo et al. 2006; Kovalchuk and Baulch 2008). F1 offspring of irradiated mice in other work showed an increase in the levels of H2AX phosphorylation (Barber, Hickenbotham et al. 2006). Further studies suggested that this increase was associated with elevated transgenerational DNA breakage rates. However, a transgenerational increase in mutations in ESTR loci was much higher than the expected alkylation damage from the ENU in another work (Vilarino-Guell, Smith et al. 2003); therefore, the mechanisms other than direct DNA damage may lead to accumulation of the ESTR mutations if the radiation is the damaging factor.

2.2.4 GI in somatic and germ cells

All the above proposals associate the origin of GI and BE with molecular change or damage. However, a defining feature of GI is that the molecular DNA damage appears in only some of the progeny of an irradiated cell in a non-clonal way and is very variable in terms of outcome. It might be concluded from this that the cause of GI is unlikely to be found by studying the intervening molecular processes. This consideration gave rise to the idea that GI is caused by the mis-regulation of the cell, that is, a failure to correctly interpret the genotype in terms of phenotype (Baverstock 2000; Baverstock 2008; Baverstock 2010). This proposal, which claims to constitute a new paradigm for radiobiology, is based on modelling the cell as a dynamical system capable of making phenotypic transitions independently of the specific nature of the damage inflicted on the DNA or other molecules. Essentially, effects (phenotypic transitions) result from stress on the processes that manage the cell cycle and the recognition and repair of damage to the genomic DNA. If these processes are overloaded phenotypic transitions akin to those involved in development and differentiation (i.e., without modification of the genotype) are triggered stochastically; the resultant phenotypes are GI phenotypes.

To summarize, the following proposals for the origin and nature of radiation-induced GI have been reviewed:

1. GI is the consequence of the tissue dysregulation with the ECM serving as the long-term damage "memory" and as the primary regulator of cellular function.
2. Related to the above is damage to centrosomes leading to the delayed effects such as spindle multi-polarity followed by aneuploidy and chromosomal instability.
3. The induction of inflammation in tissue by radiation results in raised levels of ROS leading to the GI state and these are sustained as a result of the inheritance of damaged mtDNA.
4. Telomere damage by radiation unmasks large fractions of the genetic damage accumulated during the history of the cell life due to the loss of heterozygosity and the chromosomal imbalance.
5. Chromatin marking (DNA methylation and histone acetylation), leads to altered protein expression patterns and GI and BE both *in vivo* and *in vitro*.
6. Changes in the organisation and the stability of the cellular dynamic interaction network not contingent to any specific molecular damage are proposed to explain GI and its transgenerational inheritance.

And for the bystander effect:

1. Aberrant signalling by damaged or GI cells through secreted factors into the ECM or through gap junctions between neighbouring cells to purposefully initiate a response to cellular damage.
2. The same mechanism but as a purely stochastic response to cellular damage through aberrant inflammatory signalling.

2.3 Evaluation of the Evidence and Conclusion

The phenomena of GI and BE have been clearly defined and delineated by extensive experimental work since 1992 and they allow us to draw certain conclusions as to the origins of the effects. For GI these are:

a) Target size estimations for radiation-induced GI show that the whole nucleus and/or cytoplasm, a volume much greater than the size of a specific gene, must be affected, suggesting this effect is based on a generic response to radiation (Baverstock 2000; Morgan 2003).

b) The appearance of GI in somatic cells of the offspring of irradiated fathers, who acquire the ESTR mutations in the germ cells, indicates that instability measured as ESTR mutations is related to somatic cell GI (Barber, Hickenbotham et al. 2006).
c) The unspecific nature of DNA damage as well as the detection of DNA damage in only a fraction of the cells at any period after irradiation is an indication that the inheritance of GI does not involve classical DNA-mediated Mendelean mechanism.
d) Different environmental agents including bacteria, can trigger similar systemic GI responses (Coen, Mothersill et al. 2001; Dubrova, Hickenbotham et al. 2008; Cuevas-Ramos, C et al.).

And for the Bystander Effect:

a) The BE can be seen as resulting from aberrant signalling between cells.
b) The similarity between effects seen in GI cells and those seen in BE cells is a strong indication that the two effects are related.

2.3.1 Genomic instability

The above list is used to evaluate each of the 6 proposals listed at the end of the previous section.

Point a) concerning target size eliminates proposal 2) concerning the role of the centrosome and proposal 4) regarding telomere damage on the grounds that these are small targets in physical terms, much smaller than the minimum target stipulated by target theory. As has been pointed out (Baverstock 2000) target theory is made much more complicated by repair since the effect of repair is to apparently reduce the measured physical size of the target. However, this means that for any measured target, where repair might be active, target theory will underestimate, i.e., give a minimum target size, not overestimate, the real physical target size. Moreover, proposal 4) is suitable for explaining only one aspect of GI, namely chromosomal instability.

Point b) above postulates that the GI observed in somatic cells *in vitro* and *in vivo* is the same phenomenon as the mini-satellite and ESTR mutations seen in transgenerational studies, where parental spermatogonial cells are irradiated. The key experiment here (Barber, Hickenbotham et al. 2006) indicates that offspring of irradiated mice carrying ESTR mutations in their germ cells exhibit features of somatic cell GI. For example, the mutational rate in the *hprt* locus is increased although it is inherited from the female, non-irradiated, partner. Thus, we assume that GI inherited at fusion to form the zygote is identical to GI inherited at mitosis. This characteristic serves to eliminate proposal 3) where the inheritance of GI relies on the

persistent generation of ROS from the mitochondria. Although sperm carry a small cytoplasm with minimal mtDNA this does not as a rule penetrate the zygote. As noted by Morgan (Morgan 2003) "*While secreted factors may explain radiation-induced genomic instability, bystander effects, death-inducing effects and clastogenic factors, it is difficult to imagine a scenario whereby a secreted factor could influence the reported transgenerational effects. It is unlikely that the radiation directly damages the expanded simple tandem repeats themselves or that the negligible cytoplasmic component of the mature sperm could carry a secreted factor or other radiation-induced species into the egg during fertilization*."

Remaining after these eliminations are three proposals essentially based on regulatory aspects of the cell. This is perhaps not surprising as point c) above can be taken to imply that the inheritance of GI is not contingent on the inheritance of the genotype. That a cell can apparently divide initially with no apparent damage to its DNA but exhibit radiation related damage *de novo* in later generations rules out all mechanisms based on the DNA transmission of the critical damage. This is an extremely important point where GI is invoked as a precursor to disease such as cancer. If GI is the causal event then subsequent DNA damage associated with the disease, characteristic specific chromosomal aberrations, for example, must be consequential rather than causal. Proposal 4) would therefore also be eliminated on this criterion.

These three proposals based essentially on regulatory properties of the cell, namely 1), 5), and 6) all satisfy point d) above in that there is nothing specific in the proposed mechanisms to imply that they would apply only to effects induced by radiation. DNA damage may be involved but the effects are not confined to a specific type of damage but effects induced by irradiation of the cytoplasm indicate that other damage can also be effective.

Regulation implies information and it is therefore pertinent to examine for each proposal the origin of that information. The default assumption concerning cell regulation is that based on the work of Monod and Jacob in 1961 (Monod and Jacob 1961). Essentially there are two classes of protein produced in cells, namely regulatory proteins (transcription factors TFs) and functional proteins, regulated by the action of the TFs. This concept has been elaborated into the framework of a genetic regulatory network (GRN) (Babu, Luscombe et al. 2004) and finds it most sophisticated account by Huang (Huang 2009). It has been argued (Baverstock, submitted for publication; 2010) that this model, implying as it does, a single source of information to regulate, fabricate and make functional, the cell, is a logically flawed proposal because it implies self-reference or impredicativity. However, all three of the candidate proposals invoke regulation ostensibly stemming from other than genotypic information.

Proposal 1) identifies the ECM as a regulatory agent. This idea, first floated by Mina Bissell in 1982 (Bissell, Hall et al. 1982), has most recently been elaborated by Spencer and colleagues

(Spencer, Xu et al. 2010). The ECM is alleged to “dictate cell phenotype and tissue structure”. How information to achieve this regulatory function is encoded in the ECM is not stated. The ECM is, of course, as is the case for any feature of an organism, the product of information coded in the cell. For the ECM to have regulatory control of the genotype, without a further source of information, invokes self-referral: how can the product of something regulate that same thing? In this latest publication a critical regulatory role for laminin, a basal membrane protein, is invoked on the grounds that it can induce, through interaction with the nucleus and cytoskeleton, differentiation in local cells. Laminin is a product of cells and thus, in the prevailing dogma, is the product of the GRN. For laminin to then regulate transcription in local cells independently of the genotype of those cells is impossible unless a further source of information, independent of the genotype is invoked. This is not the case for this proposal so it has to be eliminated until this point is resolved.

Proposal 5) invokes the marking of DNA and chromatin by methyl and acetyl groups respectively and pools of miRNA as regulatory mechanisms that when aberrant can lead to GI. However, the outstanding question of where the information to determine the DNA methylation, histone modification, and miRNA patterns derive has to be answered. Methylation of DNA in the germ cell undergoes extensive, although not full, removal and reinstatement of the marking pattern before and after the formation of the zygote (Santos and Dean 2004; Krawetz 2005; Rousseaux, Caron et al. 2005). No source of information (other than the genotype) has been advanced to explain how the correct reinstatement is achieved. Basically the information source that determines the correct reprogramming is not known and until it is this proposal lacks credibility. Furthermore, in a wider context Huang argues (Huang 2009) that marking has neither the locus specificity nor the stability to be the cell regulatory mechanism. This view has been recently reinforced (Deal, Henikoff et al. 2010). The initiation step of transcription resulting in the introduction of a gene product into the nucleus has been intensively studied and has been found to depend on several seemingly unrelated factors including location in the nucleus, state of chromatin in the coding sequence, position of nucleosomes in relation to transcription initiation sites, and the expression of transcription factors (Cremer, von Hase et al. 2001; Cremer and Cremer 2001; Chen and Rajewsky 2007). Chromatin marking is thus but one feature of the control of transcription. This proposal has to be eliminated.

Proposal 6) on the other hand does invoke a source of information that is independent of the genotype, namely the “state” of the cell in dynamic terms. The immediate products of transcription are, at least in higher cells, stored in inactive forms prior to activation as functional gene products. Under this proposal regulation of transcription is regarded as necessary but not sufficient to account for overall cell regulation. This, it is argued (Baverstock and Rönkkö 2008), takes place at the post-translational level and involves the dynamic interaction of the active gene products through self-organisation. This proposal will

be examined in greater detail and in the context of the other cell regulatory proposals in the next Chapter.

2.3.2 Bystander Effect

There is no dispute that the BE is a chemically mediated process and many potential molecular mechanisms have been proposed. However, of more interest is the question of why such a process exists. Is it as some have proposed a “distress signal” from a damaged cell, an attempt to protect the tissue (proposal 1 above), or a purely stochastic response to a phenotypic change induced by radiation that affects the cell’s ability to correctly communicate with neighbouring cells (proposal 2 above)?

Proposal 1) appears to endow the cell with “intelligence” so again this issue boils down to one of information. Aberrant signalling is essentially the loss of information whereas purposeful signalling implies the acquisition of information. This proposal is thus only credible when the source of the intelligence is understood.

Proposal 2) is supported by the observation that an immediately irradiated cell and a late developing GI cell are both capable of initiating the BE (E. Wright, NOTE Workshop 14 June 2010) indicating a strong connection between BE and GI and indicating that the BE is most likely to be due to aberrant signalling as a stochastic response to radiation exposure. Within the generic regulatory response process posited to underlie GI in proposal 6) the BE can be accommodated.

2.4 Conclusions.

Traditionally radiobiology has taken a “molecular target” oriented approach to understanding the effects of exposure of cells to radiation; GI and BE both presented a challenge to the prevailing paradigm in 1992 for sound mechanistic reasons based on target theory. The ensuing nearly 20 years has established these effects to be more than experimental artefacts. During that time extensive, maybe exhaustive, research efforts to place these effects into a molecular paradigm have failed, leaving as the only recourse regulatory mechanisms/processes.

Increasingly, evidence from the wider field of biology is indicating that the Central Dogma based “genetic” paradigm fails to account for observations (see for example (Anway, Memon et al. 2006; Anway and Skinner 2006; Barrick, Yu et al. 2009; Curley, Davidson et al. 2009; Yus, Maier et al. 2009). These would seem to indicate a need for a paradigm shift in biology in general. However, proposal 6) for the basis of the origin and inheritance of GI goes further

and proposes a paradigm shift from the Newtonian-Cartesian paradigm for the physics that underpins biology. This will be discussed in the next Chapter.

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Chapter 3

A model for epigenetic effects in radiobiology and its application to the Chernobyl accident

3.0 Introduction

When confronted with a radiation exposure situation one of the first responsible actions is to assess the heath risk to the exposed population. Many did this in 1986 and in subsequent quinquennial anniversaries of the accident and the estimates varied considerably even when improved estimates of the doses became available. It is clear that in some cases these estimates have not been based purely on science; politics has also played a role. For example, one argument that has been deployed is that there is no evidence of any ill health arising from doses of less than 100mGy and since few individuals exposed after Chernobyl received doses greater than 100mGy the public health implications of the accident were marginal. Similar arguments reappeared in 2006 where the WHO/IAEA discounted doses below a certain threshold as non-lethal in terms of cancer many years hence (Peplow 2006). In this they violated their own "Basic Safety Standards" (IAEA 1996) which prescribe LNT as the basis for radiation health effects. Science has also been misleading. For example, though it was recognised from the outset that releases of ^{131}I were substantial and therefore that thyroid doses would be increased, it was believed, on the basis of epidemiological studies of ^{131}I exposed patients that this isotope was not carcinogenic and therefore increases in thyroid cancer were expected to be marginal. This of course turned out to be wrong mainly because children are much more sensitive to irradiation of the thyroid and the epidemiological studies included few children. This matter was resolved by a meta-analysis of thyroid cancer in externally exposed populations which demonstrated the much higher sensitivity of children (Ron, Lubin et al. 1995). What we understand and what we think we understand, about the theoretical underpinning of the health effects of radiation are crucial in risk assessment and therefore risk management, which includes future research.

Chapter 1 of this report explains how understanding of the effects of radiation in the context of radiological protection has evolved, especially since 1986, and why it is therefore appropriate to be generating a strategic research agenda nearly 25 years after the accident to review relevant features of the accident in the light of new knowledge to assess where health risk might arise. Arguably the most significant development since 1986 has been the uncovering of genomic instability (GI) (Kadhim, Macdonald et al. 1992) and the bystander effect (BE) (Nagasawa and Little 1992). They are particularly significant because they cannot be accommodated under the classical dogma (Baverstock and Belyakov 2005). Therefore this review focuses to a large extent on this aspect of radiobiology. However, in addition evidence

has accrued to indicate that the idea that radiation only acts to initiate cancer maybe too simplistic and the more recent ideas on this subject are discussed.

In Chapter 2 the competing hypotheses to explain GI and BE are described and evaluated, GI being regarded as the more important of the two effects. While numerous molecular manifestations of these effects have been observed and claimed to be causal, judged against a set of criteria based upon the empirical properties of GI (Chapter 2.3) all are eliminated leaving only three hypotheses based on modified cell regulation as the origin of GI: i.e., focussing not on the *material* aspects of the cell but on the *process* aspects. One of the three regulatory hypotheses, namely regulation by the extra cellular matrix has been eliminated because as it stands it has a logical inconsistency. This could be resolved by identifying, if it exists, the origin of the information that the ECM uses to over-ride the genotype. Another, regulation by chromatin marking, is eliminated on the grounds that marking is unstable (Mellor 2006; Trojer and Reinberg 2006; Deal, Henikoff et al. 2010), that it is non-specific (Huang 2009) and because the origin of the information that determines the sites of marking is not known. The third hypothesis, the independent attractor model, proposes regulation from the "state" of the system (cell) in terms of its dynamics of gene product interaction. It has been argued that, in this way, it is possible to achieve some measure of unification of GI and BE at the cellular level (Baverstock 2010) and of their impact at the tissue level in terms of hereditary disease, carcinogenesis and non-cancer disease (Baverstock and Karotki). The full implications of this proposal have still to be explored, partly because the proposal does not fit neatly into the prevailing dogma that has persisted over 50 years. It is, therefore, elaborated in this chapter in more detail in order that it may be judged against the prevailing dogma.

At its most fundamental level the so called "independent attractor" hypothesis involves a change in the metaphor from that which has dominated biology since Descartes, namely the "machine" metaphor, to a dynamic metaphor such as a "whirlpool" or "the weather", where the stable states of the system (the cell) are transitory and change in response to environmental stimuli without genetic changes, that is, sequence changes in the genotype. In essence the physics that this hypothesis assumes as the underpinning of biology is the physics of *complex dynamical systems* and not that of *classical Newtonian dynamics*. This change impacts on biology in general and is in effect a paradigm change according to the definition of Kuhn (Kuhn 1970). However, the concepts are far from new and are explicated in Ludwig von Bertalanffy's book "General System Theory" (Bertalanffy 1969). This carries the implication that much of the conceptual framework that has been applied to biology in the past and especially over the last 50 years, is not applicable and probably accounts for the failure to find a mechanism for GI in nearly 20 years of research. One result that illustrates this point is in a recent publication (Barrick, Yu et al. 2009). Bacteria are grown for 20 years over 20,000 generations after the introduction to a stressful nutrient environment (reduced lactose) and assessed periodically for mutation rate, by genome wide sequencing, and adaptive fitness

relative to the founder population. While mutations increase linearly with the number of divisions (at the rate of ~2/1000 divisions) more than 80% of the increase in adaptive fitness occurs in the first 1000 divisions. The experiment therefore does not support the basic dogma that relative fitness will correlate with mutation rate. It is a basic tenet of conventional biology that genomic change underlies evolutionary adaptation. The authors reject Kimura's theory of neutral molecular evolution as an explanation. In the new approach described in herein the "state of system (the cell)" provides an independent source of information (in addition to genotype) that *informs* phenotype. Thus, under the model phenotypic change and mutation rate are only loosely, if at all, coupled. They will almost always be a result of the nonlinear interactions of the open dynamic system of the cell with the environment and its own DNA (see Chapter 3.2). Only by including this second source of information can a full picture of the operation of cell in terms of translating genotype into phenotype be understood. A useful analogy for this paradigm is a natural language which consists of a vocabulary (genotype) used in conjunction with a grammar (the independent attractor) to formulate meaning (phenotype). There are no natural languages that work solely on the basis of a vocabulary[3].

The new approach (see below) provides a potential basis for understanding the hereditary effects of radiation both in terms of classical Mendelian inheritance of sequence changes and the patrilineal inheritance of genomic instability, carcinogenesis and the causation of non-cancer disease (paper submitted for publication) mainly by including the second source of information as the primary regulator of the cell. It therefore subsumes the classical paradigm.

In addition, the absence in the prevailing dogma of any substantial consideration of radiation acting at other stages of carcinogenesis than initiation has obscured the potential for radiation to promote or accelerate the appearance of cancers it has initiated as well as spontaneous cancer latent in the population. As noted in Chapter 2 post mortem examination of victims of trauma shows the presence of pre-cancerous lesions or *in situ* tumours (Folkman and Kalluri 2004). On the basis of a random autopsy study (Harach, Franssila et al. 1985) Harach reports occult micro-cancers as being "normal" in Finnish adults affecting on average about 35% of the population. Welch and Black note the danger of over-diagnosis of cancer caused by the detection in screening programmes of "silent" disease (Welch and Black 2010). It has been proposed that *in situ* tumours are constrained in their development by surrounding very slowly dividing cells, termed "cancer stem cells" not to be confused with the normal stem cell (Enderling, Hlatky et al. 2009). The induction of GI in these cells could plausibly release the constraint and allow the development of a full blown tumour. Such a mechanism could be highly relevant to adult thyroid and breast cancer as both these tissues are known to harbour such pre-cancerous lesions by middle age. Further support for this idea comes from a recent publication that shows that the low doses in tissues surrounding a treated tumour act to

[3] To illustrate this point consider the following two sentences that contain exactly the same words: "*There were only two script writers and it is a pity one has died*" and "*There were two script writers and it is a pity only one has died*".

facilitate angiogenesis and thus promote an essential feature of tumour formation (Sofia Vala, Martins et al. 2010).

3.1 How the independent attractor approach accounts for non-targeted effects.

As stated earlier, in contrast to models based on chromosomal damage caused by radiation this model is based on the effects of radiation on the regulatory aspects of the cell. Radiobiology is about the impact of the environment, in this case ionising radiation, on the phenotype of the affected (either directly or indirectly) cells. In classical radiobiology this is assumed to take place by causing mutations and chromosomal aberrations in the genomic DNA, which modify the behaviour of the products (usually proteins) of coding sequences. In the independent attractor model phenotype is represented by an attractor state, the stability of which is contingent on the active gene products being maintained within certain ranges. This situation is formalised as a *relation* of the form applicable to each active gene products expressed in the cell:

$$\mathbf{m_{gpa}(t1) \in r_{gpa} \Rightarrow m_{gpb}(t2) \in r_{gpb}}$$

where **m** represents the activity of the gene products (gpa or gpb) and **r** the range of activity of the gene products (gpa or gpb) and time **t1** < **t2** (Baverstock and Rönkkö 2008). This can be translated as "when the activity m of gpa is in the range r_{gpa} at time **t1** the activity of gpb will be in the range r_{gpb} at a later time **t2**". For a given phenotype in a mammalian cell there would be a few thousand active gene products involved and each could have at least one and up to several tens of these relations with other active products. The remaining potential gene products (from coding in the genotype) are "silent". These relations are termed *rules of engagement* and they constitute information that is inherited at every cell division and at fusion and that regulates the cell. In a sense it could be said that the *state* of the cell contains information and this is independent of the coding sequence information in the genotype. Modification of the information coded in the relations (specifically in **r**) beyond certain limits (reflecting the robustness of the phenotype, which can be optimised by evolutionary conditioning) risks a phenotypic transition.

An important feature of cells is that major damage to the genomic DNA should be repaired before cell division and a number of strategies, such as cell cycle arrest and a battery of repair processes, have evolved to ensure, as far a possible, that this happens. Only where this is the case could stable species be sustained over many generations. Thus, damage to the genomic DNA potentially stresses the ability of the cell's transcriptional capacity to maintain the active gene products within their required ranges (**r**). It is hypothesised that genomic instability is the result of the violation of one or more relations appropriate to the established cell of a stable species, leading to an attractor transition. To illustrate this consider Figure 1 taken from (Baverstock and Rönkkö 2008), which is a cross section or

"slice" through two dimensions gpx and gpy of the state space[4] of a cell. The large circle H represents the attractor and its basin of attraction, which constitutes a domain in the state space other relevant dimensions not visible in this 2D slice) of a cell of a stable species. The *position* (coordinates x and y) is arrived at as the result of evolutionary conditioning; it is the optimum combination of active gene products for replicating the genotype within the allowed states and is contingent on the global dynamics of the system. Movement to another position in the state space, $V_{1,}$ for example, due to the violation of one or more gene product ranges (**r**) within the system, represented by vector P (not necessarily involving either gpx or gpy) entails a less optimum replicative ability. The *diameter* of the circle represents the robustness of the attractor, which is to a degree reflected in the range of gene product values (**r**gp) and in the case of attractor H it also is optimised by evolutionary conditioning. However, variant attractor V_1 is not conditioned and is thus less robust and more easily perturbed and thus liable to be perturbed and to move to V_2, for example. The attractor/phenotype transition H to V_1 exemplifies the transition to genomic instability, with the properties of being a mutator phenotype (due to the less than optimal replicative capability) and more easily perturbed (less robust) to other variant attractors as demonstrated experimentally by Falt et al (Falt, Holmberg et al. 2003).

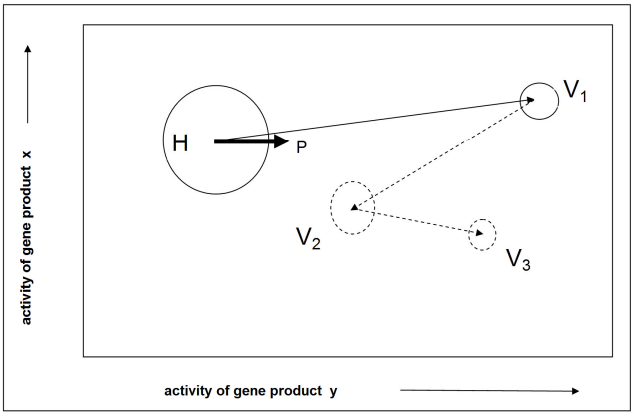

Figure 3.1
(See section above for explanation)

Thus, the impact of an environmental influence that stresses the normal processing of genomic DNA damage by the cellular processes can result in pushing an active gene product out of its appropriate range, leading to an attractor transition that does not involve any genetic

[4] State space is a conceptual tool in which the state (in this case the phenotype) of a complex dynamic system (in this case the cell) can be represented as a single point or in this case, domain. The state space is a virtual multidimensional space with a dimension for each active gene product. This, in the case of a human cell exhibiting a specific phenotype, means about 100,000 potential dimensions with a few thousand "active" (participating in the dynamics of the system), that is, at some point in the state space other than its origin (all dimensions set to zero activity). In all other respects (than its high dimensionality) state space can be equated to physical space (3 dimensions) where a single point defines uniquely a position in terms of coordinates on the x, y and z dimensions (axes).

change. The resultant cell at V_1, by virtue of its new position in the state space, that is, not the optimum position resulting from evolutionary conditioning (Baverstock and Rönkkö 2008), will be a mutator phenotype and will generate *consequential* genetic (i.e. sequence) changes in the genotype. The *cause* of this process is securely based in the initial transition that perturbed the attractor.

Phenotypic change in the absence of genetic change is the norm for cells in multi-cellular organisms and is the essence of development and differentiation. What is proposed above is simply the extension of this fundamental biological process to encompass a stochastic transition to a state (phenotype) that the system had not "anticipated" (has no pathway for). In the conventional paradigm for biology based on the work of Monod and Jacob (Monod and Jacob 1961) in 1961 and elaborated (Babu, Luscombe et al. 2004; Huang 2009) in terms of genetic regulatory networks, pathways that activate transcription factors to regulate functional proteins lead to phenotype. The necessary phenotypic transitions deployed in development and differentiation are "hardwired" into the genome through the network (Huang 2009). In such a paradigm there are no "unwired" states but the evidence from outside of radiobiology strongly contradicts this position (Kashiwagi, Urabe et al. 2006; Yus, Maier et al. 2009). In the independent attractor model regulation derives from the interaction of the active gene products, through self-organisation and although transcription is necessary it is not sufficient to fully regulate the cell.

Three features of the independent attractor model may be unfamiliar to the reader. The first is why and how are the relations governing the protein activity regarded as information, the second, how is that information inherited between cell generations and the third, why is it that transitions in the state space are discrete "jumps" rather than smooth and gradual.

At any given time the active interactions of the components in the cell constitute its "state" and where that is an attractor state, i.e. a stable dynamic state representing phenotype, the relations that give rise to it govern the system. In this sense the relations are information essentially stored in the network architecture and its dynamics. Although the relations are expressed in a material aspect of the system (the proteins) the information is not contained in the material (as in the case of the genetic code) but in the relations (interactions) between those material components. Therefore, the system state can be fully reproduced by replicating both genotype and the interaction network. For research purposes, the information on the steady dynamic states of the system can be extracted from the -ome analysis, especially proteome and interactome (Stumpf, Thorne et al. 2008), which however will represent only a snapshot of the real dynamic situation in the cell. These are self-organised states which reassemble spontaneously. A recent example is the synthesis of a bacterial genome in the laboratory by Venter et al (Gibson, Glass et al. 2010), which, when inserted into another bacterium resulted in cells capable of self-replication. As long as the process of

transcription could be initiated in the new bacterium[5], the transcription products, proteins, would be released and self-organisation would assemble a replicating attractor and therefore phenotype. In the context of the independent attractor model this experiment returns an unexceptional result.

The inheritance of the network encoded information at cell division, in contrast to the inheritance of the genotype, can be viewed as the partitioning of the "state" of a single cell, containing the operational components for two cells, into two separate cells. If the profile of proteins existing at the time of cell division (after replication of the genotype) is split into two compartments each containing a full set of the active cellular components two identical cells will result, each containing the inherited attractor encoding the information. In the case of transgenerational inheritance two attractors are inherited as pro-nuclei at fusion. The paternal pro-nucleus attractor is assumed to be maintained in the sperm by active gene products (mostly proteins) synthesised before maturity: the limited cytoplasmic component of the sperm is likely to be incapable of translation of new protein. In its initial state after fusion the zygote can be envisaged as containing two dynamical systems. If the two pronuclei derive from two stable parents they merge into one system. If one of the pronuclei is unstable they will synchronise into a single system based on the male pronucleus (according to the evidence (Barber, Hardwick et al. 2009)).

In the independent attractor model the underpinning physics differs from that underpinning the current dogma in so far as it is the physics of thermodynamically open dynamical systems (Bertalanffy 1969). In such systems stability is uniquely associated with attractor states (rather than equilibrium, the lowest available energy state) which in general have associated basins of attraction around them (see Figure 3.1) and in terms of stability the state space is "quantised" (Glendinning 1994). To move from one state to another requires overcoming the basin of attraction of the current attractor and finding another or variant attractor. The very presence of attractors in the state space imposes an architecture which proscribes stability between attractors. Thus, transitions cannot be smooth and gradual since attractors are finite and discrete regions of the state space. Most of the theoretically possible interactions between the cellular components will not result in a stable dynamic state (attractor), and thus will leave "prohibited" regions in the state space, which cannot be stably adopted by the system. Therefore, an attractor transition can involve the change in participation of the state of the system of several active gene products in a single step leading to abrupt changes in the functional capability of cells, i.e., a new phenotype that is stochastically determined and which in theory at least does not entail genetic changes.

[5] This was ensured by inserting the synthetic genome into a bacterium with an already functioning genome and selecting for the synthetic genome where upon the host bacterium eliminated the now "foreign" genome.

Due to the mutator phenotype character endowed by an attractor transition (Baverstock and Rönkkö 2008) it is pertinent to consider what role mutations (genetic changes) might have on these essentially epigenetic processes. Non-silent mutations in the genomic DNA coding sequences are conventionally assumed to affect the primary and other protein structures and generally lead to either gain or loss of function. In the independent attractor model mutations may affect the system in two ways. Firstly, mutations may impair the ability of the encoded protein to interact correctly with the proteins with which its precursor has relations. Secondly, mutations may influence the cell's ability to perform the functions it formerly performed. The former will result in the adoption of a variant attractor and thus a variant phenotype potentially, but not necessarily, involving the participation of the mutant protein. The latter will not cause an attractor transition but may change the phenotype in functional terms. Thus, there emerge two distinct ways in which mutations can initiate phenotypic changes. Firstly, *epigenetically* due to modification of the relations the mutated protein has with relevant other proteins, leading to an attractor transition and loss of genomic stability. Secondly, *genetically* due to modification of the functions of the mutated protein as they contribute to phenotype but potentially without loss of stability Of course the two can occur in combination. Thus, consequential mutations resulting from the mutator phenotype of the GI cell can stimulate further phenotypic changes. In this sense once the initiation of GI has taken place further stochastically determined transitions will follow (Falt, Holmberg et al. 2003), i.e., an irreversible train of unpredictable transitions has been set in motion. Selection will be the main influence over where it terminates.

3.2 How attractor transitions can result in health effects.

An attractor transition may involve changes in the participation in phenotype by several gene products in a single step – some gene products may be eliminated others that were not previously deployed, deployed. However, the phenotype is not a reflection (for the most part) of single gene products but the combined action of several (where a product has several relations with other products) and therefore the changes in phenotype are not "linearly" related to changes in gene product participation. This is well exemplified when some function of the cell is dependent on a complex of several proteins, for example the initiation of replication and transcription. The loss of the participation of one product will result in the loss of the others (because of the relations) and of the whole capability to perform the function undertaken by the complex. Phenotypic changes are thus "jumps" from one phenotype to another rather than a gradual change with incremental losses of function as dictated by the underlying physics.

Among the important functions that the cells of multi-cellular organisms need is to be able to signal to one another. This is exemplified by the niche concept which maintains, for example, that bone marrow stem cells are maintained in the resting state by signals from their

neighbouring cells. It is argued that the origin of the bystander effect is abnormal signalling from irradiated cells (Baverstock and Belyakov 2010) that have been destabilised (Lorimore and Wright 2003). Through this signalling, which stimulates processes such as proliferation and differentiation, tissue integrity is ensured (Barcellos-Hoff and Brooks 2001; Barcellos-Hoff 2008). Loss of the correct signalling functions by a participating cell is therefore a potential threat at the tissue level.

In somatic tissues two important outcomes of the loss of features of functionality contingent on the induction of instability can be identified. If the loss involves a loss of growth control, by, for example, eliminating cell cycle arrest, uncontrolled proliferation characteristic of malignancy can result. This process may entail (although not necessarily) the accumulation of sequence mutation damage due to the less than optimal replicative efficiency. Such consequential mutations and chromosomal damage may act to consolidate or "hardwire", the epigenetic changes that have accrued (Baverstock 2000; Prehn 2005). For this reason associations between specific mutations and translocations are observed and described as "characteristic" of particular cancers. These associations may arise due to the selective growth advantage some mutations may endow. In the case that the variant phenotype does not have a selective growth advantage it may lack a critical function, which, if it also has signalling deficiencies, can be passed to neighbouring cells resulting in a focus of abnormal tissue. Atherosclerosis may, for example, be triggered by a destabilised endothelial cell, the mal-function of which enables the penetration of the endothelial layer by inappropriate agents (Ross 1999), leading to build up of lipoproteins and local inflammation, and eventually myocardial infarction. Atherosclerotic plaque has been demonstrated to exhibit genomic instability (Andreassi and Botto 2003) as well as mutational damage (Benditt and Benditt 1973; Andreassi, Botto et al. 2000).

Studies in mice indicate that genomically unstable germ cells are transmitted down the germ line patrilinearly (Barber, Plumb et al. 2002; Barber, Hardwick et al. 2009). Explicitly, an unstable male mated to a normal female will produce unstable offspring, but the offspring of normal males mated to unstable females are normal. In this there is a clear distinction between this mode of inheritance and the Mendelian inheritance of classical sequence mutations (see Chapter 1). The explanation for this in the attractor model is to be found the physics of dynamical systems. The zygote inherits two attractors (pronuclei), normally dynamically synchronised, but in the case of the inheritance of a normal and a variant attractor, unsynchronised. Two dynamical systems sharing a common environment will synchronise (Yang 1999) and in this case the experimental evidence indicates that it is the female derived attractor that synchronises to the male. A famous historical example of dynamical synchronisation, dating back to the 1660s, is Huygens' clocks (Bennett, Scatz et al. 2002).

An important outstanding question is "is instability the basis for cancer as it is induced by radiation?" There is strongly indicative evidence that the SMT based essentially on a genetic mechanism is increasingly questioned as discussed in Chapter 1. The sharply divergent dose-rate responses of what we know to be a genetic mechanism (strong dependence shown in Figure 1.1), the inheritance of sequence mutations, and what we can derive from epidemiological observations for cancer (apparent independence[6] see Figure 1.2) might indicate that radiation induced cancer is causally related to epigenetic processes such as genomic instability. Such a conclusion is supported by long-standing evidence in favour of the argument that cancer is an adaptive process (Farber and Rubin 1991; Prehn 2005). These authors note that cancer development following exposure to carcinogens, including radiation, is a prolonged process evolving several proliferative stages, many of which are benign and in the case of liver exposed to chemical carcinogens, resistant to further environmentally induced damage during the early stages of the process. Proliferation of cells in tissue is normally strictly controlled so this is abnormal pre-neoplastic behaviour out of which neoplasms emerge. These ideas are supported by *in vitro* experiments where "cell transformation" can be induced (Kennedy, Fox et al. 1980). There is, thus, a *prima facie* case that cancer induced by radiation (especially at low doses, but see Appendix 3.1) is caused by an initial epigenetic event, namely the transition to genomic instability.

So far the discussion has concerned only initiation of disease but recent evidence has indicated that radiation may also act to promote cancer. For example, a number of relatively low dose and low dose-rate epidemiological studies have indicated linearity of dose response and returned a higher central estimate of risk than the survivors of the atomic bombings in Japan (see Chapter 1). Radiation could promote cancers in two ways, namely by accelerating the development of an already developing cancer or by activating a quiescent cancer.

Recent evidence suggests that endothelial cell migration in tissue in the relatively low dose region surrounding a cancer subject to radiation therapy is enhanced without an effect on survival or proliferation, promoting angiogenesis and the spread of metastasis (Sofia Vala, Martins et al. 2010). Evidence from a Swedish study of the effect of low doses (up to a few mSv) from fallout from the Chernobyl accident appear to show increases in solid cancer in the decade after the exposure in comparison to the unexposed population (Tondel, Hjalmarsson et al. 2004; Tondel, Lindgren et al. 2006). The short latency and low doses argue against this increase, if real, being due to the initiation of new cancers.

The post-mortem data on trauma victims showing high levels of precancerous lesions in many tissues (Harach, Franssila et al. 1985; Folkman and Kalluri 2004) supports the concept that induction of cancer is a common event but that normal tissue acts through dynamical interactions between cells, to contain any abnormal cellular activity, which is in line with the

[6] Linearity of dose dependence for malignancy implies also a lack of dose-rate dependence.

epigenetic initiation of lesions and cancer as an adaptive cellular process. Disruption of this "containment", potentially by radiation, could release the abnormal cells into growth and expansion (Enderling, Hlatky et al. 2009).

Thus, the potential of low doses at low dose rates to lead to increases in cancer by mechanisms in addition to those considered in the classical radiobiological paradigm, namely through promotion rather than initiation and through purely epigenetic rather than genetic process, have to be considered.

3.3 Implications of the in dependent attractor model for the "low dose" problem

The low dose problem is central to radiological protection and to evaluating the health impact of the Chernobyl accident. It concerns the ability of ionising radiation in the dose range up to 100mGy to cause health consequences. Low doses are "a problem" because direct measurement of risk by epidemiology is problematic and thus risk assessment must rely on extrapolation from measurements of risk at higher doses mediated by an understanding of the theoretical framework underpinning the action of radiation, namely the biological bases for radiation action. The independent attractor model is an attempt to provide such a basis.

Traditionally risk assessment for the late occurring stochastic effects of radiation has assumed that the initiating event is a relevant mutation of the DNA coding sequence. The radiation induced mutation of a specific locus in the genomic DNA occurs with frequencies between 1×10^{-6} and 1×10^{-5}/Gy/locus (for example; *hprt* mutations are induced in mice at ~3×10^{-6}/Gy (Barber, Hickenbotham et al. 2006). Thus, traditionally it is assumed that the initiation of health damaging events is a very rare occurrence. This is very much in line with the observed lifetime rates of, for example, radiation induced cancer in the atomic bomb survivors of ~10%/Gy for all cancers, given the number of cells that have been subject to exposure.

The independent attractor model proposes that the initiating event for all late stochastic effects (cancer and non-cancer disease) caused by radiation (but see Appendix 3.1 for discussion of the influence of dose) is the induction of genomic instability through a process that does not necessarily involve changes in the coding sequence and occurs at a rate considerably higher in relation to dose than does mutation induction which is off-set by protective tissue responses (see above).

The strongest evidence in favour of radiation acting through mutation is the specific locus data collected on mice. These data are the basis for radiological protection in terms of hereditary effects. Molecular genetics has established a direct relationship between specific mutations and human hereditary disease and although the mutations measured in the specific

locus data on mice do not relate to human disease the rate of induction of mutation being primarily a physical process they can confidently be “carried across” from mouse to man. As noted in Chapter 1 the mouse data exhibit a strong dependence on dose rate above 10 mGy/min implying that repair of damage is an important factor in the process (Russell, Russell et al. 1958) and can reduce the maximal rate of mutation induction by a factor ~3.6. The inheritance of these disorders is strictly Mendelian. This evidence supports the role of mutation in modifying cellular function without an underlying attractor transition.

One of the contributions to knowledge on the hereditary effects of radiation derived from the Chernobyl accident is the work of Dubrova et al on the inheritance of minisatellite mutations by the children of fathers exposed to the fallout (Dubrova, Nesterov et al. 1996; Dubrova, Nesterov et al. 1997; Dubrova, Plumb et al. 1998; Dubrova, Grant et al. 2002). These data are supported by mouse data on the inheritance of ESTR mutations by Barber et al. (Barber, Plumb et al. 2002; Barber, Hickenbotham et al. 2006; Barber, Hardwick et al. 2009). In contrast to the sequence mutations in mice the ESTR mutations are inherited patrilineally (Barber, Hardwick et al. 2009). It remains to be determined whether these mutations are sensitive to dose rate but the frequency of induction is much higher than for specific sequence mutations at high dose rates as would be expected from target theory.

However, for historical reasons it has been the “sequence mutation” model that has been “carried over” from the conventional hereditary effects to provide a mechanistic basis for radiation induced cancer at low doses. It has been argued in Chapter 1 and above, that that model, essentially the somatic mutation theory (SMT), is not strongly supported by evidence any longer and should be replaced by an epigenetically based theory which sees initiation of cancer as the induction of genomic instability mitigated by biological defences naturally derived from tissue properties (Farber and Rubin 1991; Soto and Sonnenschein 2004; Prehn 2005). The principal consequence of this step would be to emphasise the importance of individual sensitivity to the health detriment from low dose exposures, which may be very variable and depend on genetic background, lifestyle and the presence of other disease conditions etc.. In this context it is notable that the ease of induction of GI and BE by radiation is dependent on cell type. In other words this approach focuses greater attention on biology than the traditional, mainly physical, target theory based approach.

Several epidemiological and biological arguments have been cited as evidence in support of the SMT theory:

a) The presence of mutations in growth control genes in cancers, and the demonstration *in vitro* that cells with these mutated genes have a high growth rates: the accumulation of such mutations is a result of the selective growth advantage associated with cancer cells is as predicted by the attractor model and therefore cannot serve as an argument to support SMT.

b) The finding that drugs that interfere with gene products can slow tumour growth *in vivo* and *in vitro*: tumors accumulate multiple mutations some of which endow the cell with a growth advantage and are thus "selected". The use of the specific drugs blocking production or utilization of aberrant proteins, RNA or other biomolecules will change the cellular interactome (and thus the attractor) functionality. The discovery of such drugs does not move us closer to understanding of the triggering events for cancer.
c) The introduction of some mutated genes into mice lead to tumour formation: this issue is dealt with in footnote 5 on page 13.
d) Individuals with inherited mutations in DNA repair genes have a high tumour incidence: the processes underlying familial cancer might be quite different to those underlying radiation induced cancer.

In support of the traditional genetic approach to understanding carcinogenesis mutations and chromosomal modifications characteristic of a specific cancer are often cited. As noted above mutations are clearly not irrelevant in carcinogenesis but in the independent attractor model are regarded as consequential due to the reduced integrity of replication and thus increased mutation rates of the genomically unstable state. As noted above they can also serve to "hardwire" the epigenetic changes in the system. Characteristic chromosomal modifications would be expected to arise if cancer is regarded as an adaptive process and indeed under the independent attractor model would be expected to arise as the demands of the attractor for active components modulated the transcription process. In this sense the situation parallels that of the evolution of a new species with consequent chromosomal modification or in the terminology of Goldschmidt, re-patterning (Goldschmidt 1982).

In summary, adopting the independent attractor model raises mainly biological issues concerning how functional phenotypic changes to individual cells impacts upon their role in maintaining tissue integrity. Like past aspects of the low dose problem this also is a "needle in a haystack" problem because in all probability most cells rendered unstable will be benign in terms of health detriment. Rather than disease being the consequence of a rare event in terms of radiation action it would be a rare event, in biological terms, arising from a relatively commonplace event in radiological terms: exposure to radiation initiating trains of events, contingent on the host, some of which may result in disease but the majority being irrelevant. Thus, the low dose problem needs to be resolved in terms of biology and the Chernobyl accident has created a reservoir of potential subjects for research.

3.4 The implications for Chernobyl research

The assessment of the potential of the Chernobyl accident to shed much light on radiation damage to health depends critically on how radiation is perceived to cause health damage.

On the basis of the traditional genetic approach the magnitude of doses incurred would be perceived as too low to yield much research benefit. Such large populations would be required to achieve statistical significance in epidemiological studies that there would be little point in them. However, as noted above there is now evidence from some epidemiological studies of populations exposed to low dose rates to suggest that that position should be revised. Furthermore, the prospect of phenotypic change being epigenetically modified (as, for example, in the independent attractor model) adds further pressure in the same direction. In particular that model would predict that an abrupt change in background radiation levels such as caused by the Chernobyl accident would increase the initiation rate of genomic instability and hence potentially the disease rate. The potential for the release by radiation exposure of latent pre-neoplastic lesions from their developmental constraints moves the judgment even further in the same direction, namely that epidemiological studies, still of relatively large but manageable populations, may yield useful results. However, it should be noted that while pre-neoplastic lesions are common in adults they do not appear to be sensitive to radiation. For example, adults given diagnostic exposure to 131I do not show and excess of thyroid cancer (Holm, Wiklund et al. 1988)

Individual epidemiological studies alone do not shed light on causality. For this reason proposals have been made to supplement such studies with molecular studies. How this might be achieved is not difficult to see for effects with a genetic origin and indeed is the basis for the genetic hereditary effects of radiation. However, based on these undoubtedly secure risk factors the Chernobyl population is unlikely to show an increase in classical Mendelian hereditary disease.

To date attempts to relate mutations definitively with radiation as a causative agent in cancer have failed. Cancers (sporadic and radiation induced) have “characteristic” mutations but they rarely appear in more than 90% of cases. For example, the APC gene is truncated in 72% of sporadic colorectal cancers but normal in the others (Theodoratou, Campbell et al. 2008). Damaged APC cannot therefore be the “cause” of colorectal cancer. This conclusion is supported by mathematical modelling where it was shown that high APC mutation rates can be preceded by the chromosomal instability in the colon epithelia (Nowak, Komarova et al. 2002). The problem of identifying such markers of radiation induction of cancer is compounded by the high incidence rates of sporadic cancer associated with radiation induced cancers. The best possibility to examine the properties of cancer induced by radiation derives from the Chernobyl experience of childhood thyroid cancers. In the exposed populations diagnosed thyroid cancers are overwhelmingly likely to be radiation induced because the sporadic incidence is so low even though screening programmes might have been expected to enhance the sporadic incidence of cancer (Kaiser, Jacob et al. 2009). Evidence that post-Chernobyl childhood papillary thyroid cancer (chPTC) differs from sporadic PTC in terms of a higher frequency of rearrangement of the RET/PTC oncogene (Nikiforov 2002) and a reduced

frequency of BRAF mutation (Nikiforova, Ciampi et al. 2004) compared to adult PTC has been interpreted to claim that the phenotype of chPTC might differ from spPTC and thus provide a molecular marker of radiation induced disease. RET/PTC rearrangements have a role in advancing the course of carcinogenesis (Ciampi and Nikiforov 2007). However, only 20 to 40% of adult PTC exhibit this rearrangement[7]. However, an examination of *gene expression* in samples of tumour tissue stored in the Chernobyl thyroid tissue bank compared to 8 tissue samples from patients with no history of radiation (Detours, Wattel et al. 2005) indicated that there were no phenotypic differences possibly related to the origin of the PTCs and therefore no "radiation induced fingerprint". This result was confirmed in a subsequent study using more patients (exposed and unexposed) and more genes: both sets of tumours have "*the same overall expression profiles and have indistinguishable BRAF and RET/PTC frequencies*" (Detours, Delys et al. 2007). However, based on the assumption that naturally produced H_2O_2 in thyroid metabolism might be the cause of spPTC the authors examined the gene expression response of lymphocytes to radiation and H_2O_2 (data of Amundson et al (Amundson, Do et al. 2005)) and found that the response of 118 genes differed in their regulatory response to the two agents. These genes could be found in the chPTC and the spPTC gene profiles and differences in their distribution could be used to distinguish between spPTC and chPTC with a 15 to 27% error rate.

In a subsequent investigation (Stein, Rothschild et al. 2010) gene copy number and altered gene expression were measured in 10 chPTCs and compared to non-malignant cells. Overwhelmingly in the chPTCs increased copy number (amplifications) was detected despite the expectation that radiation would mostly induce deletions and translocations. Compared to normal tissue the chPTCs exhibited 242 up-regulated genes and 210 down-regulated genes. 70% of these altered genes were also found in spPTCs but 30% were unique to chPTCs. On 5 samples with results for altered gene expression and altered copy number it was possible to identify 88 genes being over-expressed and the authors speculate that further work may provide a means of distinguishing between radiation induced and sporadic tumours.

Thus, this detailed work, based on the unique material gained as a result of the Chernobyl accident provides no support for proposals that radiation induces an easily recognisable molecular signature that might be expected from a mutationally based mechanism for carcinogenesis. The most likely means of detecting radiation origin of such tumours lies in gene expression profiling or –omics, that is, process related endpoint rather than molecular signatures. The attractor model predicts that exposure to radiation, where it induces GI, will

[7] The argument is frequently made that *causality* is determined by the presence of such chromosomal anomalies and backed by evidence that when introduced into cells cultured *in vitro* they are transformed and when introduced into transgenic mice they exhibit carcinomas "reminiscent" of, or "remarkably similar" to, human thyroid carcinoma. However, it is interesting to note that in one experiment 6/11 mice with the PTC/RET rearrangement exhibited papillary carcinoma at diagnosis at age >3 months. The difficulty with this kind of argument is accounting for the discrepancies; why 60 to 80% of cases of adult PTC do not have RET/RET translocations if they are causal and why 5 mice out of 11 with the causal rearrangement did not get PTC. It seems far more likely that these are associations with the disease and are consequential in some cases but not in others.

generate such process" changes in gene expression and this was confirmed (Falt, Holmberg et al. 2003). Chromosomal changes are a signature of GI but are consequential on the underlying implications of a move from the "home" attractor associated with a normally stable species to a variant attractor with impaired replicative integrity. Thus, the dominance of gene expression alteration seen in chPTCs is entirely consistent with the attractor model and the proposal that carcinogenesis is initiated through a transition to GI and not by a mutational event.

The independent attractor model however does postulate that the initiating events arising from the Chernobyl exposure will be substantially more frequent than the specific sequence mutations the exposure might cause. This increased frequency will be offset by natural biological defence mechanisms operating at the tissue level possibly leading to *in situ* lesions (Folkman and Kalluri 2004). Thus, one approach to testing this prediction is to examine in the tissues of persons dying of trauma the prevalence of pre-neoplastic lesions and early potential non-cancer lesions (e.g., plaque in blood vessels) in relation to their exposure to Chernobyl fallout.

The most immediate priority to advance research is the generation of testable hypotheses based on the epigenetic consequences of exposure of cells to radiation where initial events are frequent and biological response is effective in limiting the effects; essentially biological rather than physical hypotheses. Faber and Rubin (Farber and Rubin 1991) have proposed that carcinogenesis is a process of adaptation. Although several proposals that carcinogenesis is an evolutionary process akin to Darwinian evolution, first proposed by Nowell (Nowell 1976), have been made mostly they are based on genetic concepts, although it is acknowledged that other factors might apply (See for example (Vineis and Berwick 2006)). However, variations in the rules of engagement allow non-genetic adaptation processes under the independent attractor model and little thought has been given to this possibility in spite of the availability of ready techniques to measure the transcriptome and proteome; the need is for plausible biological hypotheses.

However, the physical aspects should not be ignored. A potentially fruitful approach is track structure based calculations of event size distributions. Typically these have been deployed to focus on events that occur within a volume relatively small compared to the whole cell nucleus, typically 0.1 to 1.0μm. The nuclei of mammalian cells are of the order of 10 μm diameter with total cellular diameters of up to 25μm. Event size distributions in these volumes would be most relevant (Baverstock and Thorne 1998). The temporal aspect should not be ignored. While at low dose-rates events in a single cell are widely spaced on average there will be rare coincidences where two or more events occur in close temporal proximity, especially where internal emitters are concerned. The object would be to compute probability distributions of events that are putatively thought to be stressful to the cell's repair capacity.

The initial cellular responses to DNA damage occur within minutes but transcription to replenish the used gene product requires durations of the order of an hour. There seems little prospect of the Chernobyl data being appropriate to test hypotheses at this stage but theoretical and experimental studies could ultimately yield testable hypotheses where the Chernobyl exposed would be highly relevant due to the diversity in exposure conditions.

Individual sensitivity emerges as a key factor and raises rather complex questions. For example, diagnosable cancer caused by radiation exposure might be due to initiation or promotion by radiation. Cancer originating from exposure at an advanced age with a relatively short latency is likely to be due to promotion whereas later occurring cancer in those that were young at the time of the accident is likely to have been initiated by the exposure. The problem is to know which cancers are induced by radiation. However, the cancer registry data or alternatively a suitably designed lifespan study might be used to test prior hypotheses generated from non-Chernobyl derived data.

The proposal on effects inherited from irradiated parents also discusses potential approaches to assessing the health significance of genomic instability and bystander signalling. An important issue here is the question of whether the inheritance of genomic instability (mini-satellite mutations in humans) leads to health detriment. Direct human evidence is unlikely to contribute to answering this question although the proposals for family studies may produce some clues. In lieu of such direct information the results of animal experimentation and more general arguments are available.

Mice with elevated inherited ESTR mutation rates exhibit the effects of instability in their somatic cells in the form of increased mutation rates at the *hprt* locus and increased DNA strand breakage as measured by the comet assay (Barber, Hickenbotham et al. 2006). In the context of the independent attractor model this is exactly as expected as the affected offspring inherit from their father a variant attractor that applies to all their cells and gives rise to the aberrant phenomena called GI. If a similar effect occurs in humans (that is not yet established) and somatic disease is related either to increased mutation and DNA damage or more directly to the variant attractor, then it is clear that health detriment is a potential outcome. The experiment of Luning in 1976 (Luning, Frolen et al. 1976) demonstrated the ability of alpha particle induced dominant lethal mutations in mice to skip a generation (f_1) and appear in the f_2 generation. Dominant lethal mutations are a health detriment and their appearance in mice is the basis for radiological protection standards. This experiment has not been repeated but several experiments have produced results (Lord, Woolford et al. 1998; Lord, Woolford et al. 1998; Lord 1999; Barber, Hickenbotham et al. 2006) which suggest that the phenomenon is real. On the basis therefore of animal experiments the provisional conclusion would be that inherited GI is a threat to human health in hereditary terms.

The much deeper question here is “why has the phenomenon of GI evolved?”. Is it some quirk of radiobiology or is it a fundamental aspect of biology? Firstly, neither the induction of GI *in vitro* nor its induction *in vivo* is unique to radiation. Several other agents induce it in cells in culture (Coen, Mothersill et al. 2001) and other agents induce it in male mouse germ cells (Dubrova, Hickenbotham et al. 2008): it has all the properties of a generalised stress response (Dubrova 2006). It has in fact been proposed to be a fundamental feature of biology playing a central role in environmentally induced speciation (Baverstock and Rönkkö 2008). The arguments to support this position are too complex to rehearse here.

However, a picture that emerges is of a process that for the long term purposes of living systems adapting to environmental change a relatively high sensitivity to environmental stress in one gender has evolved, but which in the shorter term threatens the viability of the species. To preserve the species under relatively minor stresses has proved beneficial and mechanisms (behavioural) have evolved to ensure “true breeding”. However, under more extreme stresses these comparatively “soft” processes would be over-ridden to produce variants, which if selected would become new species. The clear conclusion here, if this argument is correct, is that as far as the species is concerned GI is detrimental (and should be eliminated) but in the much longer perspective it is a price being paid for evolvability.

On balance it would be prudent to assume that transgenerational GI is deleterious to health and that the mini-satellite mutations observed in the offspring of irradiated fathers is an indication of transgenerational inheritance in humans. Epidemiological studies must have priority to show any associations of this phenomenon with human health effects. This makes it a high priority for further research. The possible methods and populations for establishing such associations are reviewed more in detail in the specific ARCH proposals.

3.5 Conclusions

In summary, our conclusion is that many of the late somatic effects of exposure to ionising radiation (cancer, certain categories of non-cancer disease and potentially some hereditary effects) are primarily initiated by radiation induced genomic instability and not mutations of the genomic DNA. An important exception is hereditary disease based on single locus mutations. The position as to more complex multi-factorial hereditary disease has not been addressed. Cataract of the eye could be a radiation chemical effect on the crystalline protein lens or the consequences of genomic instability in the epithelial cells of the lens, which throughout life lay down the laminae that form the crystalline structure of the lens. If the former applies a threshold for the production of cataract would be expected but if the latter a non-threshold response would be expected.

The ramifications of this conclusion are far reaching for radiological protection and research on the Chernobyl accident for the following reasons:

- The transition to the genomically unstable state is irreversible and thus repair over time following the radiation insult is irrelevant to the outcome. This implies that dose responses as observed in exposed populations (through epidemiology) will be initially linear and not subject to dose-rate reductions, that is, LNT applies and DDREF = 1. Quantitatively, this dose response might be modified by biological factors following the initiation step or by differences in sensitivity among the population.

- In terms of the exposed individual absorbed dose will not necessarily be related to effect or be a surrogate for risk. Effects are contingent on, for example, the response of a single cell to stress induced in the processing of damage caused to the genomic DNA. The extent to which such stress is coped with may depend on the spatiotemporal features of the energy deposition in the cell, or biological factors, including past evolutionary experience, current lifestyle factors, or genetic/epigenetic background.

- Measures of radiation quality, such as LET or lineal energy, do not provide a continuum upon which measured RBE values can be extrapolated: RBE and therefore radiation weighting factors, are purely empirical (Baverstock and Thorne 1998).

- The potential effect of BE on tissue weighting factors as applicable to high LET radiations will need reconsideration (Baverstock and Thorne 1998).

- The link between initiation and disease/effect endpoint will not be traceable through, or characterised by, molecular damage. The GI dominated process is essentially a dysregulation of the cell and will be detected in terms of changes in gene product pattern at the active proteomic level. These changes may be approximately reflected in changes in transcriptional activity, the transcriptome, but a one to one relationship between transcriptome and proteome is not expected as the dysregulated cell is in the process of adjusting and adapting to the new regulatory regime. It is undergoing a process of adaptation.

- Cancer gives the impression of being characterised by specific mutations and chromosomal anomalies because these play a role in the growth characteristics of the affected cell and thus can be acted upon by selection. In this way the so called hallmarks of cancer (Hanahan and Weinberg 2000) are generated. These features are consequential on the dysregulation of the cell but they serve to both modify the state space of the cell and to lead to stochastic attractor transitions. In other words, as proposed by Faber and Rubin (Farber and Rubin 1991) cancer is an adaptive

process. Powerful evidence in favour of this position is to be found for thyroid cancer (Detours, Wattel et al. 2005; Detours, Delys et al. 2007; Stein, Rothschild et al. 2010).

- The position with respect to non-cancer disease is less clear. A plausible process can be advanced for the radiation induction of circulatory disease based on a transition of an endothelial (or smooth muscle) cell lining a blood vessel to the genomically unstable state and the dysregulation of neighbouring cells through the BE to form a focus of phenotypically (functionally) compromised cells incapable of preventing inappropriate penetration of the intima by, for example, lipid and lipoprotein particles, and the formation of plaque (Ross 1999).

- GI is heritable along the male germ line but it may take several generations before effects are seen. If, as is proposed above, GI is the origin of radiation induced disease then it is reasonable to conclude that the effects seen as inherited GI (ESTR mutation in mice for example), which have been shown to appear in other guises in the somatic cells (increased sequence mutation efficiency) (Barber, Hickenbotham et al. 2006), are indicative health detriment.

It should be noted that these conclusions are based on a model of the cell as a complex dynamic entity rather than the more conventional model of a mechanism or "machine". This conclusion has emerged from the remit given by ARCH to explore the biological bases for risk assessment in the circumstances of the Chernobyl accident with special emphasis on the non-targeted effects of radiation. It is our contention that the new model is better supported by the evidence than is the conventional model and that the underlying physics for such a system is considerably more appropriate than that underpinning the conventional view. A recent paper by Feinendegen et al supports this contention (Feinendegen, Hahnfeldt et al. 2008). Beyond radiobiology the work of Rosen (Rosen 1991) and its elaboration by Louie (Louie 2007) also supports this position.

The generic implication of the cell as a complex dynamic entity is that it should be treated as a system. This objective is much more daunting than it might at first sight appear. So called "systems biology" is a broad category of approaches (O'Malley and Dupre 2005) and is most commonly taken to mean computational approaches to –omics (Kitano 2002). However, if the cell is *complex* rather than *simple* (Louie 2007) the implication is that it has non-computable models (Louie 2007). The recent work of Yus et al (Yus, Maier et al. 2009) seems to confirm this.

As noted above the implications of these findings are broad and require new approaches to radiobiological research. However, the experience gained from the Chernobyl accident can make an important contribution in the following ways:

- Transgenerational inheritance of GI through the study of first and further generations and family studies on chromosomal anomalies, pregnancy outcomes, dominant lethal mutations, and signs of the premature appearance of disease typically associated with old age (It is noted that such investigations could raise important ethical issues that would need to be resolved).
- Post-mortem examination of tissues of trauma victims for frequency (cf contaminated and clean territories) of pre-neoplastic lesions or other indicators of GI and for chromosomal damage and changes in gene copy number and gene expression profiles that might constitute evidence of GI.
- There is a deficit in knowledge of event size distributions in targets comparable to the size of the cell nucleus. Track structure studies can be used to fill this gap and also determine the frequencies of unusually "demanding" (on cell damage processing capacity) events.
- Evidence that might suggest that promotion of thyroid cancer among those exposed in adulthood through the disruption of pre-neoplastic lesions is occurring.

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Appendix A3.1

Are the carcinogenetic effects of radiation induced by low doses in a different causal category to those induced at high doses?

It has been proposed that although the effects at low doses might be more effectively described under an epigenetic process involving genomic instability as an initiating event at high doses the conventional genetic paradigm will be more relevant. This appendix addresses this issue.

The above question can be addressed on two levels, namely evidence and theory.

A3.1.1 Evidence

The principle evidence as mentioned in the text is the apparent lack of a dose rate effect for cancer compared with the very marked dose rate effect seen for the inheritance of single locus mutations (see figure 1 in Chapter 1). It can be assumed that the factor of about 3.6 in the rate of induction per Gy of single locus mutations is due to the repair of damage to affected gene sequences (Russell, Russell et al. 1958). If mutations were the (causal) origin of cancer a similar dose rate effect would be expected. It is possible to compare the carcinogenetic effectiveness of near instantaneous exposures (survivors of the atomic bombings in Japan) with exposures protracted over years (Techa River for example).

Doses for the Techa River cohort range up to more than ~1 Gy with 70% in excess of 100mGy. In the latest analysis of ERR for solid cancer incidence is statistically significant ($p = 0.011$) at 0.86 ±0.37/ Gy (Eidemuller, Ostroumova et al. 2010) assuming linearity. This value is somewhat higher than a similar analysis for the survivors of the atomic bombings and is in line with the estimates derived for workers by Cardis (Cardis, Vrijheid et al. 2007) where restricting the analysis to the lower dose ranges increased rather than decreased the ERR/Sv. For leukaemia mortality, excluding CLL, the ERR/Gy in the Techa River cohort is shown in Figure A3.1 compared to the combined ALL and AML mortality in the ABS.

Thus, contrary to the dogma, the risk is higher (but not significantly) at low dose-rates than the high dose-rate risk (see figure A3.1.) In addition, the now clearly demonstrated linearity for solid cancer in the atomic bomb survivors does not support a dose-rate effect for radiation induced cancer.

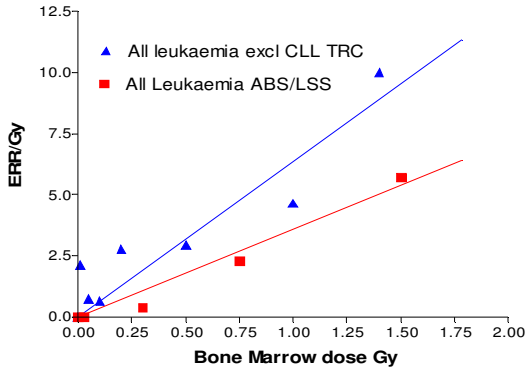

Figure A3.1: A comparison of the excess relative risks for leukaemia mortality (excluding CLL) in the Techa River Cohort, TRC, (low dose-rate) (Krestinina, Preston et al. 2005) and for all leukaemia for the Atomic Bomb Survivors, ABS, (high dose-rate) (Richardson, Sugiyama et al. 2009).

One must therefore ask why there is no evidence of repair to mutational damage if that is the causal precursor to cancer. One possible reason could be that radiation is acting as a promoter as well as an initiator in the low-does rate context and thus accelerating the development of initiated cancers. From the dose rate effect for specific locus mutations such acceleration would have to be by a factor nearly four if the effect of repair were to be neutralised by promotion. However, the distribution of leukaemia mortality after the 6 years of exposure of the TRC (Krestinina, Preston et al. 2010) is not a deviance with that observed after the atomic bombings (Krestinina, Preston et al. 2005). Secondly the linearity of the dose response for solid cancers shown in Figure 1.2 in Chapter 1 cannot be due to a promotional mechanism because the exposure is instantaneous and is indicative of dose-rate independence.

However, a dependence on dose-rate is not expected based on the independent attractor model since there is no possibility of reversing the transition from the normal or home attractor to variant attractor with repair processes.

A3.1.2 Theory

As noted in the text different metaphors apply to the conventional model upon which the SMT is based and that for the attractor model. There will be consensus that the cell should be regarded as a system when that is defined as an “assemblage of parts or components with mutual dependencies”. The issue is: is this system “simple” or “complex”. Although both words are in common usage their meanings are not always clear. A formal definition is given by Rosen (Rosen 1991) (and elaborated by his student Louie (Louie 2007)) in terms of causality (see below). A simple system (in essence a mechanism) does not have closed loops of *efficient* cause whereas complex systems have at least *one* such closed causal loop and a separate category of complex systems, organisms, have *only* closed efficient causes.

The *efficient* cause is that which brings about the *final* cause. In the analogy Aristotle drew to illustrate his arguments regarding causes, namely the commissioned sculpture, (final cause) the efficient cause is the sculptor.

To date Aristotle's final cause has been an anathema in science in general and biology in particular because it can imply teleology, namely that design or purpose is involved. However, this is not the case if the final cause of a cell or organism is viewed as stable replication, that is, the ability (of a cell/organism) to reproduce itself in both form and function in a stable manner to be a recognisable member of a *species*. This is clearly what cells/organisms do, i.e., it is biological fact and not speculation about why they are the way they are. It therefore follows that it is the *efficient* cause that brings this about and not the *material* cause although of course certain materials are required, specifically DNA with defined sequences coding for the gene products.

On this basis biology must therefore be about the efficient cause, which by definition is a *process* oriented feature of biology (in contrast to the current dogma which concentrates on *material* oriented features of biology, molecules and changes to molecules, as the rationale for biological effects). A leading exponent of the *process* or *functional* approach to biology is Robert Rosen who developed, with the aid of category theory, the "relational biology" concepts initiated by Nicolas Rashevsky. Rosen's theoretical arguments (Rosen 1991) dating back to the 1970s have been largely ignored probably in part because these concepts were eclipsed by the discovery of the structure and semi-conservative replication of DNA and also partly because category theory is "difficult".

However, Louie has elaborated on Rosen's original ideas to derive rigorously definitions of the terms "simple" and "complex" in terms of systems in general and organisms in particular (Louie 2007). In summary *simple* systems can be equated to mechanisms (and amenable to a reductionist approach) while *complex* systems are distinctly not mechanisms (and therefore not amenable to a reductionist approach) in so far as they cannot be simulated. Organisms/cells are a sub-class of complex systems. According the Louie's analysis (Louie 2007) there is a non-permeable barrier between simple and complex systems. This means that they are non-exchangeable and separate categories of system (see figure A3.2).

Since the conventional dogma, of which SMT is a component, regards biological systems as *mechanisms,* that is, simple systems, the question in the title to this appendix boils down to:
"Can the cell behave as a complex system in response to low doses of ionising radiation and as a simple system at high doses?"

According to Louie's analysis the answer has to be "no" because the barrier between the two classes of system is impermeable. Once a system is classified as "complex" it cannot be a "simple" system: it must be one or the other.

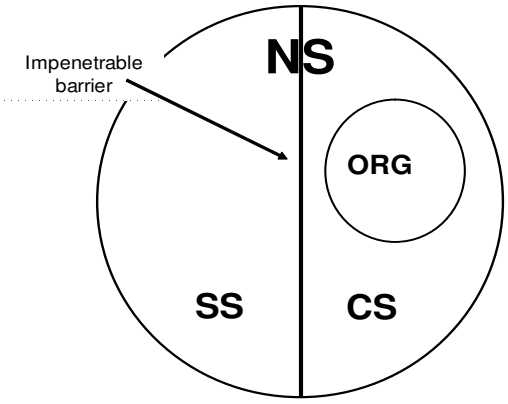

Figure A3.2. A natural system (NS) could be a simple system (or mechanism) (SS), a complex system (CS) or an organism (ORG), which would be a sub class of complex systems. There exists between SS and CS an impenetrable or impassable barrier meaning that one cannot be transposed to the other. Adapted from Louie (Louie 2007).

A3.1.3 Discussion

The evidence aspect of this question demands an explanation from the advocates of the *status quo* as to why, in the case that the outcome of irradiation being cancer, no dose rate effect is observed, whereas in the case of the confirmed mutational mechanism leading to hereditary effects, a clear dose-rate effect is observed in the dose-rate range for which there is epidemiological data for cancer. This question is entirely separate from the theoretical arguments and in our view stands as a compelling argument for there being no difference in cellular response according to dose range.

The theoretical considerations dictate that cells/organisms are complex (Louie 2007). The full argument for this, rather than that given above to show that complex and simple systems are non-exchangeable categories of system, is too complex to be rehearsed here. Essentially, the argument is that cells/organisms have only, as far as the efficient cause is concerned, closed causal loops. In other words nothing that contributes to the *processes* that bring about the final cause derives from outside the system, that is, from the environment. As the system is "open" matter and energy can be and are imported and exported from the system but the *processes* that fabricate and make functional the system are all derived from within the system. The independent attractor model envisages the efficient cause as the relations between the active gene products that form the attractor. These are provided by the process of transcription, translation and activation, all internal to the system (Baverstock and Rönkkö

2008). The independent attractor model is proposed as a dynamic material realisation of the category theory foundation for cells/organisms (Rosen 1991).

It might be argued that in transmitting hereditary disease from one generation to the next germ cells are acting as simple systems while it is being claimed that somatic cells involved in carcinogenesis are acting as complex systems. This is not so. A mutation can serve simply to change the system properties, that is, modify phenotype and this is what is observed in the inheritance of a single locus mutation. Were it is the case that a single mutation leads directly to malignancy without "processing" the damage, malignancy could be classified as the same kind of effect as single locus mutations acting as a second hit in Knudson's Two-Hit Model. Indeed, it is what happens in retinoblastoma where the Rb mutated gene is inherited. As noted in the text mutations can precipitate attractor transitions but they are a small component compared to other epigenetic processes, the induction of GI for example. Thus, single locus inheritance is fully compatible with the cell/organism being a complex system.

Essentially we are proposing that cancer, at least some non-cancer disease (circulatory disease, for example) and a category of inherited effects, are *caused*, that is, *initiated*, by radiation exposure, through an epigenetic *process*, the transition from the normal attractor state to a variant (GI) state, an event that is relatively more frequent than mutation. This proposal has major implications for radiological protection and for some of the ARCH proposals. As the GI phenotype is a mutator phenotype it generates mutations and chromosomal damage more rapidly than the normal phenotype from which it is derived. However, this molecular manifestation of GI need bear no relation to what initiated it, or indeed, what will be the future development except in one particular context. Acquired mutations and chromosomal damage can and do affect factors such as cell proliferation rate and thus, through selection, may appear preferentially in specific cancers and when introduced artificially into normal cells lead to malignancy. For example, it was reported in 1999 that human cells could be rendered malignant by introducing in a normal cell the expression of a telomerase catalytic subunit and two oncogenes *in vitro* (Hahn, Counter et al. 1999). Clearly malignancy is much more complex than this result would appear to indicate. The reason why it is relatively easy to induce the malignant state is that a relatively large amount of the cell state space and therefore cellular attractors/phenotypes is associated with uncontrolled growth, indeed uncontrolled growth could be seen as the default option for the cell of a higher organism. It follows that molecular damage is neither necessary nor sufficient to define the presence of GI still less predict future consequences.

The main implications are for so called molecular epidemiology and the molecular evolution of cancer.

A3.1.4 Conclusion

These arguments do not support a model that proposes that cells have a carcinogenic response to radiation that depends on the dose range. The absence of a dose rate dependence for carcinogenesis is powerful evidence that carcinogenesis is not initiated (caused) by a simple phenotypic change due to a mutation in any dose range between 0 and 1 Gy as is demonstrated to be the case for the inheritance of simple locus mutations.

The operation of the cell as a dynamic complex system with the observed material realisations, the basis for the attractor model, is plausible and allows the unification of the effects of radiation, in terms of Mendelian and non-Mendelian inheritance, cancer and non-cancer disease (Baverstock and Karotki 2010)

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