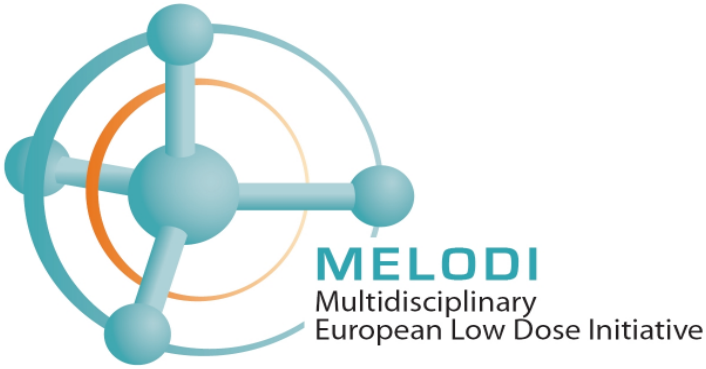

MELODI –
Multidisciplinary European Low Dose Initiative

2nd Draft of Strategic Research Agenda (SRA)

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Table of contents

1. STATUS OF THE SRA 4

1.1 Introduction 4

1.2. The early years 5

1.3. The longer term 5

2. SCIENTIFIC VISION 6

2.1.The present situation 6

2.2 The fundamental molecular interactions associated with ionising radiation and the processes leading to cancer and non-cancer diseases 8

2.3. Research Priorities 10

2.3.1. Dose response relationships for cancer 10
2.3.2. Non-cancer diseases 15
2.3.3. Individual radiation sensitivity 17

3. NEXT STEPS 19

3.1. Evolution of research areas to be exploited 19

3.1.1. Infrastructures 20
3.1.2. Education and training 21

3.2. Maintaining the SRA 22

3.3. ROADMAP 22

3.4. Major considerations 23

3.5. Consultation 23

3.6. Establishment of the Scientific Advisory Committee (SAC) 25

4. EXECUTIVE SUMMARY 25

5. REFERENCES **27**

6. Annexes **27**

6.1: Annex 1: Questions identified as being key issues for MELODI **27**

6.2. Annex 2: MELODI statement 2010 **32**

1. STATUS OF THE STRATEGIC RESEARCH AGENDA (SRA)

1.1. Introduction

The Multidisciplinary European Low Dose Initiative (MELODI) platform has the overall aim of consolidating European initiatives on researching and better understanding the health effects of exposure to low dose ionising radiation. It is intended to provide a working framework over at least 20 years.

The purpose of the present document is to present a long-term SRA for the MELODI programme. This should serve to guide the coherent integration of national low dose R&D programmes, and provide a useful basis towards the considerations of the EU when designing EURATOM calls in this field.

This will require development of new and original research lines as well as pursuing on-going research in epidemiology, disease modelling and improving understanding of molecular, cellular and tissue level mechanisms of both cancer and non-cancer diseases induced by radiation. Most research to date has concentrated on the induction of cancer but recent developments have indicated that exposed populations are also at risk of developing non-malignant diseases. The molecular biology 'revolution' has opened up the possibility of acquiring a new range of biomarkers for diseases of relevance to radiological protection. Combined with dedicated and new methods to measure (tissue) exposure to radiation, this allows the evaluation of the health risks to individuals. It is the intention of MELODI to incorporate where appropriate new technologies as well as to promote existing fields of research in a more holistic approach than has been adopted previously.

Thus, key scientific issues that should steer the SRA have been identified by the High Level and Expert Group (HLEG), namely:
(1) the shape of the dose-response for cancer
(2) the investigation of individual radiation sensitivity
(3) a consideration of induced non-cancer disease

Three further cross-cutting issues that need to be considered are:
(1) radiation quality
(2) tissue sensitivity
(3) internal emitters

The current scientific consensus is that the health effects which should be addressed are:

- Cancer – including secondary cancers
- Cardiovascular disease
- Neurological effects (cognitive effects)
- Lens opacity
- Adverse effects to normal tissues from radiation therapy

A number of overarching questions has been developed, and the areas of work highlighted in this SRA have been selected as being most likely to provide answers:

- How robust is the current system of radiation protection and risk assessment?
- How can it be improved?
- What are the areas of greatest uncertainty in radiation research and radiation protection?
- How best to prioritise questions and to identify research needs to address these questions?

1.2. The early years

The answers to the questions posed by the HLEG are expected to come from multidisciplinary low dose research in Europe. To this end, the HLEG recommended creation of a Network of Excellence (NoE) on Low Dose Research towards Multidisciplinary Integration (DoReMi). This was set up in January 2010 initially with 12 institutional partners (BfS, CEA, CREAL, STUK, IRSN, ISS, SCK-CEN, SU, HPA, HGMU, UNIPV and IC) plus 10 additional partners (UKER, GUF, UROS, UMB, NRPA, NIPH, ENEA, IES, DIT, Erasmus) in 2011 and co-ordinated by Prof. Sisko Salomaa (STUK, Finland).
DoReMi comprises 7 work packages:

WP1: Coordination and management,
WP2: Structuring MELODI,
WP3: Education and Training,
WP4: Infrastructures,
WP5: Shape of dose response for cancer,
WP6: Individual radiation sensitivity for cancer (and non cancer) and
WP7: Non-cancer effects.

1.3. The longer term

DoReMi has a planned lifespan of approximately 6 years although WP2 concerns structuring and establishing the operational tool for developing the MELODI platform to ensure longer term commitment (>20 years) to low dose research in Europe. Thus, DoReMi is a transitional initiative using EU financial support for scientific feasibility studies to facilitate and accelerate the integration process within the MELODI platform.
To achieve this, a broad concerted effort is required to develop a long-term SRA for MELODI. The SRA should be largely based on scientific consensus and integrating new research lines developed in the mid-term (6 years) transitional research agenda (TRA) of DoReMi. In order to be effective, the SRA will require periodic updating in line with future developments arising from new scientific knowledge and technologies.

2. SCIENTIFIC VISION

2.1. The present situation

The present system of radiation health risk evaluation and radiation protection is based on current scientific knowledge and societal considerations of acceptance. It is steered by the ICRP protection system (ICRP publication 103) which, for low doses, has evolved around the perceived risk of induced malignancies and, to a lesser extent, heritable effects attributable to cancer. It uses the linear-no-threshold (LNT) assumption to estimate derived from high dose radiation effects the risks associated with low doses. It is essentially a pragmatic approach for protection purposes and is in accordance with the scientific reviews of UNSCEAR and others. ICRP combine the LNT hypothesis with a judged single value of 2 as a dose and dose rate effectiveness factor (DDREF) for solid cancer induction. In reality there is no empirical evidence to support a single value for DDREF and moreover experimental data derived from both human and other mammalian species show a wide range of values dependent on parameters such as tissue or organ involvement and tumour type. The risk per unit dose is weighted for different organs and tissues by tissue weighting factors (W_T) that reflect their perceived relative sensitivities to induced malignancies. Likewise, differing effectiveness dependent on radiation quality is addressed by recommended radiation weighting factors (W_R). Radiation risks to children are judged to be 2-3 times higher than those to the population as a whole. It has to be stressed that W_T, W_R, the age value and a single dose response relationship for all cancers are simplifying judgements.

In contrast to the ICRP view, a report from the French Academies (2005) supported the opinion that there is a practical low dose threshold for induction of cancer. Adoption of this view would have significant societal implications, both financial and legal for radiological protection in Europe. There is also emerging phenomena such as transmissible genomic stability and bystander effects that may serve to modulate cancer risk relative to that predicted from the LNT model This range of candidate risk extrapolation models creates the need for the MELODI programme to address not only the risk for cancer but also for the non-cancer diseases that to date have not featured in radiation risk evaluation at low doses. Consequently new approaches are needed combining epidemiology with fundamental mechanistic studies of the processes that drive the radiation-induced health effects and individual susceptibilities. Such information will permit more biologically realistic mathematical models to be developed to extend risk evaluation to dose levels below which direct human data are unavailable.

For high radiation doses for which epidemiological studies are particularly significant the radiation protection system is reasonably well established. Nevertheless, uncertainties still exist and continue to need attention, such as:

- shapes of dose response curves for different types of cancers and non-cancer diseases;

- sensitivity variations dependent on age with possible differences between *in utero* irradiation, infants and older children and between young and old adults.
- individual radiation sensitivity and predisposition to cancers and certain non-cancer diseases;
- biological effectiveness of different types of radiation;
- sensitivity of different cell types and tissues;
- mixed radiation exposures;
- dose rate effect, including fractionated exposures;
- interactions of radiation with chemical and other environmental agents;
- effects of radionuclides and internal contamination;
- non-targeted effects of radiation.

It has been widely recognized that epidemiological studies have limitations for statistical reasons for estimation of radiation risks at low doses (<100mGy) and very low doses (<10mGy). Nevertheless it is important to extend risk estimation down to environmental exposure levels such as mGy or µGy (see also Smith 2010, Wakeford and Tawn 2010). It is now generally accepted that, as pointed out by the HLEG report, low dose risk estimations need to be based on an understanding of the mechanisms involved.

There are many reasons why knowledge about radiation induced insults from low doses and dose rates remains limited. The effects of low doses are usually much smaller than those for high doses, making it much more difficult to assess risks/effects due to inherent sensitivity limits of all methods. Furthermore, other parameters may interfere with or modulate the observable low dose effects because radiation is only one of many environmental insults producing overlapping effects. There is no particular health effect that has been identified as being unique to radiation.
To be able to determine the relationship between radiation and the effects, the quantification of radiation exposure is very important. This quantification includes characterisationof the radiation, because radiation quality and the local distribution of free radicals and damage produced contribute to the biological outcome. Not all dosimetry issues are as yet solved and improving the dosimetric quantification and characterisation can significantly decrease the uncertainty on the dose effect relationship. Some important areas for improvement are:

— research on micro- and nanodosimetry
— dosimetry of internal contamination
— Dosimetry of medical exposures
— small animal dosimetry
— Hadron and high LET dosimetry
— Biological dosimetry*

2.2. Fundamental molecular interactions associated with ionising radiation and the processes leading to cancer and non-cancer diseases.

Oxidative free radicals are produced by normal oxidative metabolism in living cells/tissue. Most of these radicals are scavenged or inactivated by cellular antioxidants and anti-radical defence systems but some may initiate endogenous cellular damage. Cells have well developed repair systems, namely base excision repair, which maintain the stability of the genome against endogenously induced oxidative DNA damage. However, antioxidant defences are limited, may be overcome by activity-mediated or environmental radiation or chemically-induced oxidative stress. Additionally, the effectiveness of anti-oxidative defences appears to depend on age and life-style. This is thought to lead to increased oxidative damage burden during the course of life, and in part contribute to the development of a number of non-cancer diseases such as Alzheimer's and Parkinson's, premature ageing which affects the central nervous system and arthersclerosis, heart disease and strokes and metabolic diseases such as diabetes, as well as different types of cancers.

Sparsely ionising radiation is generally assumed to exert in living systems direct effects (about 40%) as well as indirect effects (about 60%) due to the production of oxidative free radicals. With densely ionising radiation the majority of the DNA damage is due to the direct effects of radiation with a small contribution from indirect effects. Independent of the ionisation density of the radiation only those radicals formed close to critical targets are important due to the short lifetime of most free radicals in human cells. In terms of overall cellular damage to DNA, ionising radiation at low doses adds relatively few additional isolated oxidative lesions in addition to the plethora of those formed endogenously. However, low dose ionising radiation does induce, in addition to endogenous-like damage, low levels of DNA damage including complex DNA damage detected as clusters of lesions within one or two helical turns of the DNA by a single radiation track. These clusters of damage include double-strand breaks, some of which are complex with additional lesions close to the DSB ends, and complex damage sites which are known to be very difficult to repair and deleterious to cells, although their detection remains to be verified. An important issue is the role of the microenvironment of cells when in tissue or in 3D models as it is thought that the low level of clustered damage at low doses against the high background of isolated endogenous damage constitutes the genotoxic lesions initiating mutations and genomic instability and may result in cellular transformation and eventually cancer. Evidence is accumulating, mainly at high doses of sparsely ionising radiation, that the microenvironment of cells in tissue modifies the damage response; the effects at low dose of densely ionising radiation remain essentially unknown.

Far less is known of the induction of oxidative damage to cellular proteins and membranes by ionising radiation and their relevance to health effects. However, there is increasing evidence that oxidative damage induced in proteins and membranes may modify cellular structures and functions including mitochondrial functions, enzymatic DNA repair and also defences against oxidative damage.

From ionising radiation's interaction with living matter it can thus be inferred that direct action will induce direct and long lasting structural modifications in macromolecules (if these are not replaced or repaired). Whilst the majority of endogenously-induced oxidative damage in DNA is repaired, complex damage is difficult to repair and accompanying radiation-induced accumulation of oxidative stress in proteins including chromatin and membranes impairs important general metabolic and DNA damage recognition, repair and signalling functions. In addition to these effects, oxidative free radicals have been identified as being involved in intercellular signalling between irradiated and unirradiated cells and these communication processes may be perturbed by low dose radiation. The relevance of these perturbations to health effects of radiation remains to be resolved.

Interestingly, most normal metabolic cellular functions are based on partially reversible redox and free radical reactions. Perturbation of these processes is associated with nearly all cancers and non-cancer diseases. Thus, one of the most important future challenges of low dose research is to establish to what extent ionising radiation perturbs normal cellular metabolism at the cell, tissue and organ level, influences synergistically the DNA damage responses as well as interfers with the equilibrium of normal systemic signalling (homeostasis) of the human body and as a consequence promotes or induces pathological conditions.

In this respect, it is interesting to note that ionising radiations of different qualities vary considerably in their ability to induce direct structural and indirect radiation effects (oxidative, free radical mediated stress), and also, by definition, the rate by which free radicals and cellular damage are produced (effects of dose rate) may condition short and long term radiation effects.

The local distribution of the free radicals produced in cells and tissues as well as the pre-existing cellular oxidative stress and the available arsenal of antiradical and antioxidant defence systems (under genetic and epigenetic control) will contribute to the determination of the final biological outcome. Therefore, it is important that the basic metabolic, proliferative, genetic, epigenetic, immunological, hormonal and physiological status of cells and tissues are investigated as an important pre-determinant for understanding and quantifying low dose radiation-induced insults.

Therefore, it will be important to launch research to define quantitatively the levels of oxidative stress in cells, tissues and organs that are part of normal homeostasis and those levels that can be achieved by low dose ionising radiation exposure and if they may be regarded as precursor conditions to perturb the homeostasis for the development of cancer and non-cancer diseases. Additionally, it will be important to determine relevant molecular and structural changes induced uniquely by ionising radiation directly and that are in the long term persistent and contributing to cellular, tissue and organ dysfunction. The roles of different cell types, stem cells, progenitor and germ cells will need to be defined.

A number of questions that are key issues for MELODI has been identified. These have been derived from the HLEG report, the MELODI workshops in Stuttgart in 2009 and Paris in 2010 and responses to an early SRA discussion

document presented at the Paris workshop. The list of questions is extensive and is presented in annex 1 of this SRA, grouped by the 3 key issues of shape of the dose response for cancer, individual sensitivity and non-cancer diseases.

2.3 Research Priorities

The research priorities are presented according to the 3 key scientific issues identified by the HLEG:

- Shapes of dose response curves for cancer
- Non-cancer diseases
- Individual radiosensitivity

Some of these issues are already being addressed by DoReMi and are part of the Transitional Research Agenda (TRA) of DoReMi.

A reduction of uncertainties in these areas of major concern in radiation research and radiation protection is likely to be achieved by focussing on the following items in the forthcoming years:

2.3.1. Dose response relationship for cancer

- **Association of epidemiological research on suitable cohorts (with sound dosimetric and well-defined medical bases) with fundamental mechanistic studies to include most recent technologies (”omics” and sequencing). The priorities are the identification of appropriate molecular biomarkers and to obtain new information and a better understanding of the relationship of actual low dose (and/or low dose rate) radiation exposure to the occurrence of specific types of cancer, and the mechanisms involved for different radiation qualities as well as the development of relevant dosimetric approaches to quantify the exposure.**

Research on suitable human epidemiological cohorts remains a very high priority of research in forthcoming years. Not only promising research on existing retrospective epidemiological cohorts should be continued, but also research on new prospective cohorts should be initiated and include cohorts from cancer patient biobanks who have received radiotherapy.

Maximising the value of epidemiological studies may be aided by incorporation of suitable molecular or cellular biomarkers into studies. The ‘omics’ approach and next generation DNA sequencing technologies are promising routes to pursue. Attempts to identify suitable disease-related biomarkers are a priority alongside dosimetric biomarkers, but it has to be realised that there may not be markers specific to radiation-induced cancers. The direction would then switch to parallel studies of biomarkers of diseases per se and of exposure and thus a 3-way correlation with disease. The development of biomarkers indicative for radiation exposure, biological (early and late) effects, i.e. pre-pathological and pathological states, and radiation susceptibility are likely to constitute important pre-requisites in low dose

radiation research. To this must be added consideration of confounding factors by other agents that cause the diseases in question.

- The research priorities include for the forthcoming years:**Studies of the spectrum of damage induced (dependent on radiation quality) at the cellular, tissue and organ level and damage repairability, activation or inactivation of specific metabolic pathways, the role of genetic, epigenetic and non-targeted effects and the cell-type specific aspects of cancer induction. The mechanism of radiation-induced cancer needs clarification, in particular, the involvement of mutations (mutational theory), the contribution of non-targeted effects and radiation quality, the involvement of gene silencing, cell differentiation and stem cell development needs to be elucidated. The extent to which the sensitivity to cancer induction may develop should be studied from dosimetric studies in cohorts such as young children (example: CT scans for congenital deformities and trauma or common exposures to a large group of children such as dental cone beam CT) and screening populations.**

Research needs to continue on the dependence on track structure and microdosimetric features of the tracks' spatial distributions of energy deposition events. In particular, methods to identify and quantify complex damage sites in vivo are required. This needs to consider the interplay between the spectrum of damage induced and its repairability and kinetics in modulating the shape of the dose response curve. This feeds into improving the scientific evidence of radiation quality effects, applied by ICRP in simplified form in values of radiation weighting (w_R). Biophysical models should be based on experimental evidence and lead to reliable prediction and interplay between experiments and models.
Furthermore, a greater understanding is needed on the relationships between oxidative stress, DNA damage complexity, chromosomal damage, translocations, DNA damage signalling, perturbed cell cycle regulation, senescence and apoptosis and how the interplay of all of these contribute to cancer induction following exposure to different radiation qualities at low doses and dose-rates. Therefore, basic cellular mechanistic studies are required.
Current evidence suggests that both target cell effects and tissue (micro) environmental effects contribute to radiation carcinogenesis.The relative importance of these at differing dose levels remains a research priority. This clearly has a bearing on the relative tissue sensitivities to cancer susceptibility expressed by ICRP in simplified form as tissue weighting factor (w_T) on the basis of epidemiological data. The use of cells, 3D model systems and tissue-sample studies is likely to be important. Also, the particular radiation sensitivity of germ cells and embryonic cells at diffferent maturation or developmental stages .
The contribution of targeted and non targeted effects to cancer induction needs to be clarified. This includes the assessment of the role of delayed genomic instability, transgenerational effects and of low dose hypersensitivity. The role of perturbed immunological functions and inflammatory reactions in radiation-induced cancers has to be assessed as well.

The extent to which the sensitivity to cancer induction differs depending on developmental stage e.g., in utero, young children, adults, is an important question. In this respect, for example, repeated diagnostic X-rays (e.g., CT-scans) of premature babies and young children as well as the effect of the contrast agents of high atomic number are potentially of concern since it is expected that they are more sensitive to radiation-induced diseases than older children subjected to repeated X-ray examinations (e.g. Cone Beam CT scans in dentistry). Indeed, cumulative exposure of children during early childhood (e.g. to correct congenital deformities) may also present a concern. A typical example of such risk category may be the young children with craniofacial anomalies (such as cleft palate), who may easily receive up to 10 CT scans before cleft closure.

- **The use of susceptibility biomarkers should also enable the importance of individual radiation sensitivity to be determined as well as age and developmental specific effects for low dose cancer risk.**

- **Bio- and data banking will be important for analysis and follow-up for such combined epidemiological and fundamental research. It may become necessary to create a dedicated biobanking infrastructure within the MELODI programme**

Establishing the range of human radiation disease sensitivity and the contribution of genetics is an important issue. Studies of migrant populations indicate that environmental factors as well as genetic factors affect cancer risk. Until appropriate molecular / cellular biomarkers are identified biobanking of material has to be undertaken. This should be considered as a workpackage in any newly initiated epidemiological study. Also, the extent to which existing biobanks (STORE, GENEPI) can be used needs evaluating.

- **Well-designed studies on effects of different types and qualities of radiation, of low dose rate, dose fractionation and chronic exposure from external radiation and/or internal contamination are another important priority.**

In fact, extensive research is still needed aimed at understanding the impact of physical parameters such as radiation quality and the dose rate (and/or fractionation) on those aspects of cell responses which could be relevant in risk estimates for cancer induction. For this, also the effects of dose delivered by internal emitters will need particular attention. It would be most profitable and relevant to prioritise epidemiological studies on internal emitters that have the statistical power to quantify excess radiation related disease. These could include: actinides and tritium in the nuclear industry and radium, radon and thorium exposures in mining industries. Diagnostic, and possibly therapeutic, applications of radiopharmaceuticas in nuclear medicine are possibly a rich source of experimental studies because planned, monitored and controlled delivery of radiopharmaceuticals gives scope for precise dosimetry follow-up. In

this research single cell, 3D and tissue, organ and animal models should be used as well as the molecular/biomarker epidemiological studies.

Also here, the identification and the nature and number of 'target' cells at risk for specific cancers in humans are important questions (for example: thyroid cancer). Another example is the glandular tissue in the breast as opposed to the adipose tissue. The amount and distribution of glandular tissue is very variable among women but new techniques may allow patient specific quantification and improved dosimetry. In Europe, breast cancer screening programmes are implemented in most Member States and known to be very well monitored, providing potentially very useful dosimetric data. The target cells are likely to be stem cells or at least relatively early progenitor (little differentiated) cells. In this area studies with short-range internal emitters are likely to be of particular value due to their characteristic tissue distribution leading to localised delivery of dose. Of necessity such studies will also consider radiation quality issues.

- **Animal studies are in general useful complements for assessing specific mechanistic studies for low dose/low dose rate radiation induced human cancers.**

For example, more effort is required to determine whether good animal models can be employed for some cancers, other than AML. Furthermore, appropriate protocols with transplantable tumours and pre-cancerous cells should be useful to specify the importance of cellular micro-environmental responses.

The information collected by molecular studies on the multiple pathways involved may then become part of an overall systems biology approach which will allow modelling of biological dose responses for both cancer and, non-cancer diseases.

- **Suitable cohorts (retrospective and prospective) for this type of study are CT scansor Cone Beam CT in children, cohorts from patients treated with radiotherapy in childhood or adulthood who later developed a second cancer as adults. Other reliable cohorts are from Chernobyl, the Mayak workers, uranium mining and other contaminated sites where relevant biomaterial is available and accessible.**

Among the well-controlled medical cohorts one prospective cohort to be considered should be that of radiotherapy patients with a good survival prognosis. These are at risk of out-of-field low dose induced second cancers particularly with newer modalities of delivering radiotherapy (IMRT, Hadron therapy, IGRT). Whilst treatment field doses are reasonably well specified, further effort needs to be given to improving the dosimetry for the out-of-field tissues and organs. Other cohorts such as children exposed to CT scans with possible long term follow-up, population cohorts undergoing screening with X-ray examinations (example: breast cancer screening, colorectal cancer with virtual CT colonoscopy) and occupationally exposed individuals such as interventional cardiologists and aircraft crews are groups where individual dosimetry can be

reasonably well defined. A typical cohort could be children born with craniofacial defect, easily receiving up to 10 Cone Beam CT scans during early childhood to allow functional and esthetic defect closure. Such a study would benefit from better individual dosimetry than many of the previous and on-going retrospective studies such as A-bomb survivors, Chernobyl and South Urals (Mayak) populations. For most of these groups there is a continuing need for retrospective dosimetry and modelling. Suitable modelling will bridge the gaps between epidemiological and radiobiological studies and should allow the quantification of low dose health risks.

The Fukushima di-ichi powerplant incident has served to remind us that many nuclear incidents involve, in the most part, people being exposed to low radiation doses. Such incidents provide opportunities for scientific study of radiation – associated disease risk as and when the immediate consequences have been adequately addressed. MELODI partners are willing to collaborate with Japanese scientists in such studies. It is also apparent that there is value in considering development of recommended protocols for use in post-emergency situations that will optimise the gathering and compilation of radiation disease risk related information.

Suggestions for way forward

Taking into account the limits/advantages of the different existing cohorts in terms of size, duration of follow-up, data quality and completeness, and availability of additional information, no single cohort could provide a satisfactory dose-response relationship adjusted to smoking and other occupational carcinogens. The discrepances in biomonitoring and medical surveillance between countries are an issue and should be addressed in the protocol, as well as the authorisations of national ethical committes and local trade unions.

The population of workers involved in the nuclear fuel cycle could provide a very good opportunity to evaluate the possibility of constructing a combined cohort (compatbility of databases), with a precise reconstruction of past exposures to insoluble uranium oxides and other exposures (availability of data, job exposure matrix construction), including the feasibility of biological sampling and biomarkers testing (legal and logistic prcedures), and the collection of additional information (hypertension, serum cholesterol levels…) from the occupational medical files. Its particular interest would be to test the feasibility to collect blood samples directly from exposed workers, including those, who have been exposed in early years of nuclear industry to relatively high levels of radiation, from both intake of radionuclides and external radiation, and are still alive. These workers would be considered of high prioriy for biological sampling and specific follow-up (both retrospective and prospective) with the aim of launching epidemiological studies involving approaches from molecular biology and toxicology. Such studies have a potential to address short-and long-term pathologies of a cancerous and non-cancerous nature.

2.3.2. Non-cancer effects

Compared with cancer much less information is available on effects of low and medium dose radiation exposures in producing long term consequences such as cardiovascular dysfunction, neurological alterations, lens opacities, or effects on other physiological functions. In order to tackle these important aspects, there is an urgent need for multidisciplinary approaches bringing together radiation biosciences with disciplines such as cardiology, ophthalmology and neurology that to date have had little or no involvement with ionising radiation research. Most urgent issues to resolve are the possible induction of cardiovascular, lens opacities and neurological (cognitive) impairments by low/medium dose ionising radiation. Feasibility studies carried out in the DoReMi project and elsewhere in EC programmes are likely to orient the different lines of future research in these areas.

- **Epidemiological and fundamental mechanistic studies should be undertaken in order to determine the dose-effect relationships (absence or presence of thresholds) for the induction of cardiovascular, lens opacities and neurological (cognitive) impairments. For this, suitable cohorts (some retrospective already existing cohorts, most prospective) with sound dosimetry and medical control have to be identified and/or set up**

It has long been realised that high radiation doses have the potential to cause effects such as circulatory diseases, lens opacities and cognitive impairment but such non-cancer effects at low doses cannot be readily explained by the mutational theory (DNA paradigm) underlying the extrapolation of cancer risk from high to low doses (LNT). Thus it has been traditionally assumed that the non-cancer effects and diseases show a threshold at doses that are well above the levels of exposure typically encountered in the public environment, at work or from medical diagnostics. However, some epidemiological evidence as well as various tissue responses and non-targeted effects recently observed at low doses call for new experimental (mechanistic) and epidemiological studies that address the extrapolation issue.
Thus, in addition to the cancer surveillance, non-cancer endpoints have to be included in prospective epidemiological studies proposed above under the dose response sub-heading.

- **For each of these pathologies the age and developmental-specific mechanisms involved should be determined. Particular attention has to be put on the involvement of specific tissue, and overall metabolic, hormonal, immunological, inflammatory (tissue micro-environmental) status in the different pathological responses.**

Generally speaking, it is likely that the underlying mechanisms involve as starting points (initial events) radical formation and radical (oxidative stress) induced lesions are similar to those implicated in cancer. However,

subsequent stages involving different tissues, metabolic, hormonal, immunological, inflammatory and tissue micro-environmental responses are likely to be rather specific and different from those identified for cancer. Thus it is important to attract new disciplines into the field of radiation research bringing in existing knowledge of mechanisms involved in the development of these conditions.
The observation that inflammatory response is a predisposition to malignancy and may be a risk factor for the development of many other clinical conditions lends support to the hypothesis that radiation injury may predispose to a range of health consequences wider than previously thought. Thus, it is important to establish the impact of immunological status on radiation-induced pathological responses that lead to non-cancer diseases.

- **Research on cardiovascular disease should be carried out on low and medium doses of ionising radiation (as a follow up and extension of the previously more high dose oriented EU project CARDIORISK).**

- **Research on lens opacities needs to be launched combining, if possible, epidemiological research and mechanistic studies on the dose response relationship including acute and chronic exposures to ionizing radiation. The development of CT dosimetry tools for brain perfusion CT and other (repeated) brain CT examinations may allow accurate monitoring of this cohort of patients.**

- **Research on neurological disorders and cognitive dysfunctions induced by low dose ionising radiation should be undertaken in order to determine low dose and dose-rate related responses.**

Impaired IQ caused by irradiation of the foetus, particularly during the critical weeks 8-15, is well known and has led to foetal diagnostic radiology being replaced by ultrasound. Prospective epidemiology is therefore no longer feasible. However, considerable interest was generated when a recent study on the effect of low doses of ionizing radiation in infancy reported cognitive impairment function in adulthood. This suggestion of a second time window during infancy, when diagnostic radiology is undertaken requires investigation. Also, exposure of premature babies should be of concern.

- **Animal studies are essential complements of all these studies, in particular they are needed for the analysis of low dose acute and chronic effects from external as well as from internal (contamination) exposures.**

Given the ethics constraints on research with humans, the use and development of animal models is essential. This is particularly so for studying chronic exposures due to external irradiation or the intake of radionuclides. Recent

animal experiments have suggested the possibility of links between ingestion of low doses of radionuclides and effects on unsuspected biological targets, such as the central nervous system, liver and major metabolic functions. Chronic uranium exposure at low doses led to molecular and cellular effects on metabolisms of xenobiotics, vitamin D, cholesterol and iron. Behavioural and cognitive effects were also reported after chronic uranium exposure at low doses, in addition to the well-known nephrotoxic effect of uranium. In human populations, uranium has been shown to affect bone metabolism. There is also some evidence that chronic contamination by cesium-137 could affect cardiovascular functions. The extent to which these and other effects are due to radiation as opposed to chemical toxicity needs to be established. Radioactive contamination studies should be backed up by investigations on the effects of particle size, distribution and specificity including research on nanoparticles. Furthermore, concerning external irradiation, recent animal studies have reported a high sensitivity of the brain and cognitive impairment after low doses of X-irradiation during the perinatal period; in particular, in neurite outgrowth and in neuron connectivity.

2.3.3. Individual radiation sensitivity

Clinical evidence from diagnostic and therapeutic uses of ionising radiation clearly shows that individuals respond differently to ionising radiation. From a radiation protection point of view, it is thus very important to identify radiation sensitive individuals and to understand the mechanisms involved. However, research in this area is difficult.

Ideally, this should be a consideration (workpackage) within any newly initiated epidemiological study such as those suggested above for investigating cancer dose response. However, just as with the biomarkers for disease/exposure, there is also an urgent requirement for identifying biomarkers, gene markers and phenotypic traits to indicate specific radiation risks in individuals.

To date there are no well established assays, although it is likely that in the current framework of DoReMi such biomarkers will be developed.

In the interim, biobanking of suitable material from relevant epidemiological cohorts is again a necessity. However, at present it is not clear what to store because it is not known what markers will emerge. Furthermore, there are formidable ethics restrictions placed on this type of research in Europe and of course logistical limitations (especially for cohorts of children) on what may be collected from human subjects. For the time being, it seems appropriate to store retrievable DNA (frozen or possibly fixed blood cells) and samples from blood serum or even skin biopsies. Moreover, it will be important that a substantial proportion of the cohort is available for recall in the event that an eventual individual sensitivity biomarker requires some other, as yet unknown, assay that cannot be performed with the banked material. From this, the importance of setting up prospective study cohorts of persons such as children exposed to low radiation doses and with long life expectancy is paramount.

The following issues are of high priority:

a. **For the detection of individual sensitivity, it is essential to set up suitable (dosimetrical and medical) cohorts that are well controlled together with appropriate infrastructures allowing concomitant fundamental research (molecular studies) to be carried out using most recent technologies. Research should cover the following items: sensitivity of different cell types (stem cells and progenitor cells) in different types of tissues, establishing the range of radiosensitivity, redox profiles (oxidative stress), genetic (SNPs, sequencing) and epigenetic profiles, the DNA repair capacity, capacity to undergo radiation-induced death, the immunological, hormonal, inflammatory, general health status of radiation sensitive and resistant individuals, latencies for different pathologies (cancer, non-cancer diseases).**
b. **Using such well-defined cohorts, knowledge on genes and genetic polymorphisms (DNA repair, cell cycle checkpoint genes, oncogenes, genes of DNA and general metabolism, hormonal and immune responses etc.) as well as epigenomic imprints should be sought in order to define their roles in individual low dose radiation responses.**
c. **This knowledge can then be used to define sensitive subpopulations in the cohorts and the effects of confounding factors such as age, sex, gender, lifestyle, physiological and reproductive status, and concomitant exposures to other physical, chemical or infectious agents or from mixed radiation fields as well as the amount of radiation sensitive tissue (example the amount of glandular tissue versus adipose).**

Some endpoints have shown promise in the field of markers for individual radiosensitivity e.g., G2 sensitivity, dicentric chromosomes or micronuclei and may need further study. However, to date, all assays have fallen short of being reliable individual predictors. For example, when applied to groups of normal or over-responding radiotherapy patients a bi-phasic profile can be seen but there is a considerable overlap. Applying some newer assays for markers of radiation exposure (γH2AX, 53BP1) and specific DNA repair activities (RAD50, MRE11) have shown some promise for indicating intrinsic individual radiation sensitivity and repair capacity, and this work should be encouraged. Some tests (e.g. MRE11) may prove to be predictive for long term cancer risks in human. These newer cytological and molecular assays have to be applied on a large scale for validation. It is possible that an analysis based on a compilation and integration of results from several markers will emerge as the most reliable way to specify an individual's sensitivity.

At present, it is unclear to what extent inflammatory and immunological factors are involved in modulation of individual radiation responses. Likewise, the degree to which non-targeted radiation responses differ between individuals needs to be

examined. Ultimately it needs to be established the extent to which individual sensitivity is dependent on genetic background in contrast to the role played by potentially modifiable lifestyle factors and measures such as the amount of radiosensitive tissues (example: the amount of glandular tissue in the breast). From this will emerge the potential usefulness of embarking on programmes of systematic genetic profiling of individuals within cohorts such as radiation workers.

Within the framework of individual radiosensitivity, it is likely that developmental stage is an important factor. Thus differing responses to exposure in utero, in childhood and in adulthood should, where possible, be factored into studies of mechanisms.

Inclusion of various functional assays for radiation sensitivity in epidemiological studies will increase statistical power for identifying risk factors in later genome wide association studies. Moreover, additional functional cohorts could come from human longevity studies (cancer susceptibility and radiation response), cancer susceptible individual and radiation therapy patients with aberrant responses.

d. Combined epidemiological and animal model studies should be useful in identifying risk variants.

In-bred laboratory animal models cannot represent the intrinsic variability of a human population. However, they can be useful for validation purposes. Specific endpoints can be examined and specific modifiers can then be further explored using suitable animal models (e.g. for congenital malformation risks or for cancer risks like osteosarcomagenesis (RB1), mammary tumours (Aps) and medulloblastoma (ptch or thyroid rRET-PTC) cancers).). Radiation quality and dose-rate effects should be considered as well.

3. NEXT STEPS

3.1. Evolution of research areas to be exploited

It is clear from the above questions relating to key issues that the research cannot be adequately undertaken by only extending already existing fields in radiation research such as radiation physics, dosimetry, radiation chemistry, radiation biology, radiation therapy and diagnostics, radiotoxicology etc. New lines of research have to be developed based on recent achievements arising from areas outside of radiation research to broaden thinking with a view to a new dynamism. For example, there have been in recent years many discoveries on specific metabolic functions and pathways, homeostasis, signalling mechanisms, stem cell biology, cellular stress, proliferation, genetics, epigenetics, systems biology, toxicology, genotoxicology, physiology, pathology, immunology, inflammation research, hormone research, research on cell death (apoptosis, mitotic catastrophy, autophagy), central nervous system, recognition and behavioural effects, embryology, teratology, molecular markers for imaging,

effects of nanoparticles (nanotechnology), heredity, transgenerational transmittance, diseases (medical treatments and diagnosis of cancer and non-cancers, …).

3.1.1. Infrastructures

For low dose and low dose rate research, the current provision of suitable infrastructures (cohorts, radiation facilities, data-and biobanks, platforms for high through put analyses) within Europe is a limiting factor. A strategy for the upgrading of infrastructures should be given a high priority.

- Most important is the establishment of suitable cohorts that allow at the same time molecular and medical follow-up. A number of potentially informative cohorts drawn from industrial and nuclear workers, medically exposed groups and residential radon exposures have been suggested in section 2.3.1 of this document. Initiating studies of prospectively followed-up cohorts is strongly recommended and therefore mechanisms have to be set up to ensure the study subjects' continued availability for research. Harmonisation of the collected data and of the methods of collecting them and dedicated dosimetry has to be strengthened.

  Including molecular and mechanistic studies in the surveillance of followed-up cohorts is essential. This requires suitable cell and tissue banking facilities with a harmonised approach across Europe. In this respect there already exist the STORE and BBMRI infrastructures which MELODI should look to exploit.

  A large proportion of the work within MELODI will require ethics approval. This is a notoriously slow and resource-intensive procedure that can add considerable delays to starting projects. Moreover, the multi-institute / multi-national nature of the studies often means that all progress is delayed until the final partner has secured approval. Overriding ethics issues need to be sorted out and settled by consensual interaction with the national ethics committees. MELODI should consider how a Europe-wide infrastructure to facilitate ethics approval could be created.
- Suitable radiation sources able to deliver low and low dose rate irradiation to cells, tissues and whole animals (both external beam irradiators and internal radionuclides) are needed together with associated laboratory facilities and dosimetry capabilities. DoReMi WP4 has begun work to identify and/or develop such facilities that can be made available to MELODI.

  The existing and forthcoming radiation facilities including microbeams, devices for alpha, beta, gamma, x-ray; neutron, proton exposure as well as facilities for low dose rate exposures have been listed by DoReMi. and MELODI is asked, wherever necessary, to help in getting access.

- Bio- and databanking appear to be important requisites that have from the start to accompany classical and molecular epidemiplogical research. Obviously, banking will be needed in order to collect and to keep relevant biological samples from retrospective and prospective epidemiological studies as well as from animal studies. This will be an important (permanent) source for present and forthcoming fundamental research using specific, newly developed molecular biomarkers defining radiation damage and exposure as as well as pathological changes and disease. Links to existing facilities supported by national or EU funds (see STORE project) have to be established. Ensuring free access to these data-and biobanks and the long term maintenance of these will be an important organisational and financial challenge of MELODI.

- Analyses platforms *for high throughput 'omics' exist in several institutions in Europe. Access is usually possible via collaborative projects or through direct individual contracting. MELODI should seek support for this type of collaborative and integrative efforts in low dose research and facilitate access to forthcoming high level sequencing activities (often done commercially) in the framework of defined low dose radiation research projects.*

3.1.2. Education and training

In recent years, many European member states have lost key competences and are no longer capable of independently retaining their current research activities in radiation sciences, with implications for effectively fulfilling operational and policy needs and obligations. Thus, specific programmes aiming at knowledge management across generations have to be designed in order to achieve sustainable continuity and development. Important aspects to be considered are:
(1) the underlying scientific programmes have to address questions that are attractive to both young scientists and faculties of universities as well as to the management of research organisations.
(2) the attractiveness of the field has to be increased by a multiple approach implemented from summer schools to master degrees, PhD and post-doctoral European research training programmes.
(3) In the long term, such programmes cannot be successful unless they do provide job opportunities to young scientists.
(4) In the present situation, sustainability of such programmes can only be achieved by a long-term commitment of funding bodies.
The MELODI platform does effectively respond to these needs and aims at establishing an integrated approach to education and training of research and teaching at Universities and non-university research organisations. Existing elements of education and training activities in this domain such as the European MSc course should be strengthened, making it compliant with the Bologna Process which creates the European Higher Education Area (EHEA) and is

based on the cooperation between ministries, higher education institutions, students and staff from 46 countries, with the participation of international organisations. At present, only a few universities in Europe will have the resources to offer a full educational programme at the basic as well as the advanced level of subjects such as radiation biology and radiation physics. Thus, an integrated approach is needed.
In line with this, the following steps are to be implemented:

- Audit of radiation courses in Europe (undertaken by DoReMi) to establish a European course (and/or summer school) in radiation biology and radiation protection with conventions with European universities and institutions.
- Identification of stakeholders able to provide long term sustainability.
- Proposition of EU calls directed to education and training that promote new ways of setting up new multidisciplinary interactive courses. These must be Bologna compliant and based on solid conventions with leading universities and research organisations and allow inclusion of most recent research developments in the field of low dose radiation research and the evaluation of radiation health risks.

3.2. Maintaining the SRA

It is essential that the SRA is periodically revised in order to take account of new developments, achievements of research from DoReMi, feedback from the consultation processes and the progressive unrolling of the SRA targets.
Revision should include a statement of achievements in terms of scientific ground covered, the establishment of multidisciplinary and multinational teams and the development of infrastructures.
MELODI should organise on a permanent basis a team tasked with this review process and the team should make input, initially yearly, to the MELODI workshops. The Scientific Committee of MELODI should review and approve successive revisions of the SRA and pass them to the Governing Board for formal endorsement. At this point, it may be circulated to all stakeholders and organisations as a support to the continued integration of national R&D programmes and to the formulation of R&D calls.
Revision should be made regularly every year taking into account most recent developments. The timing and frequency of the revision needs to be well adapted to the main stream of budgetary procedures, including the EURATOM call process.

3.3. ROADMAP

The discussions on the first draft of the MELODI SRA have been further substantiated in the 2nd MELODI Workshop October 18-20, 2010 in Paris. In

spite of that, the Working Group considers it still premature to outline already a ROADMAP for MELODI at this stage more detailed than that presented by HLEG in January 2009. Part of the projected low dose programme is realised by the DoReMi TRA covering the next 6 years and involves important scientific feasibility studies and putting into place important aspects of essential infrastructures and new approaches to education and training. From the above SRA it is clear that intrinsically, most prioritized research items will have to follow a more or less preset time scale. Thus, it is evident that the search for suitable biomarkers for defined radiation exposures (internal or external), predictions for sensitivity, the initiation of pathological pathways and for final pathological outcomes will come first in the research on radiation biology networking. Some of these biomarkers are expected to stimulate molecular epidemiological studies and the establishment of suitable prospective or retrospective cohorts (i.e., prospective cohort of CT scans in children, induction of secondary cancers in out-of-radiotherapy-field sites). This research work will be accompanied by fundamental and mechanistic studies on the specific low dose and low dose-rate radiation effects together with their relationship to perturbation of cellular and tissue homeostasis and the induction of cancers and non-cancers. This fully integrated research will highly rely on the input from non-radiobiological research disciplines such as toxicology, immunology, inflammatory research, physiology, pathology, genetics, epigenetics, cardiology, neurology, ophthalmology etc. (see SRA). It is expected that in the long term a systems biology approach together with well-defined epidemiological studies will allow mathematical modelling and the evaluation of low dose health risks.
Taking into account feedback from the MELODI GB, the outcomes of the 2nd MELODI workshop and the input of the MELODI SC, the scientific community and the stakeholders the MELODI Roadmap should then (end 2011) give the forseeable timing of the different research lines as well as a possible financial sustainability programme.

3.4. Major considerations

- MELODI needs to promote multidisciplinary integrated low dose research in Europe
- Positive efforts are required to bring in ‘new blood’. These comprise specialists with skills in research areas that previously have not been associated with ionising radiation
- Sustainability of infrastructures, education and training
- Ongoing interaction and communication with stakeholders and the public.

3.5. Consultation

A working group of experts constituted by MELODI will list important domains of low dose research, prioritize scientific questions relevant for low dose radiation risk research and assess the corresponding research needs in the light of present EU funded research and other international programmes. In order to

promote a high degree of multidisciplinary integration, covering as wide a range of scientific areas as possible, a large consultation is foreseen of the general scientific community through specific MELODI (and also DoReMi) workshops and expert group meetings. E-mail contacts, the DoReMi and MELODI Web sites, contacts to the members of the MELODI Scientific Committee and to experts in complementary fields (not yet necessarily involved in radiation research) should be explored. In fact, DoReMi and MELODi workshops open to a large scientific community will be held to attract new scientific competences, new players and provide a driving force in the field. Additionally, links to other relevant European research projects will be sought. If necessary, specific working groups will be created to develop strategies to deal with very specific research items and issues (for example, new technological and ethics problems).
For the selection of actual research needs as well as topics for future scientific calls a hierarchy will be established based on priorities and consideration of presently funded projects to pre-defined criteria including relevance, feasibility, sustainability, expected outcomes etc.
A regular survey and mapping of national scientific research (as well as education and training activities) launched by MELODI will be required to identify original and novel research lines relevant to low dose research but as yet not realised. Further, the availability and sustainability of suitable infrastructures, education and training as well as modes of interaction and communication with stakeholders and the public will need to be developed.

Thus, it is recommended that there should be

- Fully interdisciplinary working group meetings held to develop, refresh and update based on on-going research and re-appraise the research areas and priorities
- Discussion forums set up to attract fundamental scientists
  (from radiation physics, medical physics, dosimetry, biophysics, radiation chemistry, toxicology, imaging, physiology, immunology, cancer research, DNA repair, genetics, oxidative stress, epigenetics, molecular signalling, developmental research, nanotechnology, inflammatory and immunological research, ‘omics’, protein research, miRNAs, systems biology, medicine).
- A series of MELODI sponsored mixed forums-conferences-seminars-colloquia should be held on e.g.
    - molecular intra- versus extracellular signalling/ immunologica responses
    - cellular damage/ epigenetics
    - nanotechnology/toxicology/internal emitters
    - ‘omics’ and systems biology
    - stem cell research/ cancer/non-cancer
    - infrastructures, radiation facilities, omic centres, animal research
    - the establishment of cohorts and their dosimetry
    - training, degree courses, regulators, researchers etc

3.6. Establishment of the Scientific Committee (SC)

The MELODI Governing Board will establish a Scientific Committee essentially composed of experts with well-founded reputations embracing a wide range of disciplines and competences. These are to cover both the existing branches of radiation biomedical sciences and the new research areas identified and presented in this SRA as important for radioprotection and radiobiology projects and thus most attractive for the MELODI programme.
In order to ensure regular updating and some continuity in the work on the SRA, members of the present MELODI SRA working group may also be active members of the Scientific Committee. Of course, the MELODI governing Board is going to formally invite and appoint the SC members who should not represent their specific institution or country, but should be serving the cause of MELODI as individual experts.

4. EXECUTIVE SUMMARY

The SRA Working Group was tasked to develop a long term strategic research agenda to guide the coherent integration of national low dose R&D programmes, and to facilitate the process of preparing EURATOM calls in this field. The SRA builds on the initial considerations of the HLEG that formulated overarching questions concerning specified key issues. The SRA supplements a transitional research agenda (TRA) composed within the DoReMi network of excellence that considered shorter term research needs and priorities over the time scale of the first 6 years. The SRA attempts to take this forward to structure and establish the operational procedures for development of a long term commitment (>20y) to low dose research in Europe.

The SRA thus attempts to look beyond the TRA and to define the broader concepts and directions that low dose research should achieve taking account of the needs of national and international stakeholders and, very importantly, the public perceptions and anxieties concerning low dose irradiation.

As a general scheme one may consider radiation-induced events at the level of cells or the whole organism as follows: Energy deposition by different types of radiation in living systems will create perturbations in homeostatic equilibrium (metabolism) as well as reversible or irreversible damage (structural changes) which may be detectable at the molecular level by sensitive physical, chemical and biological methods. It should be possible to identify specific biomarkers directly linked to radiation exposure. Additionally there is a high priority for research aimed at being able to define individual radiosensitivity and, for this, reliable markers and accurate patient specific dosimetry are essential. For health risk assessments, the most important question will be which type of radiation exposure, radiation dose and dose-rate will give rise to a pathological outcome such as cancer and non-cancer diseases in the short or long term. Also, at this

level of investigation, it should be possible to identify specific biomarkers that can predict or are precursors of pathological developments towards defined diseases. The overall scientific challenges will be to define the borderline between normal metabolism, normal physiological responses and a disease-prone perturbed metabolism being a precondition of pathology that may be induced by low dose ionising radiation.

Priorities that need to be addressed concern fundamental mechanistic research ranging from radiation track structure and the deposition of energy in biologically important molecules; the resultant homeostatic perturbations and the steps in the cellular and tissue metabolic pathways that eventually lead to disease pathologies.
In fact, the main priorities are here the step-wise elucidation of the mechanisms of radiation-induced (oxidative) stress responses and their impact on radiation-induced cancers and non-cancer diseases. To achieve this a holistic approach is proposed starting with radiation-specific effects, radiation-induced molecular, biological and pathological effects involving a systems biology approach as well as molecular epidemiology and mathematical modelling in order to come up with more solid low-dose health risk assessments. The pathologies considered are outlined in the report where the need is stressed for the MELODI platform to involve a constellation of classical and emerging technologies in a highly multidisciplinary approach. Improved dosimetry will be needed to achieve a lower uncertainty on the dose-effect relationships. Elucidating the shapes of low-dose response relationships, resolving the question of thresholds and establishment of dosimetric tools in individuals (also as part of a cohort) is paramount to resolving questions of risk for both populations and individuals. Much is known about radiation-induced cancer in humans and animal models but this needs to be pursued particularly at low doses. More recently, the scientific community has realised that low radiation–induced health effects range well beyond cancer. The priority non-cancer areas that need to be brought into focus are cardiovascular, neurological and ophthalmic. The SRA notes that this will require input from disciplines, clinicians and scientists where there has been little or no prior involvement in radiation research and from radiology departments having large databases of medical exposures, where new dosimetry tools could estimate the required organ doses with a sufficient accuracy.

This SRA represents a point early on in the MELODI programme, indeed prior to research outcomes from the DoReMi network. The programme will evolve; some lines of enquiry will emerge showing great promise whilst it will become apparent that others are less likely to yield answers to the key issues. Thus, it is essential to keep the SRA under review and periodic reassessment and revision. Revision is necessary to take account not only of specific research achievements but also the feedback from a wide ranging consultation with the scientific community and in particular from the MELODI workshops and the Scientific Advisory Committee.

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6. Annex

6.1 Annex 1: Questions identified as being key issues for MELODI

These are grouped according to the three key issues specified by the HLEG and have been collated from the HLEG report and discussions at the MELODI workshops in Stuttgart, 28-29 September 2009 and in Paris 18-20 October 2010 and responses to the first draft of the SRA discussion document received after the 2nd International MELODI workshop in Paris.

1. Shape of the dose-response curves for cancer

- **Mechanisms**
  - What is the dependence on track structure, spatial distribution of energy deposition events? – interplay between spectrum of damage induced and its repairability in modulating the shape of the dose response curve.
  - What is the dependence on dose rate and LET?
  - What are the **molecular biomarkers** that can be validated and used in molecular epidemiology to define pathological impact and disease? – both cancer and non-cancer

- Are **molecular biomarkers** available or may suitable biomarkers be developed for radiation-induced carcinogenesis (molecular signatures) in animals and humans and as biological dosimetry of human exposure?
- What is the implication of irradiation of **stem cells** in carcinogenesis?
- Can the processes underlying radiation-induced carcinogenesis be **modelled** for different types of cancers?
- To what extent does the sensitivity to cancer induction differ for exposures during various **developmental stages** (e.g. *in utero*, young children, adults)?
- What is the effect of radiation quality and the sensitivity for **different tissues** for radiation-induced carcinogenesis and disease?
- What are the mechanisms underlying the appearance of secondary cancers or out-of field low dose radiation effects in humans?
- Can good **animal models** be developed to analyse radiation-induced cancers other than acute myeloid leukaemia or are good animal models available?
- What is the impact of **non-targeted effects** on radiation-induced carcinogenesis? - cellular signalling at low dose and low dose rate; adaptive responses to radiation
- What is the relationship between **oxidative stress**, DNA damage complexity, chromosomal damage, translocation, DNA damage signalling, perturbed cell cycle regulation, senescence, apoptosis and the induction of cancer (and non cancer diseases) by radiation?
- What is the role of **epigenetic** effects including chromatin remodelling on health effects induced by radiations of different quality?
- What is the impact of **immunological status** (systemic factors) on radiation-induced pathological responses (inflammation, cancer, non-cancer)?

Dosimetry

- How can the information on dosimetry and biokinetics of **internal emitters** be improved to understand radiation-induced short and long term effects?
- What are the most important **radionuclides** to focus on (scoping of internal emitter studies) to gain better understanding of their short term radiotoxicity and long term effects (cancer and other pathologies)?

Omics and systems biology

- How can research based on ‘omics’ contribute to a systems biology approach to processes underlying radiation-induced carcinogenesis and non cancer diseases? - Involvement in homeostasis?
- How can ‘omic’ approaches enhance our understanding of the effects of radionuclides?

Epidemiology

- What are the cohorts that can be used for molecular epidemiological approaches to understand low dose radiation effects (cancer, non cancer)?
- Is it possible to launch an epidemiological study on low dose induced second cancers?

- Is it possible to launch an epidemiological study on out-of field low dose radiation effects in humans?
- Can existing biobanks (STORE, GENEPI) be used in molecular epidemiological studies?
- Can specific epidemiological studies be conducted to reveal and analyse specific radiation responsive cancer prone tissues?
- Can feasibility studies be performed on non-cancer effects (cardiovascular, lens opacities, neurological effects)?

2. Non-cancer effects

The system of radiological protection is mainly based on excess risk of cancer induced by ionizing radiation. The main data on stochastic effects have been derived from situations with a very short exposure at a high dose rate, like Hiroshima and Nagasaki populations. Much less information is available on effects of internal exposures or long term consequences on non-cancer effects such as cardiovascular dysfunction, neurological alterations, lens opacities, or effects on other physiological functions.
In order to tackle these important asoects there is an urgent need for multidisciplinary approaches: cardiology, neurological aspects, toxicology, dosimetry, radioecology, embryology, bioinformatics and biomathematics, pharmacokinetics…

Mechanisms

- What are the mechanisms involved in radiation-induced **lens opacities**?
- What are the mechanisms involved in radiation-induced **cardiovascular** effects?
- What are the mechanisms involved in radiation-induced effects on the **central nervous system** (neurogenesis) and **behavioural** changes?
- Are these mechanisms consistent with stochastic or deterministic dose responses?
- What are the mechanisms involved in radiation-induced effects on the **digestive system**?
- What are the mechanisms involved in radiation-induced effects on reproduction and **trans-generational** effects?
- What are the mechanisms involved in radiation-induced effects on the **immune** system (inflammation, immunodeficiency)?
- How can systemic effects be distinguished from organ specific effects?
- What is the impact of **non-targeted effects**?
- What is the impact of radiation quality, dose and dose rate, acute and chronic exposure?
- What are the age, gender, population and temporal effects?
- What is the possible impact of synergistic and interactive effects with other agents?

Concerning the mechanisms involved in tissue responses it has to be noted that for many years great effort has been focused on cell level responses to radiation (e.g. study of DNA mechanisms) which have contributed to an

understanding of low dose effects and individual radisensitivity. However, this has not greatly increased our understanding of low dose responses that may involve other processes than repair. Certainly, non-targeed effects are to a large extent independent of repair processes, and tissue or system level responses such as the development of cardiovascular disease almost certainly do not require efficient repair to develop.

To better understand tissue responses, the key questions are:

- To what extent are in vitro experiments on single cell types relevant in predicting responses of more complex tissues and organs to low doses, e.g. are the biological responses (radiation sensitivities) uniform amongst different cell types and between tissues?
- Is there a long term adverse tissue response at low doses in tissues other than cardiovascular/cerebrovascular tissue and lens (bone, brain etc.)?
- Are the risks of impairment of system level response adequately known at low doses (e.g. immune competence, cognitive ability, reproductive capacity, osteogenic regeneration?
- Does the known genetic predisposition to cancer risk extend to non cancer risk of tissue level responses at low doses? If so, which biological pathways are influenced by these genetic factors?

Epidemiology

- Do **confounding factors**: diet, smoking and many other lifestyle factors, plus genetic and epigenetic factors, multi-stress exposures contribute to non-cancer effects?
- What are the main non cancer diseases to be considered after low dose radiation exposure?

Are there suitable cohorts available? (out-of-field exposures in radiation therapy, CT scans, nuclear medicine patients, interventional cardiologists, dentists, staff preparing radiopharmaceuticals (PET imaging), workers exposed to alpha emitters (Mayak), uranium miners and others (fluorspar), aircrews).

3. Individual radiation sensitivity

An “overriding priority” is for the research to include ethical considerations.

Mechanisms

- What is the evidence that individual sensitivity plays a significant role towards cancer and **non-cancer pathologies** through modulating radiation response to exposures at low dose and dose rates? - Links to cancer predisposition.
- Are there **genetic and/or epigenetic modifiers/biomarkers** available that allow determination (monitor, predict) of individual sensitivity to radiation, cancer and disease development?
- Which mammalian and non-mammalian systems should be able to validate candidate **biomarkers** related to individual radiation sensitivity?
- To what extent are **inflammatory and immunological factors** involved in individual radiation responses?

- To what extent do **non-targeted radiation responses** differ in different individuals?
- What are the factors involved in individual sensitivity and dependent on **genetic background**, age, gender and lifestyle?
- Can a multilevel approach using cells in culture, tissue cultures, non mammalian and mammalian models help to analyse individual sensitivity?
- Can an **'omics'** approach help to elucidate individual sensitivity and be used to develop a systems biology approach?
- Can omics help to define tissue weighing factors?
- How do **stem cell and progenitor cell** biology contribute to individual radiation sensitivity and tissue responses?
- Do **genetic or epigenetic modifiers** of radiation responses affect individual radiation responses similarly at low and high LET radiation?
- Is individual radiation sensitivity dose rate dependent?
- Can risk assessments for individuals be developed on the basis of molecular indicators for cancer and disease? – leading to **genetic profiling** of individuals?
- Are mechanisms and factors governing cancer susceptibility independent of dose rate and radiation quality, or are there differences in the degree to which risk modifiers contribute to individual risk at different dose rates and radiation qualities?
- Can the magnitude of individual sensitivities be quantitatively assessed and compared?
- Can non-mammalian and animal models contribute to a better understanding of the mechanisms involved in individual sensitivity?

Epidemiology

- Are there cohorts available or which can be set up that allow establishment of direct links between molecular experimental studies and epidemiological studies ("molecular epidemiology") on individual sensitivity?
- Are there cohorts available or may be set up to allow modelling of individual sensitivity responses?
- Can populations at risk be identified and distinguished by biological markers?
- Can realistic cohorts be designed including low dose exposures and protracted exposure scenarios (medical imaging cohorts, nuclear workers, flight crews) that allow detection of individual sensitivity by available **biomarkers**?
- Can **biomarkers, gene** markers and phenotypic traits indicate specific radiation risks in human individuals? -Ethical problems to be considered

6.2. Annex 2

MELODI Statement
(18 November 2010)
on a
Short- to medium-term research agenda for R&T projects to improve the scientific basis for radiation protection in Europe

The following statement of the MELODI governing board provides information on re- search priorities, which are currently being refined into a first edition of the Strategic Research Agenda of MELODI, elaborated on the basis of the concepts developed by HLEG, of the transitional research agenda of the Network of Excellence DoReMi, of the proceedings of the 1st MELODI workshop (28-29 September 2009, Stuttgart), and of the 2nd MELODI workshop (18-20 October 2010, Paris).

The research calls by EURATOM for R&T projects (Research and Training) have been led by the policy goals to improve radiation protection standards in Europe, and to prioritise and focus European research efforts to achieve maximal impact on scien- tific knowledge and avoid duplication of research, with a view to consolidating the current scientific basis for the system of radiation protection, as recommended by ICRP and defined in the Basic Safety Standards. These goals are fully supported by MELODI.

The present radiation protection system is mainly based on scientific knowledge from epidemiological studies, which have played an important role in assessing the magni- tude of radiation risk in the dose range down to about 100 mSv. Epidemiological studies continue to contribute to low dose risk research and particularly on risks of low dose rate chronic exposures, risks from internal emitters and non-cancer risks.

However, further refinement of low dose risk estimates will necessitate the close association of epidemiological with experimental mechanistic studies. For example, by using suitable molecular and cellular biomarkers, the value of future epidemiological studies (molecular epidemiology) for radiation risk research is expected to be maximised. To achieve maximal value, robust and reliable biomarkers are required for exposure, for effects on the cellular and tissue level and, most importantly, for radiation-associated diseases. The research should always include a realistic assessment of the associated uncertainties.

Experimental evidence suggests that both effects in target cell and effects and responses in the tissue environment (microenvironment) contribute to radiation-induced disease. The relative importance of these effects for different radiation qualities, at different dose levels and exposure conditions (acute, chronic, fractionated) in relation to different diseases is currently not sufficiently understood. Experimental work can best contribute by seeking dose levels where disease-associated effects are or are not observed, either in target cells, in the tissue environment or in the interacting system of both (tissue, organ or

organism).
The experimental and epidemiological studies require a commitment to collect and sustainable archiving of biological materials and data.
Mathematical and computational modelling of experimental data will allow a better understanding of radiation track structure and mechanisms of radiation effects at the level of the DNA, other intracellular targets, at the level of target cells and the tissue environment. These modelling efforts together with those using animal and epidemiological data including systems biology approaches will provide further insights into biological effectiveness effects of radiation quality.
According to MELODI, priorities for forthcoming and long-term future research should take into account the need to investigate effects of ionising radiation of different qualities on radiation-induced cancer and non-cancer diseases as well as on individual variation of radiation risks. All efforts should include a careful dosimetric approach.
The long-term priorities include the following areas:
(1) for radiation-induced cancers and non cancer diseases
- Identification, establishment and continued follow-up of suitable cohorts of radiation exposed people for epidemiological studies related to cancer and non-cancer effects.
- Identification, development and validation of biomarkers for radiation exposure, effects and disease.
- Continuing development of suitable whole animal as well as human cellular models (including somatic stem cells) for radiation carcinogenesis and non- cancer diseases which bear clear relationships to human diseases.

(2) for radiation-induced cancer
- Examination of the impact of low dose and low dose rate radiation effects on pathways/processes contributing to carcinogenesis, This involves the under- standing of the relationship between early and late effects, targeted and non- targeted effects as well as the role of delayed genetic instability.
- Identification of the nature and number of target cells at risk for a specific cancer in humans.

(3) for radiation induced non cancer diseases
- Examination of the impact of low dose and low dose rate radiation effects on pathways/processes contributing to cardio-vascular disease.
- Identification of the nature of target cells at risk for specific non-cancer diseases in humans.
- Examination of the impact of low dose and low dose rate radiation effects on pathways/processes contributing to cerebro-vascular disease and cognitive function.

(4) for individual and general health and radiation protection issues
- Understanding the impact of inter-individual variation of radiation risks in relation to cancer and non-cancer diseases, and how this might impact on dose response relationships in populations.
- Clarification of the contribution of radiation effects in target cells as well as radiation effects and responses in the tissue environment and interaction between both target cell and tissue environment at different dose levels to

the development of radiation-associated diseases.

- Examination of the impact of low dose and low dose rate radiation effects on immune function.
- Understanding of the effect of age-at-exposure on radiation risk.
- Better understanding of the risks of internal emitters following internal contamination with radionuclides.

MELODI is currently in the process of structuring all these priorities within a Strategic Research Agenda (SRA). In view of the most recent developments, MELODI recommends that short- to medium-term priorities (funding period 2011/2012) should be given to:

- Quantification of the role of ionising radiation in cardio-vascular and cerebrovascular disease development after low dose (< 500 mSv) irradiation.
- Development of suitable biomarkers for exposure (immediate post radiation as well as long term after exposure), for cellular and tissue effects and for radiation associated leukaemia, solid cancers and non-cancer diseases.The biomarkers should be usable for molecular epidemiological studies of cancer risk below a cumulative dose of 100 mSv and for non-cancer risk studies below 500 mSv, respectively.
- Clarification of the role of effects in target cells and in the tissue environment in a dose range with clear focus on low doses. This includes the development of suitable tissue, organ and animal models for the identification of tar- get cells and the interaction between target cells and tissue environment as well as the utilisation of stem cell approaches.
- Identification and analysis of suitable epidemiological cohorts if available with archived biomaterial to improve low dose radiation risk assessment by reducing uncertainties especially for the age- and gender-dependency of radiation risk and including those uncertainties contributed by exposure assessment. These may include cohorts exposed to internal contaminations.