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Working Party on Research Implications on Health and Safety Standards of the Article 31 Group of Experts

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FOREWORD

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Under the terms of the Treaty establishing the European Atomic Energy Community, the Community, amongst other things, establishes uniform safety standards to protect the health of workers and of the general public against the dangers arising from ionizing radiation. The standards are approved by the Council, on a proposal from the Commission, established taking into account the opinion of the Group of Experts referred to in Article 31 of the Treaty. The most recent version of such standards is contained in Council Directive 96/29/Euratom of 13 May 1996 laying down basic safety standards for the protection of the health of workers and the general public against the dangers arising from ionizing radiation.

The European Commission organises every year, in cooperation with the Group of Experts referred to in Article 31 of the Euratom Treaty, a Scientific Seminar on emerging issues in Radiation Protection – generally addressing new research findings with potential policy and/or regulatory implications. Leading scientists are invited to present the status of scientific knowledge in the selected topic. Based on the outcome of the Scientific Seminar, the Group of Experts referred to in Article 31 of the Euratom Treaty may recommend research, regulatory or legislative initiatives. The European Commission takes into account the conclusions of the Experts when setting up its radiation protection programme. The Experts' conclusions are valuable input to the process of reviewing and potentially revising European radiation protection legislation.

In 2008, the Scientific Seminar discussed *Emerging evidence for radiation induced circulatory diseases*. Seven scientists actively working in the field of radiation induced circulatory diseases presented current knowledge. They reported on evidence of such diseases among patients treated with radiotherapy, and on epidemiological evidence in the atomic bomb survivors, in radon exposed miners, and in nuclear industry workers in the UK and in the Russian facility Mayak. In addition, the status of ongoing research on biological mechanisms of radiation induced diseases was given. The presentations were followed by a round table discussion on *Policy implications and research needs*, in which renowned scientists in the areas oncology and cardiology, as well as representatives from a regulatory authority and from a workers' union participated actively.

The Group of Experts discussed this information and drew conclusions that are relevant for consideration by the European Commission and other international bodies.

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CONTENTS

1 EVIDENCE OF CIRCULATORY DISEASES AMONG PATIENTS TREATED WITH RADIOTHERAPY EMITTERS

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Radiotherapists have known for a long time that blood vessels in the high dose volume of radiotherapy may after latencies of many years, develop atherosclerosis and cause thrombotic events. Radiation-induced thrombosis of the carotid arteries and subsequent stroke has been the most commonly reported late circulatory disease after radiotherapy of head and neck cancer. A recent report by Dorresteijn (2001) described the clinical experience of a cohort of 367 patients treated with radiotherapy for head and neck cancer at the National Cancer Institute in Amsterdam**.** Fourteen cases of stroke occurred between 1 and 20 years later. The mean latency was 10 years. In this relatively young population with a mean age of 63 years at the time of the stroke, the rate of ischaemic stroke was significantly increased by more than a factor of 5, compared to an age matched normal population. In those patients who were followed for more than 10 years, the risk of stroke was even ten times higher than in the general population! Thus, there appeared to be good epidemiological evidence that exposure of major arteries to high radiation doses is an independent risk factor in human vascular disease.

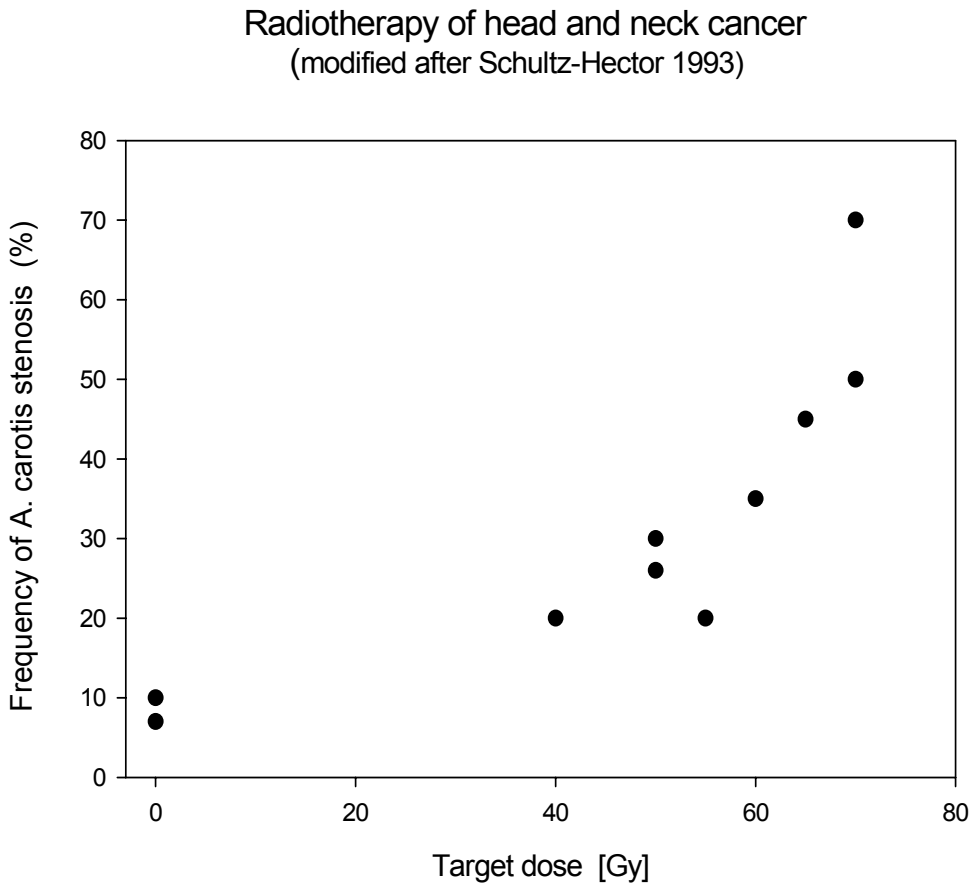
Yet the situation is not as simple as it may appear from the Amsterdam data**.** A few weeks ago, at the annual meeting of the American Society for Therapeutic Radiology and Oncology, abstract #89 by Huang et al. (2008) described the results of the largest epidemiological analysis yet of the rate of strokes after radiotherapy of head and neck cancer. This study was based on about 100.000 eligible patients with head and neck cancer documented in the Surveillance, Epidemiology, and End Results (SEER) cancer registry data base which covers approximately 10 % of all US cancer patients. Nearly 10,000 of those patients were hospitalised within 10 years after cancer treatment because they suffered a stroke. 10% of the patients who were treated with radiotherapy developed a stroke compared to 7.5% of the patients who were treated surgically. This is an increase by 33%, still a large increase but nowhere near the 500% identified in the Amsterdam study. This dramatic difference of the results from the Amsterdam study and the recent SEER study can probably be attributed to the fact that patients who develop head and neck cancer generally also have a very high risk of developing a stroke. This would be expected from their particular life style which favours the development of both, head and neck cancer and stroke, alike, as both share major risk factors such as excessive alcohol consumption, smoking and others.

One of the most comprehensive, critical reviews of the older clinical data has been published by Schultz-Hector in 1995. It is based on nearly 100 clinical reports, most of which are concerned with high dose exposure to the carotid or to the coronary arteries. These arteries frequently develop atherosclerotic changes spontaneously, but they are also often exposed to doses between 40Gy and 70Gy in the treatment of common cancers such as head and neck cancer, breast cancer and lymphomas. More informative than the clinical diagnosis of stroke are the results of non-invasive imaging investigations in those patients which revealed a rate of approximately 25% of significant stenosis of the carotid arteries. A report from

Nijmegen (Dorrestijn et al., 2005) demonstrated that the thickness of the irradiated arterial walls progressively increased during the follow-up period.

For our discussion today, the most important result of the review by Dr. Schultz-Hector is the dose response relationship which she drew from the collected data and which was based on the prescribed dose in head and neck cancer patients (figure 1). The dose dependence of severe stenosis rate at doses >40 Gy is proof of causality. Yet the high base rate and the frequent and variable exposure of people to other known risk factors makes any extrapolation to low radiation dose levels very uncertain.

Figure 1: The dependence of the frequency of stenosis of the arteria carotis after radiotherapy for head and neck cancer. Each point represents the result of one published report. Modified after Schultz-Hector et al., 1995.



Also the findings from non-invasive imaging investigations support the important role of radiation exposure for the development of atherosclerotic changes. The radiation-induced atheroma plaques nearly always occur throughout the very high dose volume which received the target dose, i.e. the same dose as the treated cancer. On the other hand, the spontaneous stenosis of the unirradiated patients nearly always is restricted to the bifurcation.

In addition to the carotid artery, radiation-induced atherosclerosis has also been described for the coronary arteries in the heart. The most convincing evidence for a role of radiation exposure in its pathogenesis, besides the well known metabolic and dietary factors, comes from those patients who developed coronary heart disease at a very young age. Coronary

heart disease below the age of 41 is very rare unless several strong risk factors are present. Yet Dr. Schultz-Hector found reports on more than 40 patients who developed coronary heart disease at this young age who had received radiotherapy which included part of the heart but who did not present with the usual risk factors.

The latency until the manifestation of the radiotherapy-associated atherosclerosis which becomes manifest as stenosis can be very long. Even 10 years after radiotherapy with high radiation doses, no more than half of the stenosis have become clinically manifest.

Whether radiation-induced stenosis of the major arteries such as carotids leading to stroke, or of the coronary arteries leading to myocardial infarction are indeed a problem radiation protection needs to be concerned with, cannot be answered from these radiotherapy data, despite the fact that the A-bomb survivor data suggest a linear dose dependence of stroke at doses more than one order of magnitude lower than those reported by the radiotherapists. It is for this reason that the CARDIORISK project included this problem in its work-programme. Yet, there can be no doubt that this problem is bound to become a serious issue in modern radiotherapy. New techniques in clinical radiotherapy, in particular stereotactic radiotherapy require careful re-consideration of radiation doses to the major blood vessels (Nieder et al. 2006).

The clinical importance of radiation-induced heart disease was recognised later. Initially it was mainly related to radiotherapy of Hodgkin's disease. Based on the follow-up studies in Hodgkin's disease patients, it was concluded that radiation-induced heart disease may assume three different clinical manifestations which is pericarditis, myocardial insufficiency and ischaemic heart disease. These different clinical manifestations have different latency distributions and, also show different dependency on dose-volume relations. (Table 1)

Table 1: Clinical manifestations of radiation-induced heart disease

1	radiation-induced pericarditis may occur if a large proportion of the heart (>30 %) receives a dose of >50 Gy. The mean latency is approximately 1 year
2	radiation-induced myocardial damage may be diagnosed at lower mean doses to the heart. The mean latency is >5 years
3	the risk of radiation-induced cardiovascular disease begins to increase 10 years after irradiation and is progressive with time. A significant increase of risk of cardiovascular disease has been observed after mean heart doses lower than 10% of the generally accepted tolerance dose to the heart of 40-50 Gy fractionated exposure.

In recent years, the high rate of ischaemic heart disease in Hodgkin's patients which usually occur more than 10 years after radiotherapy has attracted particular attention. The Amsterdam cohort study on more than 1200 Hodgkin's disease patients by Aleman (2003) is a good example of such a study (Table 2).

Table 2: Long-term cause specific mortality of 1261 young Hodgkin's disease patients after 13-35 years of follow-up (data from Aleman et al, 2003)

Overall number of deaths	534
Hodgkin`s disease	291
Second cancer	116
Cardiovascular disease	50
Ischaemic heart disease	19
Myocardial infarction	16

The most important message is that despite the large numbers of radiation-induced heart failure and a similar number of radiation-induced second cancers, the main problem remains the failure to control the primary cancer. With very few exceptions, this is the message of most studies on cardiovascular risk in radiotherapy patients: the main risk after radiotherapy is recurrence of the treated cancer.

The classical treatment fields as introduced half a century ago by Kaplan in Stanford and Musshoff in Freiburg for mediastinal Hodgkin's disease (the mantle field) leads to doses of up to 40 Gy in large parts of the heart. Vordermark et al (2006) was among the first to use modern treatment planning methods to reconstruct, in retrospective, dose distributions in Hodgkin's patients many years after treatment in order to relate findings of functional imaging of the hearts of irradiated patients to those dose distributions. The results of the functional imaging investigations cause concern, in particular the unexpected high frequency of vascular, mostly microvascular perfusion changes. Modern radiotherapy of Hodgkin´s disease is very different, with more emphasis on chemotherapy and on giving lower radiation doses to smaller volumes, i.e. those which are clinically involved by malignant disease. No studies have been presented on the results of functional imaging in patients who were treated more recently with the new protocols.

It is only since the early nineties that the heart has been found to be a critical organ in other areas of radiotherapy and in radiation protection. The observations made since the early 1990s of a significant dose dependent increase in cardiovascular mortality among the Life Span Study cohort of the Japanese A-bomb survivors (latest up-date by Preston et al., 2003) stimulated a number of studies in radiotherapy patients. The Stockholm group reported the first convincing evidence that, compared to breast cancer patients treated by surgery alone, breast cancer patients treated with post-operative radiotherapy revealed a dramatic increase in mortality from ischaemic heart disease. (Rutqvist et al 1992). This finding initiated a large number of more studies into the cardiovascular radiation risks associated with post-operative radiotherapy of breast cancer patients. The same group in Stockholm also published the first study into the pattern of blood perfusion in hearts of breast cancer patients treated with radiotherapy. They reported that about 50% of the patients had new scintigraphic defects which they related to radiation damage to the micro-circulation (Gyenes et al., 1996).

Despite these reports in the early nineties of the last century, it is only very recently that radiotherapy-associated cardiovascular disease has been recognised by radiation oncologists as a significant clinical problem. The first time that radiotherapy-induced heart disease was given a special symposium at an international meeting of radiation oncology was only two years ago at the German Congress of Therapeutic Radiology and Oncology in Dresden. However, the awareness of the seriousness of this problem has spread rapidly. This is documented for example by the fact that at the recent meeting of the American

Society of Therapeutic Radiology and Oncology (ASTRO) as many as 22 presentations dealt with radiation exposure and radiation risk of the heart in radiotherapy.

This sudden interest of the radiotherapy community in very late occurring radiation damage to the heart was stimulated by two major reports on the increase of the rate of myocardial infarctions and other ischaemic heart diseases after post-operative radiotherapy of breast cancer. In these patients, part of the heart is exposed to the target dose of 40 to 50 Gy, while the mean organ dose usually is only a few Gy given in very small fractions. After correction for fractionation effects using the linear quadratic model and the α/β ratio determined in experimental studies in the rat heart of 1 – 3 Gy, equivalent single doses to the total heart are about 1 – 2 Gy and thus very similar to the heart doses in the A-bomb survivors who developed fatal radiation-induced heart disease (Schultz-Hector and Trott, 2007).

The Surveillance, Epidemiology and End Results cancer registry data base provide unrivalled opportunities to study the effects of radiotherapy on radiation-induced cardiovascular diseases. They have repeatedly been analysed. Probably the first to compare the risk from radiotherapy according to whether the breast cancer had affected the left or the right breast was Paszat et al. in 1998. Also using the SEER data, the Oxford group of Darby et al. (2005) demonstrated the most significant evidence that the risk continuously increased with time after radiotherapy (Table 3).

Table 3: The risk of cardiovascular disease after post-operative radiotherapy of breast cancer (data from Darby et al., 2005)

Study design	308861 women included in the SEER programme who were treated for breast cancer between 1973 and 2001. 115165 (37%) had received radiotherapy as part of primary treatment. The response criterion was death from cardiovascular disease in relation to the laterality of the breast cancer (left versus right).		
Results	1. Of those 4130 women who died after >10 years, 1721 (42%) died from breast cancer, but 894 (22%) died from heart disease 2. Post-operative radiotherapy of left-sided breast cancer was associated with a 44% higher risk of death from cardiovascular disease compared to right-sided breast cancer 3. Mortality from radiation-induced heart disease increased with time after radiotherapy.		
Results of the patient group with >20 years follow-up			
Time after diagnosis Years	Cardiac deaths		Mortality ration Left vs. right
	Left	Right	
< 5	230	180	1.19
5 – 9	189	145	1.21
10-14	157	106	1.42
>15	234	145	1.58
At no time was there a difference of the cardiac mortality ratio left vs. right breast cancer in those patients who did not receive radiotherapy			

In the total cohort of more than 300.000 women who are recorded in this data base as being treated for early breast cancer between 1973 and 2001, about 115.000 received post-

operative radiotherapy as part of the primary treatment. Of those 4.130 women who died more than 10 years after radiotherapy, 1.721, that is 42 % died from recurrent breast cancer, but 894, that is 22%, half as many as from recurrent cancer, died from heart disease. Whereas the risk of death from recurrent breast cancer was the same after left- or right-sided cancer, the risk of death from heart disease was higher by 44% in those women who had cancer of the left breast than in those women who had cancer of the right breast. In absolute numbers, 359 women with right-sided breast cancer and 535 women with left-sided breast cancer died from heart disease. This is an excess of 176 deaths of which 44 are due to myocardial infarction and 72 from other ischaemic heart disease. All of this excess of fatal heart disease has to be attributed to the higher radiation dose to the heart in patients with left-sided breast cancer. In the 1970s, the mean heart dose for right-sided breast cancer from the tangential fields was in the order of 5 Gy, but for left-sided breast cancer this was about 10 Gy. The 5 Gy higher dose, given in fractions of <0.25Gy, after correction for fractionation is equivalent to an additional single dose of about 1.5Gy which could be regarded as the cause of the increased risk of cardiovascular death by 44%. In this study design, each patient was her own control - no other epidemiological protocol can provide such perfect control. The excess risk is not significant in the first 10 years after treatment but its significance and its magnitude increases progressively with follow-up time.

Because both surgical and radiotherapy procedures changed dramatically over the analysed period of time, the two decades between 1973 and 1982 and between 1983 and 1992 were also analysed separately. Confidence limits for the later period are large, but there is little evidence that the advances in radiotherapy techniques decreased the excess relative risk of radiation-induced heart disease significantly.

The second large data base used to investigate the risk of fatal radiation-induced heart disease after radiotherapy of breast cancer is that of the Early Breast Cancer Trialists´ Collaborative Group (EBCCG). This data base is particularly valuable as it is based on a large number of randomised clinical trials. The analysis of the cause specific mortality among 20.000 women at 10 to 20 years after primary treatment for breast cancer clearly demonstrated the superb effectiveness of adjuvant radiotherapy not only to reduce the risk of loco-regional treatment failure from 30% to 10%, i.e. by a factor of 3. (Table 4).

Table 4: Ratio of breast cancer deaths and non-breast cancer deaths in breast cancer patients treated with or without radiotherapy (data from EBCCG 2005)

		Follow-up	
		10 years	20 years
breast cancer free survival	with radiotherapy	63.4%	53.4%
	without radiotherapy	60.4%	48.6%
non-breast cancer-free survival	with radiotherapy	90.2%	73.8%
	without radiotherapy	89.2	69.5%

Also the risk of death from breast cancer, including death from distant metastasis was significantly reduced. However, this clinical benefit relating to death from cancer did not translate into any survival benefit because it was offset by a statistically significant increase of deaths from cardiovascular disease. These have to be ascribed to inadvertent irradiation of the coronary arteries, the carotid arteries and the micro-vasculature of the heart.

Also single institution studies such as those performed in the Netherlands Cancer Institute by Hooning et al.(2006 and 2007) provided important additional information, in particular with regard to treatment details. Whereas post-operative radiotherapy after mastectomy increased the risk of cardiovascular death two-fold, no increase was observed after post-operative radiotherapy when the surgical procedure was breast conserving surgery. This difference may be ascribed to different radiotherapy techniques leading to different dose volume relationships. Yet, a later study by the same group (Borger et al., 2007) did not find a significant influence of irradiated heart volume on cardiovascular radiation risk. It is becoming increasingly clear that although the large studies such as the SEER studies and the EBCCG studies were crucial in identifying and quantifying the importance of the problem, they cannot help solving the problem. The key problem of which anatomical structures are important for the risk and define dose response relationship can best be investigated in smaller but more detailed studies. The most important of those studies is the Radiation Associated Cardiovascular Events study, the RACE study.

Also radiotherapy of non-malignant disease has been shown to be a significant cause of radiation-induced heart disease. (Carr et al., 2005; Table 5)

Table 5: Cardiovascular mortality after radiotherapy for peptic ulcer (data from Carr et al., 2005)

Study design	Cohort study on 1470 patients treated between 1936 and 1965 for peptic ulcer with radiotherapy compared with 1568 patients treated with drugs			
Radiation doses	Radiation doses to the stomach were 8 – 18 Gy in fractions of 1.5 Gy Radiation doses to the heart were 1.6 – 3.9 Gy in fractions of 0.33 Gy			
DOSE DEPENDENCE OF CARDIOVASCULAR RISK				
Heart dose				
Absolute	Equivalent single dose *)	Number of patients	Cardiovascular deaths	RR
0 Gy	0 Gy	1568	484	1.0
1.6 Gy	1.2 Gy	363	94	1.0
2.3 Gy	1.4 Gy	384	97	1.2
2.8 Gy	1.7 Gy	341	114	1.5
3.9 Gy	2.2 Gy	382	121	1.5

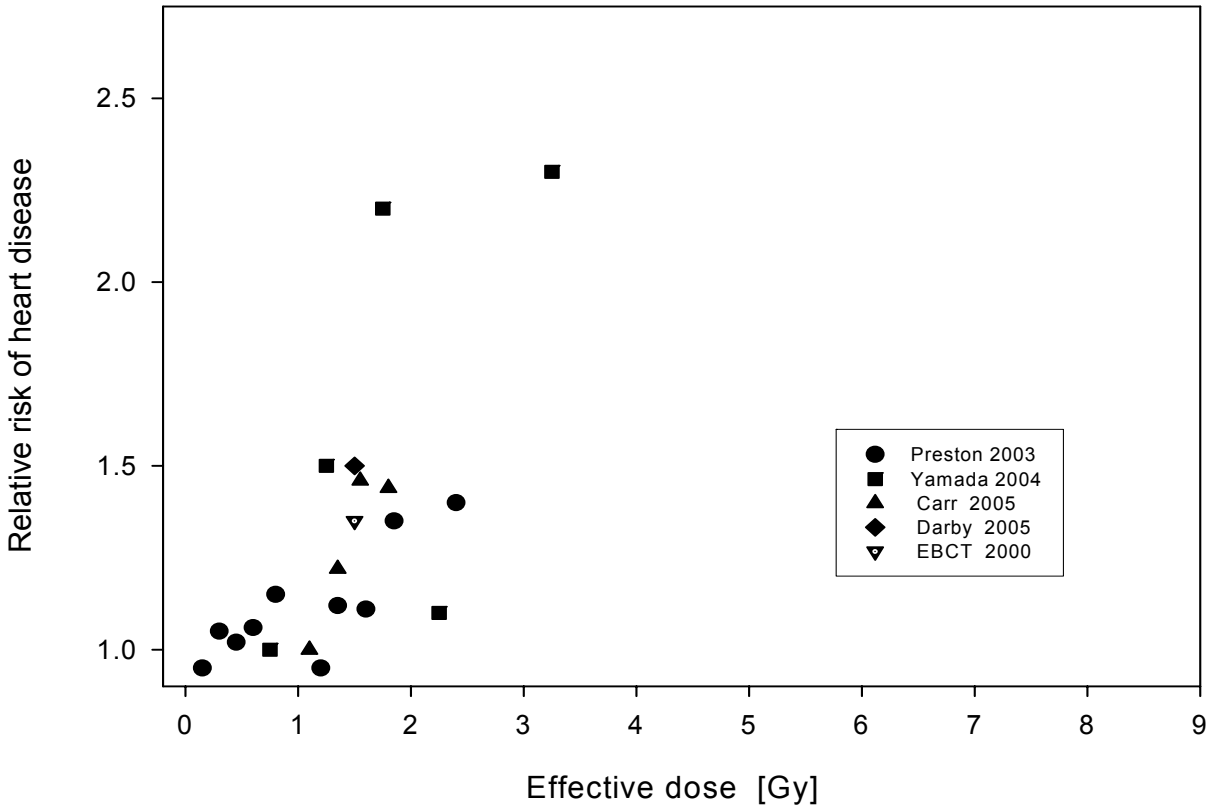
*) corrected for fractionation with the linear quadratic equation using an α/β ratio of 2 Gy

Between 1936 and 1965, nearly 1500 patients, suffering from peptic ulcer received fractionated radiotherapy to the stomach with a total dose between 9 and 18 Gy to reduce gastric secretion of hydrochloric acid. A similar number of patients suffering from the same disease but treated with drugs were selected as control group. After a latency of >10 years, mortality from coronary heart disease was significantly increased in the radiation group by 24%. Moreover, a significant relationship between the mean heart dose and the relative risk of mortality from coronary heart disease was calculated.

In all radiotherapy studies and scenarios, there is pronounced heterogeneity of doses within the heart. It has been demonstrated already by the Stockholm group ten years ago that dose

and volume appear to be important parameters defining cardiovascular radiation risk (Gyenes et al., 1998). As a first approximation we compared the results of the different studies, including the A-bomb survivor studies, by relating the reported relative risk of cardiovascular mortality to the estimated mean heart dose, but correcting the given dose for fractionation using the linear quadratic model which is the generally established standard procedure in clinical radiation oncology**.** (Figure 2).

Figure 2: The dependence of the relative risk of cardiovascular disease incidence (Yamada et al., 2004, A-bomb survivors) or mortality (all other studies) on the mean heart dose corrected for fractionation with the linear quadratic model. (Preston data on A-Bomb survivors, Carr data on radiotherapy for non-malignant disease, Darby and EBCCG data on radiotherapy for breast cancer. modified after Schultz-Hector and Trott, 2007).



Despite the great differences in dose distribution between all studies, the results of all studies fit surprisingly well to a common dose response relationship if the LQ-corrected mean heart dose is used as denominator of dose. This does, however, by no means prove that the mean heart dose is the relevant criterion for the estimation of cardiovascular radiation risks.

Current and planned research on radiation-induced cardiovascular disease in radiotherapy patients, particularly in the RACE project, concentrates on the relationship between local dose and risk, i.e. the determination of the dose at the site of damage development and thus the identification of the anatomical structures which are the targets that trigger damage development. Closely related is the question how the heart dose is to be reported and limited or constrained in radiotherapy and in radiation protection. Is it the mean heart dose, or the maximum heart dose, or the dose in particular anatomical structures of the heart, such as the

left anterior descending coronary artery which in most cases receives the highest radiation dose in radiotherapy of breast cancer? This is presently the most important issue in research on cardiovascular radiation risks, particularly in radiotherapy.

The RACE study (www.race.ki.se) is a large case control and a case/case study on those breast cancer patients from the Danish and the Swedish cancer registries who later developed severe heart diseases. Through linkage of cancer registry data and hospital discharge codings, many hundred women were identified who developed myocardial infarctions and other ischaemic heart diseases after being cured from breast cancer. The case control study with 1000 cases and 1000 controls, i.e. matched breast cancer cases but without heart disease, aims at identifying mainly the radiation dose relationship of cardiovascular risk. In contrast, the case/case study concentrates on the relationship between the localisation of the myocardial infarction or of the ischaemic lesion, and the anatomical dose distribution in the heart in the individual patient in order to define the target for dose definition and to suggest underlying mechanisms..

These aims of the RACE study require enormous effort to reconstruct, from stored treatment plans, the individual anatomical dose distributions. Several publications on this aspect of the problems by the RACE project, one published (Taylor et al, 2007), others submitted demonstrate that this is difficult but possible.

The individual mean heart doses and the doses for each of the three coronary arteries were estimated based on the individual stored radiotherapy charts which often also included photographs of the treatment fields and drawings of the actual dose plans. These individual doses will form the basis of the on-going case control study. A wide range of doses to the heart and the three coronary arteries were determined**.** (Table 6)

Table 6: Mean doses to the heart and the left anterior descending arteria coronaria in patients treated with left tangential radiotherapy for breast cancer (data from Taylor et al., 2007)

Mean dose	Number of patients	
	Heart	Coronary artery
<1 Gy	2	0
1 – 2 Gy	31	1
2 – 3 Gy	14	11
3 – 4 Gy	3	5
4 – 6 Gy	0	8
6 – 8 Gy	0	5
8 – 10 Gy	0	6
10 – 12 Gy	0	5
12 – 14 Gy	0	6
14 – 16 Gy	0	2
21 Gy	0	1

The greatest source of variability in cardiac dose estimation for any particular treatment plan was found to be the effect of differing patient anatomy, e.g. heart position in relation to breast, body fat and shape of the thorax. Nevertheless, the difference in heart dose produced

by anatomical variation was smaller than the difference produced by different radiotherapy regimes. Calculated mean heart doses changed very much over time. They were highest in the seventies and have continuously fallen since and continue to do so. This is due to changes in target definition, changes in treatment technique, and probably mostly due to growing awareness of the potential problem of radiation-induced heart disease for breast cancer patients, most of whom have a mean life expectancy of more than 20 years after cure, long enough to experience the clinical manifestation of their radiation risk.

The scientists working in the RACE project are confident that the wide range of coronary artery doses, as well as mean heart doses, with detailed information on morbidity and mortality from heart disease in the RACE study should provide solid clinical and dosimetric data for the development of reliable dose response relationships for several cardiac endpoints and several cardiac structures. These results are expected to enable the prediction of future cardiac risks associated with current and evolving radiotherapy regimens.

This approach is a model for future research into other normal tissue damage probabilities and also on second cancer risk in current and emerging treatment modalities in radiation oncology which is the main aim of the new ALLEGRO project which will start early next year.

Future clinical studies in radiotherapy patients could link the results of the mouse studies with the results of the epidemiological studies. The most promising approach are clinical studies based on modern non-invasive imaging procedures such as SPECT, PET and CT/PET.

Some recent studies using SPECT or PET imaging of micro-vascular perfusion demonstrated perfusion defects already within 6 - 12 months after breast cancer radiotherapy**.** (Table 7)

Table 7: Myocardial perfusion and other functional studies in the hearts of 36 young breast cancer patients 6 – 10 years after radiotherapy (data from Seddon et al., 2005)

Functional abnormality	Left-sided breast cancer	Right-sided breast cancer
perfusion defect	17/24	2/12
irreversible defect	10/24	0/12
abnormal wall motion	8/24	0/12
myocardial damage	10/24	0/12
coronary artery injury	10/24	0/12

More clinical studies are in preparation with the aim of relating those changes in functional imaging and their gradual development to the individual dose distribution.

Research in the field of cardiovascular radiation risks in radiotherapy has to integrate, as much as possible, clinical and epidemiological research with experimental studies in vivo and in vitro to analyse and to answer the critical open questions**:**

1. Is there a dose threshold of increased risk? Does the latency to clinical manifestation depend on dose as is suggested by experimental data? In other words? Is there a dose dependence of incidence or rather a dose dependence of damage progression rate?

2. What is the clinical nature of cardiovascular disease induced by different radiation doses and dose distributions to the heart? Is the pathology after low radiation doses different, or the same but developing more slowly, compared to that after high radiation doses?

3. In the radiotherapy studies, there are pronounced dose inhomogeneities within the heart. Which part of the heart is most radiosensitive and should be chosen as a reference point for tolerance doses in radiation oncology or for effective dose to be corrected with an organ weighting factor in radiation protection?

The current CARDIORISK project aims at addressing some of these questions experimentally in mice after local heart irradiation.

I conclude that clinical, epidemiological and functional imaging studies in radiotherapy patients have great potential to provide some essential evidence which could help to assess vascular and, in particular, cardiovascular radiation risks not only in radiation oncology but also after exposure to intermediate and to low radiation doses in radiation protection.

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2 EPIDEMIOLOGICAL EVIDENCE FOR CIRCULATORY DISEASES – NON-OCCUPATIONAL EXPOSURE

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2.1 Abstract

It has now been known for a number of years that an excess risk of blood circulatory system disease exists among the Japanese survivors of the atomic bombings of Hiroshima and Nagasaki, which is associated with the radiation dose received from the explosions. That moderate and high doses of radiation can increase the risk of circulatory disease, and other non-cancer diseases, as a result of deterministic tissue damage is unremarkable; but an unanticipated finding is that as follow-up has increased and data have accumulated the dose at which an excess risk of circulatory disease can be discerned has decreased so that now a raised risk is apparent below 2 Gy and the dose-response is compatible with a linear no-threshold relationship. A linear no-threshold dose-response implies an underlying stochastic biological mechanism (or mechanisms) and has important ramifications for radiological protection because the risk of circulatory disease would represent a significant proportion of the overall risk of radiation-induced mortality and morbidity. However, currently the data for the survivors are also consistent with a threshold dose of ~0.5 Gy, and 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 not possible to confidently select an appropriate dose-response model and the consequent implications for the risk (if any) at low doses. The data for circulatory disease among the Japanese atomic bomb survivors must be considered with other scientific evidence to permit an appropriate interpretation of the increased risk. However, the repercussions for the understanding of blood circulatory system disease in general, and for radiological protection in particular, of an increased risk of heart disease and stroke at low doses are sufficiently profound that studies of the bomb survivors and other exposed groups, together with research into potential biological mechanisms, needs to progress with some urgency.

2.2 Introduction

The epidemiological study of the Japanese survivors of the atomic bombings of Hiroshima and Nagasaki in August 1945 represents the “Gold Standard” for the investigation and quantification of the risks to health of exposure to ionising radiation, and it is upon the

evidence provided by this study that the risk estimates that underlie radiological protection are primarily (but not solely) based. Updates of the follow-up of the health of the Japanese atomic bomb survivors are periodically conducted by scientists from the Radiation Effects Research Foundation (RERF), a joint Japanese-US organisation based in Hiroshima and Nagasaki.

The main means of investigation is through the Life Span Study (LSS), which follows a cohort of ~87 000 survivors, ~48 000 of whom were non-trivially exposed to radiation during the explosions. The LSS cohort was established during the Japanese national census of October 1950 and includes individuals of both sexes and all ages drawn from a "healthy" general population, although men of an age when they could serve in the military would be of a lower proportion than in peacetime, and wartime conditions in Japan in 1945 would have led to a certain degree of malnourishment. Unlike those who are exposed to radiation for medical reasons, the survivors were not selected for irradiation because they were ill, or suspected of being ill, but were exposed because they were in the wrong place at the wrong time. Hence, selection biases that could be present among medically irradiated groups are absent from the Japanese atomic bomb survivors. However, other selection effects could be present in the LSS cohort, in particular biases arising from a "healthy survivor effect" whereby those entering the LSS had to be alive in October 1950 and have survived the difficult conditions in the aftermath of the bombings, and the health experience of this subgroup may not be typical of the entire group that survived the direct consequences of the explosions.

Extensive work on determining the organ-specific doses received by each survivor has been conducted, the most recent evaluation producing the DS02 doses. A wide range of doses, predominantly from a brief exposure to penetrating γ-rays although with some neutron component, was experienced by the LSS members. It is inevitable, however, that some uncertainty must result from this retrospective dose reconstruction exercise, and it has not proved possible to derive reliable doses for some survivors exposed in complex circumstances (for example, where individuals were in an environment that offered many opportunities for radiation shielding and reflection). Mortality and cancer incidence among the LSS cohort has been comprehensively studied and risk coefficients (excess risks per unit dose of radiation received as a consequence of the bombings) derived.

In addition to the Life Span Study the Adult Health Study (AHS), a subset of the LSS consisting of ~20 000 survivors, was established in 1958. Members of the AHS are given biennial health examinations, and this allows disease morbidity to be investigated for a variety of conditions. Just over half of the members of the AHS were present in Hiroshima or Nagasaki during the bombings and have DS86 doses assigned (using the dosimetry system that was in use before the DS02 was established), and attended two or more health examinations.

It has been known for several years that an excess radiation-associated risk of non-cancer diseases exists among the Japanese atomic bomb survivors, in particular a radiation-associated risk of blood circulatory system disease – cardiovascular disease (ischaemic (coronary) heart disease, hypertensive heart disease and valvular heart disease), cerebrovascular disease (stroke) and peripheral vascular disease. In this paper we examine the evolution of the evidence for a radiation-associated risk of blood circulatory system disease among the Japanese atomic bomb survivors and its interpretation.

2.3 The Evolution of Evidence

The first indication of an excess risk among the Japanese atomic bomb survivors of diseases other than cancer was reported in 1972 by Jablon and Kato who examined mortality during 1950-1970 in LSS Report 5. Jablon and Kato (1972) found that

> "Males showed no evidence of radiation effect on circulatory system disease mortality; but females did. Among females the mortality ratios for the entire 20-year period were elevated in all dose groups from 10 rads [100 mGy] up, and especially so above 50 rads [500 mGy]. Two of the differences are significant."

However, the data for mortality from blood circulatory system diseases presented by Kato *et al.* (1982) in LSS Report 6, covering deaths during 1950-1978 showed an overall variation with dose that was unremarkable, and no mention was made of the risk in particular subgroups of survivors, such as females.

Meanwhile, non-cancer morbidity in the AHS for the period 1958-1978 was examined by Robertson *et al.* (1979) and again by Kodama *et al.* (1984). They found that the incidence of myocardial infarction (heart attack) increased with radiation dose for females in Hiroshima, but a similar effect was not observed in Hiroshima males or in survivors from Nagasaki. The positive finding for Hiroshima females was considered to be tentative.

LSS Report 11, Part 3, by Shimizu *et al.* (1992) examined in detail non-cancer mortality among the Japanese atomic bomb survivors using deaths during 1950-1985 and DS86 doses. For mortality from all diseases other than cancer (and diseases of the blood), the just over 20 000 deaths for the entire study period displayed statistically significant upward curvature with dose, and a threshold dose-response model with a threshold at 1.4 Gy (90% confidence interval (CI): 0.6, 2.8) provided a significantly better fit than a linear no-threshold model; the excess of non-cancer mortality was confined to high doses in excess of ~2 Gy. When mortality was examined by three successive periods (1950-1965, 1966-1975 and 1976-1985) and by two age-at-time-of-bombing (ATB) groups (<40 years and ≥40 years), it was found that the excess in mortality was greatest in the younger ATB group in the latest period. Indeed, for the older ATB group in the earliest period the dose-response was J-shaped, i.e. a relative risk (RR) less than 1.0 in the 0-3 Gy dose range, indicative of a possible "healthy survivor" selection effect. Considering the data as a whole, mortality from circulatory disease showed a statistically significant increase with dose, largely due to heart disease rather than cerebral stroke, but for the <40 years ATB group during 1966-1985 the significant increase also extends to stroke. The excess in mortality at high doses in the <40 years ATB group and 1966-1985 period was also apparent for digestive disease and, to a lesser extent, respiratory disease. For all non-cancer mortality the RR at 2 Gy was estimated to be 1.06 (90% CI: 1.02, 1.09), and for the <40 years ATB and deaths during 1966-1985 subgroup the RR was 1.19 (90% CI: 1.10, 1.29).

Sposto *et al.* (1992) investigated whether the increase in non-cancer mortality with dose might be explained by death certificate misclassification. Of ~3000 cancer deaths and ~10 000 non-cancer deaths that occurred among the Japanese atomic bomb survivors during 1961-1975, just over one-third and about one-quarter, respectively, were the subject of *post-mortem* examinations. A comparison of the classification of death at autopsy with

that registered on the death certificate found that 22% of cancer deaths and 3.5% of non-cancer deaths were wrongly classified on the death certificate. However, Sposto *et al.* found that this level of dose-independent misclassification reduced the ERR at 1 Gy by ~20% only, from 0.063 to 0.0495, and that the increase in the risk of non-cancer disease with dose remained highly statistically significant. The authors observed that a comparatively small dependence of cancer misclassification upon dose would have a relatively large influence on the non-cancer dose-response, but that there was no clear evidence for such dependence.

Non-cancer disease incidence among the Japanese atomic bomb survivors during 1958-1986 was studied by Wong *et al.* (1993) using data from the AHS. Overall, there was little indication of an effect of dose upon circulatory disease, but given the results of Shimizu *et al.* (1992) for LSS mortality, Wong *et al.* examined circulatory disease morbidity among those <40 years ATB during 1966-1985, which resulted in a RR for myocardial infarction at 1 Gy of 1.40 (95% CI: 1.00, 2.30), while for 1968-1986 the RR at 1 Gy was 1.57 (95% CI: 1.26, 2.76). No dose-related effect was found for those ≥40 years ATB. Wong *et al.* commented,

> "The two [LSS mortality and AHS incidence] results together indicate that both the fatal and nonfatal forms of coronary heart disease are possible consequences of exposure to high level of ionizing radiation."

In LSS Report 12, Part II, Shimizu *et al.* (1999) considered just over 27 000 deaths from diseases other then cancer and blood disorders during 1950-1990, 54% of which were due to circulatory diseases. Statistically significant linear trends with dose were found for circulatory (both stroke and heart disease), digestive and respiratory diseases. Shimizu *et al.* found evidence of a decrease in the rate of non-cancer mortality during 1950-1960 among those receiving low-to-moderate doses (<2 Sv), resulting in a J-shaped curve, which they attributed to a "healthy survivor effect". As a consequence, they derived risk coefficients for the period 1966-1990, since the selection effect had largely disappeared by the mid-1960s, and reported a 10% increase in the non-cancer mortality rate at 1 Sv. The shape of the dose-response was, however, unclear, the data being statistically compatible with linearity, upward curvilinearity and a threshold at ~0.3 Sv, so that the excess risk at low doses (if any) is uncertain.

Shimizu *et al.* (1999) observed that confounding is less likely to provide an explanation for a statistical association when small areas are considered because the resulting study groups are likely to be more homogeneous in terms of potential confounding factors, and when the analysis was confined to those ~61 000 survivors exposed within 3 km of the hypocentre the dose-response was still significant and with a slope similar to that for the entire cohort. Indeed, a raised risk was also found for ~3000 survivors exposed within 0.9-1.2 km of the hypocentre. Nonetheless, these subgroups received generally moderate-to-high doses, so cast little light on the risk at low doses. When possible confounding factors such as smoking and physical activity, obtained from mail surveys and interview data, were used to adjust the dose-response for non-cancer mortality their inclusion had little effect upon the risk coefficient, the adjusted estimates being within 10% of the unadjusted estimates. For example, the ERR/Sv unadjusted for smoking status was 0.083 whereas the adjusted ERR/Sv was 0.079. Shimizu *et al.* concluded:

> "Taken together, the strong indications of a dose response even when analyses are limited to proximal survivors and the minimal impact of some potentially important confounding factors for which mail survey data are available suggest that the association between noncancer mortality in the LSS is unlikely to be an artefact of confounding."

Currently, the most recent study of non-cancer mortality among the Japanese atomic bomb survivors is presented in LSS Report 13 by Preston *et al.* (2003), who investigated the influence of radiation exposure upon almost 32 000 deaths from non-cancer diseases (excluding blood disorders) during 1950-1997. Again, the presence of a "healthy survivor effect" in the early years of follow-up had to be taken into account, the selection effect producing a highly statistically significant J-shaped dose-response for non-cancer mortality during 1950-1967. However, the selection effect had essentially disappeared by 1968, the dose-response for 1968-1997 showing no statistically significant deviation from linearity. As a consequence, Preston *et al.* confined their analysis of non-cancer mortality to the period 1968-1997, presenting results with and without an adjustment for a small, but statistically significant, variation in the background rate with distance from the hypocentre. Although the data are consistent with a linear no-threshold dose-response for non-cancer mortality, the evidence for any effect below around 0.5 Sv is equivocal: formal analysis provided no evidence against a no-threshold dose-response, although the data could not exclude a threshold of ~0.6 Sv. Preston *et al.* concluded:

> "These results suggest that radiation effects on LSS noncancer mortality 25 or more years after exposure can be adequately described by a linear dose-response model with risk increases of about 14% per Sv."

A statistically non-significant difference in ERR coefficient for non-cancer mortality was found between men (ERR/Sv = 0.11 (90% CI: 0.04, 0.18)) and women (0.17 (90% CI: 0.10, 0.24)), and a non-significant decrease of ERR with age-at-exposure was also apparent: -0.15 (90% CI: -0.36, +0.11) per decade increase of age-at-exposure.

Table 1 shows how the ERR coefficient for all non-cancer mortality of 0.14 Sv^{-1} (90% CI: 0.08, 0.20) breaks down by various major causes. Circulatory diseases (both heart disease and stroke) are significantly elevated, but respiratory and digestive diseases are also significantly associated with radiation dose. This lack of specificity of the non-cancer effect could be a reason to doubt a causal interpretation of the association of circulatory disease with radiation dose, although an increase in risk for other non-cancer diseases is a possible explanation that needs to be considered.

Table 1. Cause-specific estimates of the excess relative risk (ERR) coefficients for mortality from non-cancer diseases during 1968-1997, applying a linear no-threshold dose-response model to data for the Japanese atomic bomb survivors in the Life Span Study (LSS) (Preston *et al.*, 2003).

Non-cancer Disease	ERR.Sv^{-1} (90% Confidence Interval)
Heart disease	0.17 (0.08, 0.26)
Stroke	0.12 (0.02, 0.22)
Respiratory disease	0.18 (0.06, 0.32)
-Pneumonia	0.16 (0.00, 0.32)
Digestive disease	0.15 (0.00, 0.32)
-Cirrhosis	0.19 (-0.05, 0.50)
Infectious disease	-0.02 (<-0.2, 0.25)
-Tuberculosis	-0.01 (<-0.2, 0.4)
Other diseases	0.08 (-0.04, 0.23)
-Urinary diseases	0.25 (-0.01, 0.60)
All non-cancer	**0.14 (0.08, 0.20)**

Of the 14 459 non-cancer disease deaths among the proximal survivors during 1968-1997, 273 are associated with radiation-exposure based upon the linear, no-threshold dose-response. Of these 273 non-cancer disease deaths, 165 are due to circulatory disease (101 from heart disease and 64 from stroke), 57 from respiratory disease, 27 from digestive disease and 24 from other non-cancer diseases. By way of comparison, Preston *et al.* (2003) predicted that during 1950-1997 there were 440 radiation-associated deaths among a total of 9335 deaths from solid cancers among the Japanese atomic bomb survivors.

Yamada *et al.* (2004) considered non-cancer disease incidence among the bomb survivors during 1958-1998 using data from the Adult Health Study. DS86 doses were used for 10 339 survivors, almost half (5035) of whom were found to have raised blood pressure, although a linear increase in hypertension with dose did not achieve formal statistical significance: RR = 1.04 (95% CI: 0.99, 1.09). However, the RR at 1 Sv was significant under a quadratic dose-response model: 1.03 (95% CI: 1.00, 1.06). Neither other cardiovascular diseases nor cerebrovascular diseases showed a significant variation with dose, although the various circulatory diseases exhibited a tendency to increase in incidence with dose; but when attention was concentrated on myocardial infarction during 1968-1998 among those under 40 years ATB the increased RR for the incidence of heart attack at 1 Sv was significantly raised under a quadratic dose-response model, at 1.25 (95% CI: 1.00, 1.69). Of interest are the results obtained when adjustment for tobacco smoking and alcohol drinking were carried out for quadratic dose-response models: for hypertension the risk at 1 Sv increased in statistical significance, at 1.03 (95% CI: 1.01, 1.06), while for myocardial infarction during 1968-1998 among those <40 years ATB the risk at 1 Sv was no longer significant, at 1.17 (95% CI: 0.97, 1.56).

Data collected at the biennial examinations conducted as part of the Adult Health Study have permitted the investigation of clinical and sub-clinical factors that could be indicative of underlying biological mechanisms that may be responsible for the statistical association between circulatory disease and radiation dose. For example, Wong *et al.* (1999) reported a difference in mean total serum cholesterol levels between exposed and unexposed subjects in the AHS during 1958-1986; Sasaki *et al.* (2002) found an association between radiation exposure and blood pressure measured during 1958-1986; Yamada *et al.* (2005) reported an association between radiation exposure and aortic, but not carotid artery, atherosclerosis from examinations made of Hiroshima residents during 2000-2002. Elevated levels of the pro-inflammatory cytokines IL-6, CRP, TNF-α and INF-γ, but also increased levels of the (generally) anti-inflammatory cytokine IL-10, have been observed in the Japanese atomic bomb survivors (Hayashi *et al.*, 2003, 2005; Kusunoki and Hayashi, 2008). There is also a dose-related elevation in erythrocyte sedimentation rate and in levels of IgG, IgA and total immunoglobulins in this cohort, all markers of systemic inflammation (Hayashi *et al.*, 2005) and consistent with the hypothesis that inflammation initiates the cardiovascular disease process (Ross 1999a, b). It has been proposed that infections play a role in circulatory disease (Gura, 1998; Ridker, 1998), so it is of interest that certain T-cell and B-cell population numbers are known to vary with radiation dose among the bomb survivors (Kusunoki *et al.*, 1998). Given the implied involvement of the immune system in cardiovascular disease, this suggests that the whole body dose (or possibly the red bone marrow dose) might be the most relevant dose with respect to the risk of circulatory disease. The atomic bomb survivors also demonstrate dose-dependent decreases in levels of $CD4^+$ helper T-cells (Hayashi *et al.*, 2003); decreased levels of helper T-cells have also been found in blood samples from Japanese atomic bomb survivors with myocardial infarction (Kusunoki *et al.*, 1999).

Little (2004) examined the non-cancer mortality data from the LSS for 1968-1998 with respect to the nature of the dose-response and its dependence upon dosimetry uncertainties. In particular, Little investigated four dose-response models for heart disease and stroke: linear threshold, quadratic threshold, linear-quadratic threshold and power of dose. There was no statistically significant non-zero threshold dose, and no significant departure from linearity, for either heart disease or stroke.

Tatsukawa *et al.* (2008) used AHS data to study circulatory disease incidence during 1978-2003 among ~500 survivors exposed *in utero* as compared with ~1000 survivors exposed as children (<10 years ATB). Increases in the RR with dose were reported for hypertension and for myocardial infarction or stroke among the *in utero* group, but these increases were far from being statistically significant. Among those exposed as children, however, the increase in the incidence of hypertension with dose was of borderline statistical significance and the RR of myocardial infarction or stroke at 1 Gy was highly significant, at 1.72 (95% CI: 1.24, 2.40). It should be appreciated when considering these results that the *in utero* exposed group was still under 60 years of age at the time of the study and therefore yet to enter the age of greatest risk from circulatory disease.

Currently, researchers from the RERF are analysing the latest data on non-cancer disease mortality and incidence in the LSS and AHS. The additional deaths and cases may shed further light on the risk of circulatory diseases and other non-cancer diseases among the Japanese atomic bomb survivors, in particular the nature of the dose-response at low doses.

2.4 Summary of the current position

It has now been known for a number of years that the Japanese atomic bomb survivors have experienced an excess risk of blood circulatory system disease that is associated with the radiation dose received from the explosions. That moderate and high doses of radiation can increase the risk of circulatory disease is not especially surprising since the relevant tissues could have received long-term damage as a result of such doses. What is surprising is that as follow-up has increased the dose at which an excess risk of circulatory disease can be detected has decreased so that now a raised risk is apparent below 2 Gy and the dose-response is compatible with a linear no-threshold relationship. A linear no-threshold dose-response has clear implications for the nature of the underlying biological mechanism(s) and substantial implications for radiological protection because the risk of circulatory disease would represent a significant proportion of radiation-induced mortality and morbidity. However, currently the data for the survivors are also consistent with a threshold dose of ~0.5 Gy, and 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 not possible to confidently select an appropriate dose-response model and the consequent implications for the risk (if any) at low doses.

There are a number of factors that complicate the interpretation of the risk of circulatory disease among the Japanese atomic bomb survivors. First, the radiation-associated risk of non-cancer diseases is not confined to circulatory disease but is also apparent for respiratory and digestive diseases, and linear no-threshold dose-response models are also compatible with the data for these other non-cancer diseases. Although it is possible that radiation increases the risk of a range of non-cancer diseases, especially at high doses, the lack of specificity in the apparent action of radiation does raise doubts over a causal interpretation of the findings. Further data may clarify the position. Second is the influence of the "healthy survivor" selection effect upon the results. There is no doubt that this selection effect has a substantial impact upon the data for the early years of follow-up, which is why data for the period prior to the late-1960s are frequently excluded from analyses of non-cancer diseases. However, this does raise the question of the full impact of the exclusion of these early deaths and cases upon risk estimates, and how much influence a residual selection effect may have upon later data. In combination with the *post hoc* selection of particular diseases and age groups, this must cast some degree of doubt over the interpretation of the reported associations with radiation dose. Further, although some adjustment for potential confounding factors has been carried out in analyses, it cannot be claimed with confidence that these adjustments can exclude confounding as at least contributing to the associations with radiation dose. Thus, at present, bias and confounding cannot be reliably eliminated as possible explanations for the radiation-associated risk of non-cancer diseases among the bomb survivors; but neither can a direct cause-and-effect interpretation.

In conclusion, as data on non-cancer diseases among the Japanese atomic bomb survivors have increased, the evidence for a raised risk of blood circulatory system disease at low doses has grown. However, the data are consistent with both a linear no-threshold dose-response and a threshold at ~0.5 Gy and, in the absence of an appropriate biological mechanism to explain an increased risk at low doses, it is difficult to see how a reliable distinction can be made between these possibilities from the bomb survivor data alone. It is clear that the data for circulatory disease among the bomb survivors must be considered with

other scientific evidence to permit an appropriate interpretation. However, the repercussions for science and radiological protection of an increased risk of circulatory disease at low doses are sufficiently profound that studies of the bomb survivors and other exposed groups, together with research into potential biological mechanisms, needs to progress with some urgency.

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3 EPIDEMIOLOGICAL EVIDENCE FOR CIRCULATORY DISEASES – OCCUPATIONAL EXPOSURE

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3.1 Abstract

This paper commences with a short overview of studies of occupational radiation exposure and circulatory disease risk and highlights limitations of these studies. It then describes a database of workers at the Mayak Production Association in Russia and summarises a new analysis of circulatory disease morbidity and mortality among these workers in relation to radiation exposure. The Mayak findings are compared with those from previous studies and future research needs are outlined.

3.2 Summary of findings from studies published up to mid-2008

Circulatory disease risks have been considered among several types of radiation workers. These studies have been considered in reviews by McGale and Darby (2005, 2008), UNSCEAR (2008) and Little et al (2008). As will now be described, the findings from these studies have been mixed.

3.2.1 Radiologists and radiologic technologists

Several studies have examined long-term mortality among workers exposed in the medical field. Information on individual doses was generally missing from these studies, particularly for exposures received many years ago. Consequently, analyses have been conducted according, for example, to the period during which radiation work is likely to have commenced, since doses have tended to fall over time. A study of around 90 000 radiologic technologists in the United States found higher mortality from circulatory disease among those who started work in the first half of the 20th century when compared with those who started later, having adjusted for potential confounding factors such as smoking and alcohol consumption (Hauptmann et al, 2003). Within the former group of workers, annual doses might have ranged up to 0.25 Gy per year; research to reconstruct doses is in progress (Simon et al, 2006). However, similar trends in mortality over time have not been seen in studies of radiologists in the United Kingdom and the USA (McGale and Darby, 2005). Furthermore, circulatory disease mortality among these workers was often less than expected from national

rates and, in the case of early UK radiologists (Berrington et al, 2001), was less than that among other medical practitioners.

3.2.2 Radon-exposed miners

Studies of circulatory disease among miners exposed to radon in the course of their work have given mixed results (UNSCEAR, 2008). By far the largest study was of 59 000 German uranium miners (Kreuzer et al, 2006). This showed no association between cardiovascular disease mortality and cumulative exposure to radon, external gamma radiation or long-lived radionuclides. In contrast to some other occupational studies, the miners received internal as well as external radiation exposures. However, doses to the heart and arteries are likely to have been fairly low (roughly <100 mSv on average), particularly when compared with doses to the lung. Furthermore, it has not been possible in these studies to adjust for potential confounding factors.

3.2.3 Nuclear workers

The largest cohort studied to date consisted of about 275 000 workers from 14 countries (Vrijheid et al, 2007). The findings for circulatory disease mortality from this analysis were consistent both with no raised risk and with a risk of the size seen in the Life Span Study (LSS) of Japanese atomic bomb survivors. However, the statistical power of this analysis was low, owing to the relatively short follow-up (the average age at end of follow-up was 46 years) and the relatively low mean cumulative external dose (20.7 mSv).

In the United Kingdom, McGeoghegan et al (2008) examined non-cancer mortality among about 42 000 radiation workers at British Nuclear Fuels plc (BNFL). Compared with the international study of Vrijheid et al (2007), the mean cumulative external dose was higher (53.0 mSv) and - in spite of the much smaller cohort of workers (many of whom were in the international study) - the numbers of circulatory disease deaths did not differ greatly between the two analyses because of the longer follow-up of BNFL workers. In contrast to the international study, the BNFL cohort included workers with internal exposures, although the analysis focussed on external doses. Whilst circulatory disease mortality was less than expected from regional rates, there was a statistically significantly increasing trend in risk with increasing external dose. However, the interpretation of this finding is complicated by heterogeneity in the estimated trend between sub-groups of workers. Other than for a proxy measure of social class, it was not possible to adjust for potential confounding factors in the above two analyses.

Other analyses of mortality among nuclear workers have involved smaller numbers of deaths and/or formed part of the afore-mentioned international study. Ivanov et al (2006) have analysed circulatory disease *morbidity* among Chernobyl recovery operation workers in Russia. The findings from this analysis and from the studies of nuclear workers mentioned above are summarised in Tables 5 and 6.

3.2.4 Limitations of studies to date

There have been two main types of problem with the occupational studies conducted to date. First, there is scope of bias or confounding in many of the studies, due to:

- The “Healthy Worker Effect”, which complicates comparisons with national mortality rates;

- The reliance solely on mortality data in most studies, which may lead to the misclassification of specific types of circulatory disease; and/or

- The general lack of information on known risk factors for circulatory disease, such as smoking, alcohol consumption,

The second problem relates to low statistical power, in that many of the studies restricted in terms of cohort size, length of follow-up and/or range of doses. Furthermore, the Japanese A-bomb survivors LSS suggests that the raised risk of circulatory disease would be lower – in relative terms – than that for cancer (Preston et al, 2003); in particular, that this relative increase would be less than 10% for doses below 0.5 Sv, so making detection and quantification difficult. Nevertheless, it is important to recognise that, because circulatory disease is so common, a small relative risk may represent an absolute excess risk similar to that for cancer.

3.3 Background to the Mayak worker cohort

The Mayak Production Association (PA) was the first Russian nuclear facility and is located 10 km from the city of Ozyorsk in the Southern Urals. Mayak PA started operation in June 1948 and included all the plant necessary to produce weapon-grade plutonium: reactors, radiochemical plant, plutonium plant and auxiliary plants.

From the first days of operations at Mayak, a special system of personnel medical observation was undertaken which included an obligatory pre-employment medical examination and routine medical examinations of all the workers based on a common standard program. This system of medical observation of Mayak personnel health allowed a unique archive of primary medical data to be accumulated. As a consequence of the long-term and careful storage of medical follow-up data, a unique medical-dosimetric database entitled “Clinic” for the cohort of Mayak workers chronically exposed to radiation was established in the late 1990s. Quality control checks were an essential part of the creation and maintenance of the medical-dosimetric database and continue to be performed on a regular basis.

The structure of the “Clinic” database and the basic data currently contained in the database have been described in detail elsewhere (Azizova et al, 2008). The main blocks of data are: identification and passport data; work history and dosimetry data; medical history; vital status; data on initial health status at the pre-employment medical examination; clinical data for the whole period that the worker was resident in Ozyorsk, including morbidity data; and data on reproductive function and family etc.

The “Clinic” medical dosimetry database forms the basis of research to estimate risks of morbidity and mortality from circulatory diseases up to the end of 2000 in the cohort of workers first employed at the main facilities of Mayak PA in 1948-1958 in relation to external and internal radiation, whilst allowing for age, gender and non-radiation risk factors (Azizova et al, 2009a,b). This work has been conducted within the framework of the SOUL project, supported by the European Commission’s 6th Framework Programme (Euratom) and the Federal Medical Biological Agency (Russian Federation).

3.4 Characteristics of the study cohort

3.4.1 Study population

Characteristics of the study cohort are given in Table 1.

Table 1. Characteristics of the study cohort.

	Number	%
Workers included in the cohort	12210	100.0
Females	3552	29.1
Migrants from Ozyorsk	6557	53.7
Vital status known	10789	88.4
Died	5685	52.7
Died in Ozyorsk	3009	52.9
Autopsy performed	1948	34.3
Autopsy performed in Ozyorsk	1868	95.9
Cause of death known	5317	93.5
Alive as of 31 December 2000	5104	47.3
Alive and lived in Ozyorsk as of 31 December 2000	2548	49.9
Medical documentation (morbidity data) available	11597	95.0

The cohort included 12210 individuals, 29.1% of whom were females. As of 31 December 2000, about half of the study cohort had migrated from Ozyorsk and vital status was known for about 88% of workers. Among those with known vital status, 52.7% of them died and 47.3% were alive. It should be emphasized that follow-up data for Ozyorsk residents are nearly complete. The autopsy rate was about 34% for the whole cohort, and about 96% of all autopsies were conducted for workers who died in Ozyorsk. Cause of death was known for about 93% of workers known to have died. Morbidity data were available for 95% of cohort.

3.4.2 Dosimetry

Individual monitoring of exposures to external gamma doses was conducted from the beginning of operations at Mayak. Regular monitoring of internal exposure among those who worked with transuranium radionuclides began later, during the 1960s. The results of individual monitoring of external and internal exposure were recorded in individual dosimetric cards and journals. The data containing in these documents formed the basis for the dosimetric database for “Mayak” PA workers. The history and stages of establishment of the dosimetric database, the main principles of its organization, structure, and methods of external and internal dose estimation are presented elsewhere (Vasilenko et al, 2007a,b; Bess et al, 2007; Smetanin et al, 2007a,b; Khokhryakov et al, 2000; Khokhryakov et al, 2003; Lyzlov et al, 1996; Vasilenko et al, 2001).

The recent dosimetric system – *Mayak-Doses 2005* – which was created in the framework of Russian-American radiation health effects research sponsored by DOE’s Russian Health Studies Program and conducted under the authority of the Joint Coordinating Committee for Radiation Research (JCCRER), formed the basis of the dose estimates used in this study.

Annual external gamma doses are available for 99.9% of workers in the study cohort. The average total external gamma dose for the whole employment period was 0.91±0.01 Gy (range 0-5.92 Gy) for males and 0.65±0.01 Gy (range 0-5.70 Gy) for females.

Plutonium body burden was measured (and estimates of internal doses were subsequently derived) only for 30% of workers who were in contact with transuranium radionuclides. Therefore analyses of internal exposures were restricted to monitored workers. The absorbed dose to liver was used in analyses of internal exposure as surrogate for dose to blood vessels/heart; although these doses would differ, they should be highly correlated. The average absorbed liver dose from internal alpha exposure was 0.40±0.02 (range 0-17.90 Gy) for males and 0.81±0.13 Gy (range 0-127.82 Gy) for females.

3.4.3 Quality control

As mentioned above, quality control checks were obligatory and conducted on a regular basis. For this analysis, specific checks were conducted on:

- identification of the worker cohort;
- dosimetry;
- non-radiation risk factors;
- follow-up.

These checks showed a high level of data accuracy and completeness, e.g.:

- Level of data loss was only about 2.5%;
- Expert reviews of samples of circulatory diseases diagnosis showed high levels of diagnostic verification (98.8% for acute myocardial infarction and 94.9% for stroke);
- It is estimated that only 1.7% of cases of circulatory diseases were missed in the database;
- A comparison of smoking and alcohol consumption data from different sources showed good agreement (93-95%).

Errors that were identified in the course of these checks were corrected.

3.4.4 Follow-up

The start of follow-up was the date of first employment at one of the main plants of Mayak PA. The end of follow-up was the earliest of:

- 31 December 2000;
- Date of first diagnosis of circulatory disease (for the morbidity analysis);
- Date of death;
- Date of migration from Ozyorsk (for the morbidity analysis);
- Date of last known vital status.

3.4.5 End points

The effects studied were ischemic heart diseases (IHD; ICD9: 410-414) and cerebrovascular diseases (CVD; ICD9: 430-438). Table 2 shows the numbers of deaths or cases and corresponding numbers of person-years for analyses of risks of circulatory disease morbidity and mortality.

Table 2. Numbers of deaths or cases and corresponding numbers of person-years for analyses of risks of circulatory disease morbidity and mortality.

	Number of cases	Number of person-years
Mortality - IHD	1495	443350
Mortality - CVD	753	443350
Morbidity - IHD	3751	205249
Morbidity - CVD	4418	197344

3.5 Methods for analysing risks

The analyses involved calculating relative risks (RRs) for categories of one or more factors, having adjusted for other variables. These relative risks were calculated by maximum likelihood, using the AMFIT module of EPICURE (Preston et al, 1993). 95% confidence intervals for the RRs and p-values from tests of statistical significance were obtained via likelihood-based methods, using AMFIT. Attention was initially directed to non-radiation factors, following which measures of radiation exposure were analysed with adjustment (through stratification) for non-radiation factors. Analyses of internal radiation exposures were restricted to workers known to have been monitored for possible plutonium exposure.

In addition to the categorical analyses, models for trends in disease rates with level of radiation exposures were also fitted to the data, using Poisson regression methods. These models again were fitted using the AMFIT module in EPICURE. In particular, the excess relative risk (ERR) (i.e. the relative risk minus 1) was modelled by a linear trend with external or internal dose, with adjustment for non-radiation factors.

Sensitivity analyses were conducted to examine the impact of:

- modifying the set of non-radiation factors for which adjustment was made in the analyses of radiation factors;
- restricting the mortality follow-up (like that of morbidity) to Ozyorsk, because some migrants were lost to follow-up and because of lower autopsy rates among those who left the city;
- adjusting for internal dose in analyses of external dose and vice versa;
- using various lag periods for external and internal doses.

Furthermore, examination was made of how radiation risks might vary by gender and between plants at Mayak.

3.6 Analysis of non-radiation risk factors

It is known that circulatory diseases are multifactorial diseases. For this reason, mortality and morbidity risk analyses initially examined known non-radiation factors such as gender, age, smoking, hypertension, etc. These analyses showed the impact on mortality and morbidity from circulatory diseases in the study cohort of factors such as:

- Gender
- Age
- Hypertension

- Increased body mass index
- Smoking.

Such factors were taken into account in the subsequent analyses.

3.7 Analysis of radiation risk factors

3.7.1 External exposure

Table 3 shows that the risk of morbidity was statistically significantly increased for workers with a total dose from chronic external gamma ray exposure above 1 Gy for IHD, and above 0.5 Gy for CVD, when compared with doses less than 0.5 Gy.

Table 3. Relative Risks & 95% CI for categorical analyses of external dose.

	IHD (vs. <0.5 Gy)	
	0.5 - 1.0 Gy	> 1.0 Gy
Mortality	0.92 (0.78, 1.08)	1.11 (0.96, 1.30)
Morbidity	1.02 (0.92, 1.13)	1.20 (1.09, 1.32)
	CVD (vs. <0.5 Gy)	
	0.5 - 1.0 Gy	> 1.0 Gy
Mortality	1.15 (0.92, 1.43)	0.99 (0.80, 1.24)
Morbidity	1.14 (1.04, 1.25)	1.60 (1.47, 1.75)

There was a statistically significant increasing trend in IHD morbidity with increasing total external dose (Figure 1). There was also a statistically significant increasing trend in CVD morbidity with increasing total external dose (Figure 2).

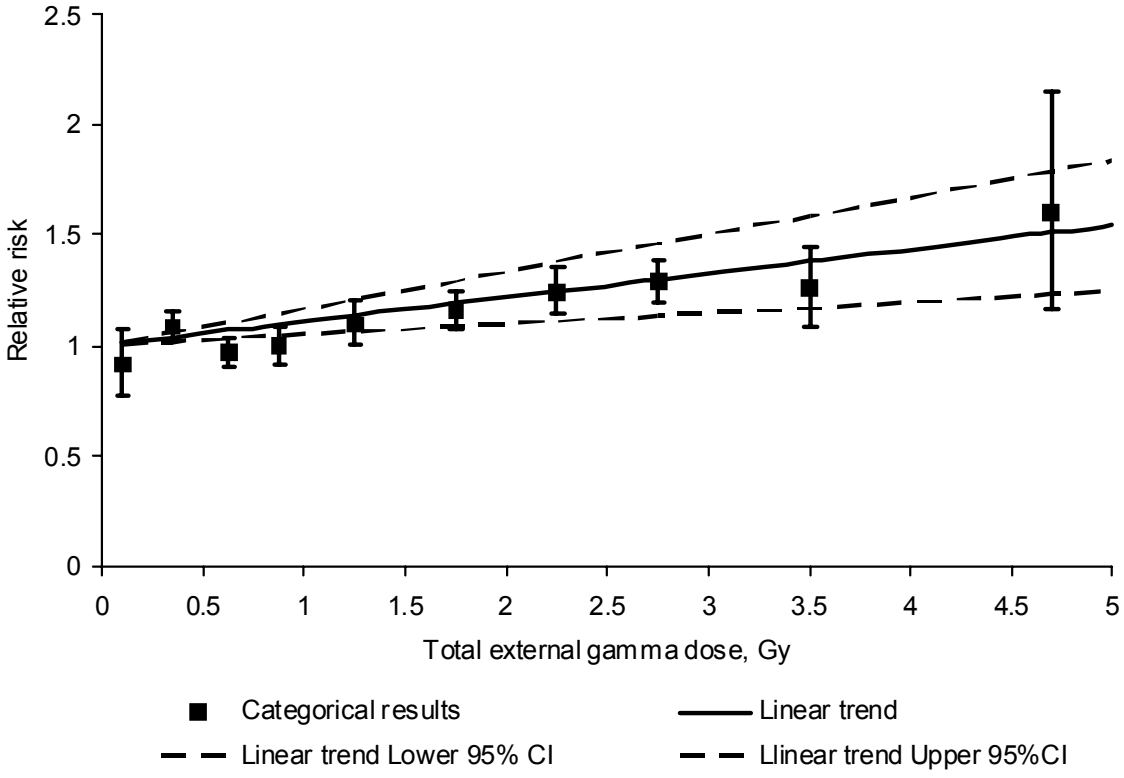

Figure 1. IHD morbidity in relation to total external gamma dose.

ERR/Gy = 0.109 (95% CI 0.049-0.168)

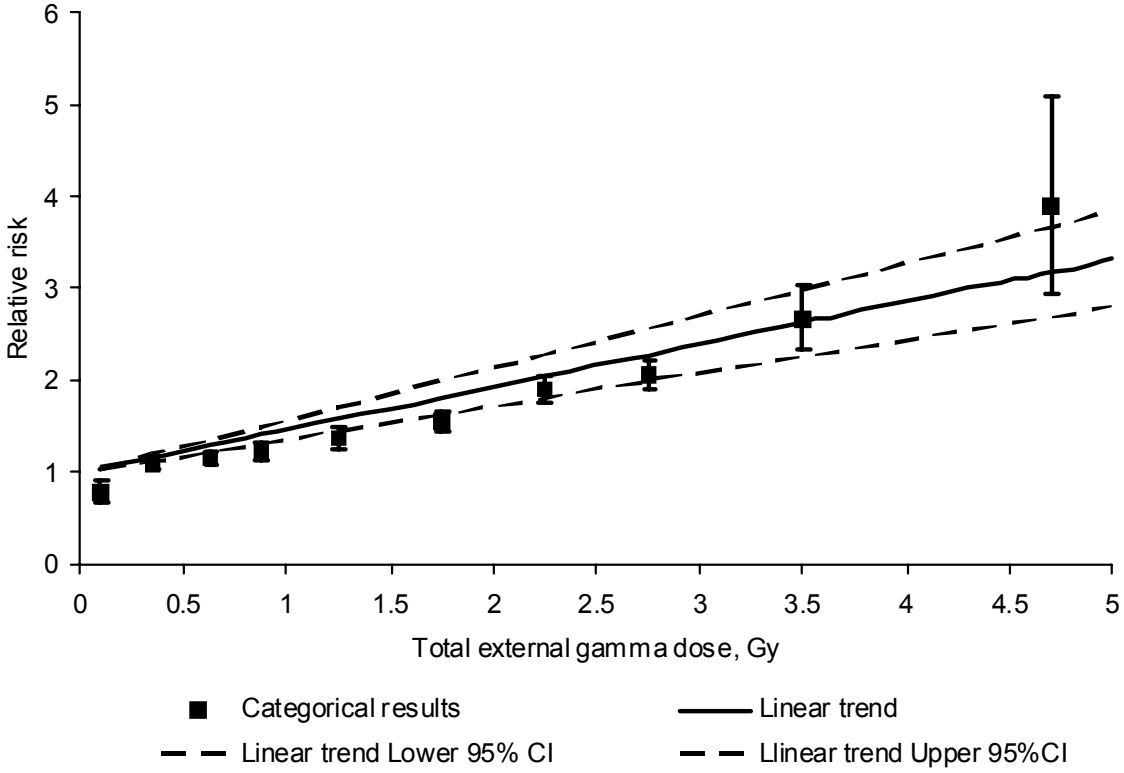

Figure 2. CVD morbidity in relation to total external gamma dose.

ERR/Gy = 0.464 (95% CI 0.360-0.567)

The findings for IHD morbidity and CVD morbidity did not vary greatly when adjusting for extra non-radiation factors or for internal dose or when using different lag periods.

The raised risk of IHD morbidity was seen mainly in males, but the findings were consistent across genders. However, the risk of CVD morbidity was statistically significantly raised among both males and females.

3.7.2 Internal exposure

As seen from Table 4, risks of both mortality and morbidity from IHD and CVD were raised among workers with total absorbed doses to the liver from internal alpha exposure above 0.1 Gy, when compared with workers monitored for such exposures who had lower doses.

Table 4. Relative Risks & 95% CI for categorical analyses of internal liver dose.

	IHD (vs. <0.1 Gy)	
	0.1- 0.5 Gy	> 0.5 Gy
Mortality	1.33 (1.08, 1.64)	1.59 (1.16, 2.19)
Morbidity	1.17 (1.06, 1.30)	1.23 (1.04, 1.45)
	CVD (vs. <0.1 Gy)	
	0.1- 0.5 Gy	> 0.5 Gy
Mortality	1.40 (1.02, 1.92)	1.05 (0.61, 1.80)
Morbidity	1.23 (1.13, 1.35)	1.58 (1.35, 1.85)

There were statistically significant increasing trends with increasing total internal alpha dose to the liver in IHD mortality (Fig.3) and in CVD morbidity (Fig.4).

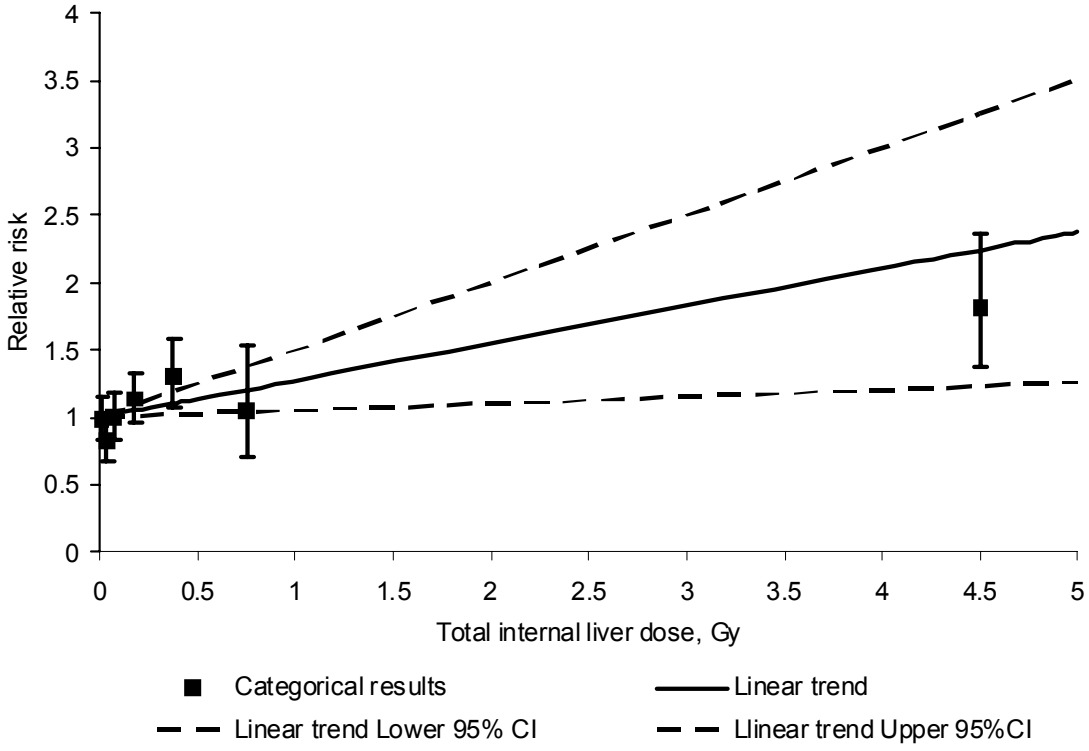

Figure 3. IHD mortality in relation to internal liver dose.

ERR/Gy = 0.275 (95% CI 0.050-0.501)

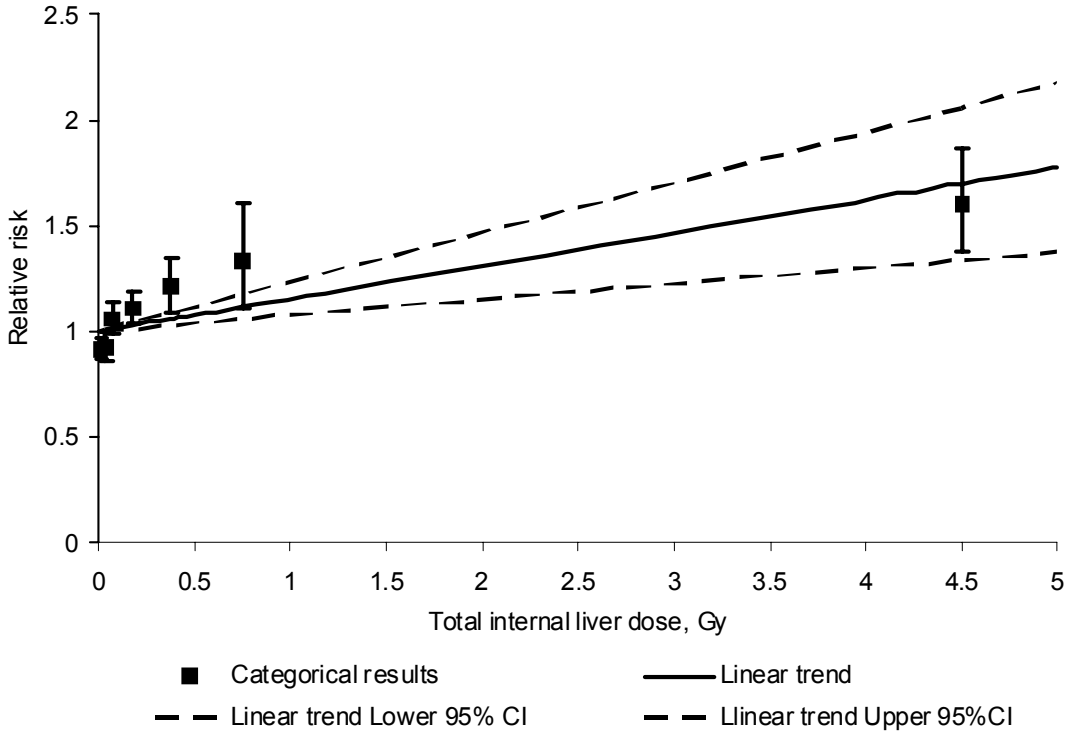

Figure 4. CVD morbidity in relation to internal liver dose.

ERR/Gy = 0.155 (95% CI 0.075-0.235)

For IHD mortality, there was little change in the internal dose results when adjusting for extra non-radiation factors or using different lag periods. However, the ERR/Gy was lower and not statistically significant after adjusting for external dose. For CVD morbidity, there was little change in the internal dose results when adjusting for extra non-radiation factors or for external dose. The ERR/Gy for CVD morbidity increased with increasing lag period and raised risks were seen separately among workers at the radiochemical plant and at the plutonium plant.

3.8 Comparison of findings across studies

Table 5 shows IHD findings from various studies of external radiation exposure. It can be seen from this Table that the estimates of the ERR/Gy from the various studies are mostly consistent. In particular, the morbidity findings for Mayak workers are consistent with morbidity and mortality results for the Japanese atomic bomb survivors. The wide confidence interval from the international nuclear worker study means that these results are not greatly informative, whilst the morbidity data reported by Chernobyl recovery operation workers – although yielding a trend estimate higher than that found here – are consistent with the Mayak findings. The only finding that is inconsistent with the Mayak results arises for workers at BNFL in the UK (McGeoghegan et al, 2008), where a higher estimate of the ERR/Gy was found.

Table 5. Comparison of IHD findings from various studies of external exposure.

Cohort	Mean cumulative dose (Gy)	Mortality or morbidity?	Number of deaths or cases	ERR/Gy
Japanese A-bomb survivors: LSS	0.20	Mortality	4 477	0.17 (90% CI 0.08, 0.26)
Japanese A-bomb survivors: Adult Health Study	0.57	Morbidity	1 546	0.05 (95% CI -0.05, 0.16)
Mayak workers (this study)	0.84	Mortality	1 495	0.07 (95% CI -0.02, 0.15)
Mayak workers (this study)	0.84	Morbidity	3 751	0.11 (95% CI 0.05, 0.17)
Nuclear workers (international)	0.018	Mortality	5 821	-0.01 (95% CI -0.59, 0.69)
BNFL workers (UK)	0.053	Mortality	3 567	0.70 (90% CI 0.33, 1.11)
Chernobyl recovery operations workers (Russia)	0.109	Morbidity	10 942	0.41 (95% CI 0.05, 0.78)

Table 6 shows cerebrovascular disease findings from the same studies. Whilst the estimated ERR/Gy for mortality among Mayak workers is consistent with the A-bomb survivor findings, otherwise the occupational studies point towards higher risk estimates. In particular, the precise estimate for cerebrovascular disease morbidity among Mayak workers is consistent with the morbidity estimate for Chernobyl recovery operation workers and the mortality estimates from the international and BNFL studies. Although the latter two findings are not statistically significant, expanding the BNFL analysis to include deaths with CVD as a

contributory cause (rather than solely as underlying cause) did achieve statistical significance (ERR/Gy 0.66, 90% CI 0.17, 1.27) (McGeoghegan et al, 2008).

Table 6. Comparison of CVD findings from various studies of external exposure.

Cohort	Mean cumulative dose (Gy)	Mortality or morbidity?	Number of deaths or cases	ERR/Gy
Japanese A-bomb survivors: LSS	0.20	Mortality	3 954	0.12 (90% CI 0.02, 0.22)
Japanese A-bomb survivors: Adult Health Study	0.57	Morbidity	729	0.07 (95% CI -0.08, 0.24)
Mayak workers (this study)	0.84	Mortality	753	-0.02 (95% CI -0.12, 0.07)
Mayak workers (this study)	0.84	Morbidity	4 418	0.46 (95% CI 0.36, 0.57)
Nuclear workers (international)	0.018	Mortality	1 224	0.88 (95% CI -0.67, 3.16)
BNFL workers (UK)	0.053	Mortality	1 018	0.43 (90% CI -0.10, 1.12)
Chernobyl recovery operations workers (Russia)	0.109	Morbidity	12 832	0.45 (95% CI 0.11, 0.80)

In interpreting the results for occupational studies shown in these tables, it should be remembered that the Mayak analysis is the only one in which adjustment was made for factors such as smoking and alcohol consumption.

3.9 Conclusions and future research needs

Raised risks of circulatory disease have been found among workers at Mayak PA in relation to *external radiation dose*, having adjusted for non-radiation factors and internal dose, and *internal radiation dose*, having adjusted for non-radiation factors and (in the case of CVD morbidity) for external dose.

The risk estimates for external radiation are generally compatible with those from other large occupational studies and/or the Japanese A-bomb survivors. However, more powerful information is needed on the effects of protracted exposures at lower doses, whilst allowing for non-radiation factors. Among Mayak workers, this topic is currently being addressed by:

- expanding the cohort to include workers employed after 1958, who tended to receive lower doses than earlier workers;
- extending the period of follow-up until 31 December 2005; and
- considering diagnostic medical exposures.

3.10 Acknowledgements

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4 INVESTIGATION OF BIOLOGICAL MECHANISMS OF RADIATION INDUCED CIRCULATORY DISEASES

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4.1 Introduction

The cardiovascular system has long been regarded as radioresistant organ because of the extremely low proliferative activity of endothelial cells and connective tissue cells (Lauk and Trott 1990) and the postmitotic state of cardiac myocytes.

With increasing success of radiotherapy and longer follow-up times, however, a variety of cardiovascular symptoms were observed:

- Early radiation techniques for Hodgkin's disease, involving doses in excess of 50 Gy to the anterior pericardium, were associated with pericarditis occurring at months to years following radiation. These effects could be avoided with the advent of more modern radiotherapy techniques and will therefore receive no further consideration in this paper.

- Older autopsy studies in radiotherapy patients reported diffuse fibrotic alterations of the myocardium, and valves which also appear to be less frequent in recent times.

- Increasing evidence, as summarised by Trott in this issue, associates radiation with an increased incidence of arteriosclerosis (Adams et al 2003, Schultz-Hector and Trott 2007, Little et al 2008). Heart and coronary arterial doses associated with RT treatment of Hodgkin's disease or breast cancer can reach >40 Gy (McGale and Darby 2005). In recent years, however, there is growing epidemiological evidence of excess risk of cardiovascular disease at much lower radiation doses and occurring over much longer intervals after radiation exposure in the Japanese atomic bomb survivor Life Span Study (LSS) cohort (Wong et al 1993, Preston et al 2003, Yamada et al 2004) as well as other cohorts of medically or occupationally exposed individuals (McGale and Darby 2005, Cardis et al 1995, Howe et al 2004, Ivanov et al 2006, McGeoghegan et al 2008). However, at present the epidemiological evidence available for an effect of moderate and low doses is suggestive rather than cogent (Little et al 2008). Thus, in the absence of a firm biological mechanism, caution is required in the interpretation of the statistical associations. On the other hand, a causal relationship can by no means be excluded, and therefore further research is urgently required to better understand the nature of the epidemiological associations.

Risks associated with the exposure to moderate and low doses and dose-rates of ionizing radiation have generally been assumed to be dominated by cancer risks in the directly exposed individuals.

The mechanisms by which low doses of ionizing radiation cause cancer are reasonably well understood, being fundamentally driven by mutational damage to DNA (UNSCEAR 2000 Report), although a role for non-DNA targeted effects cannot be ruled out (Morgan 2003). The pathogenesis of radiation induced cardiovascular disease after <10 Gy on the other hand, has not been studied in detail.

In this paper we review the available evidence and currently ongoing radiobiological research for a causal interpretation of the epidemiological associations between low and moderate dose radiation exposure and cardiovascular disease with special emphasis on possible biological mechanisms.

4.2 Analysis of published experimental data

4.2.1 Radiation induced arteriosclerosis

Clinical symptoms of coronary heart disease or peripheral arteriosclerosis are not specific to radiation and in the individual patient it is therefore not easily possible to attribute symptomatic arteriosclerosis to radiation as opposed to other causes. Early animal studies on cardiovascular radiation effects on the other hand, were not designed to look at arteriosclerosis, I.e. coronary arteries or peripheral arteries were not systematically analysed. Furthermore, laboratory rodents are known to be fairly resistant to arteriosclerosis – possibly due to the absence of any life-style associated risk factors.

Therefore, a causal relationship has only been more firmly established by recent studies:

Stewart et al. (2006) have studied radiation-induced arteriosclerosis in an arteriosclerosis prone mouse model carrying a genetic defect in lipoprotein metabolism, the APOE-/- mouse. In this mouse strain the low density lipoprotein receptor APOE is impaired, leading to elevated plasma cholesterol levels and development of arterial lesions. The APOE-/- mouse is the most extensively studied animals model of human arteriosclerosis.

Local radiation of the carotid arteries of APOE-/- mice with a single dose of 14 Gy caused an earlier onset and faster growth of arteriosclerotic plaques in the irradiated vessels, while it had no effect on cholesterol levels or atherosclerotic lesions in non-irradiated arteries. Histology revealed increased signs of plaque instability such as intra-plaque haemorrhage or macrophage accumulation in the irradiated carotid arteries. Since plaque instability and disruption is strongly associated with clinical symptoms of arteriosclerosis, this finding is particularly relevant. These studies were then extended to a clinically relevant fractionated radiation (20x2 Gy) as well as to a lower single dose of 8 Gy (Hoving et al 2008). The results show that fractionated irradiation as well as a lower dose of 8 Gy accelerated the development of atherosclerosis in ApoE-/- mice and predisposed to the formation of an inflammatory, thrombotic plaque phenotype. The inflammatory plaque alterations were less pronounced after 8 Gy as compared to 14 Gy, suggesting a dose effect. This study is the first

to reveal a causal relationship between radiation and arteriosclerosis, indicating radiation as an independent risk factor of arteriosclerosis.

This conclusion is strongly supported by an unpublished study in radiotherapy patients (Russel et al, unpublished), where arterial biopsies from within and distant to therapeutic radiation fields are being compared in a careful histological analysis.

4.2.2 Myocardial capillary network

There is both experimental and clinical evidence implying clinically relevant radiation effects on the myocardial microcirculation:

In two clinical studies, myocardial perfusion was studied after breast cancer radiotherapy revealing inhomogeneities and local defects. In a longitudinal study, 114 patients underwent SPECT (single photon emission computed tomography) cardiac perfusion scans before and at 6, 12, 18 and 24 months after radiotherapy for left sided breast cancer. The incidence of new perfusion defects were increased with follow-up time as well as irradiated heart volume. A follow-up study (Prosnitz et al 2007) showed that lesions were persistent at 3 to 6 years after radiation. None of the patients in either study had symptomatic heart disease. It is conceivable, however, that a lower than normal local perfusion should predispose to development of tissue damage secondary to arteriosclerotic changes.

In experimental studies on radiation effects in the rat heart, clinical heart failure occurs at dose dependent latent times after high single doses of > 15 Gy. Heart failure is due to focal myocardial degeneration, which in turn in preceded by a decrease in capillary density (Fajardo 1971, Lauk 1987). Long-term experimental studies showed, that a single dose of 15 Gy, which does not cause any clinical symptoms of radiation-induced heart disease in rats, is causing a moderate but significant decrease in capillary density which is persistent after more than 600 days following heart irradiation.

After 20 Gy, morphometric capillary counts start to decrease at 2-3 weeks following radiation. At about the same time, there is a wave of increased endothelial cell proliferation (Lauk and Trott 1990), a focal loss of the endothelial cell marker enzyme alkaline phosphatise as well as ultrastructural signs of endothelial cell activation (Schultz-Hector and Balz 1994). Capillary loss is a likely experimental, i.e. morphological correlate of the perfusion defects observed in radiotherapy patients. Whether or not these findings have any relevance to lower than therapeutic radiation doses and whether or not they do aggravate functional consequences of coronary artery disease is not known and will be addressed in ongoing studies (see below).

4.2.3 Endothelial cells

Both, macrovascular and microvascular radiation effects involve the endothelium and pro-inflammatory signalling cascades. A great number of studies have investigated radiation effects on endothelial cells in vitro and a few studies have confirmed some of these observations in vivo (summarized in Schultz-Hector and Trott 2007). Doses of >0.5 Gy appear to cause transient over-expression of a adhesion molecules such as E-selectin, PECAM and ICAM-1, of cytokines including IL-6, IL-8 (van der Meeren 1999), TGF-ß (Milliat et al 2006) as well as increased release of the prothrombotic von Willebrand factor (Boerma et al 2004). The transcription factor NFkB, which also plays a pivotal role in the pathogenesis of arteriosclerosis in the absence of radiation, has been shown to be involved in radiation

induced up-regulation of adhesion molecules (Hallahan et al 1998). Furthermore, foam cell formation could show to be activated by radiation independent from endothelial cells (Katayama et al 2008). The relevance of these findings to large vessel arterial endothelium in vivo is not known; the factors involved, however, indicate the possibility of radiation enhancing several steps in the molecular pathogenesis of arteriosclerosis.

Furthermore, observations of pro-inflammatory radiation effects are restricted to doses of > 0.5 Gy. For lower radiation doses there is experimental and clinical evidence of an opposite, anti-inflammatory effect (Rödel et al 2008). It would be important which variables besides radiation dose are responsible for opposite effects of different dose ranges of radiation.

Endothelial cell activation and lymphocyte adhesion observed in myocardial capillaries in vivo after experimental heart irradiation with 20 Gy, is consistent with the pro-inflammatory signalling phase described above and could be involved in initiating endothelial cell proliferation.

4.2.4 Conclusions from published data

In conclusion, radiation to the cardiovascular system is associated with acceleration and de-stabilization of arteriosclerotic lesions and may also cause a decrease in myocardial perfusion. The pathogenesis of both effects needs to be further investigated; pro-inflammatory endothelial cell reactions could play a role in the initiation of both. Observation of perfusion effects are limited to high doses and should be studied at lower doses. The endothelium apparently plays a pivotal role. However, endothelial effects are very difficult to analyze: in situ measurements may lack in sensitivity and specificity while observations in cell culture or in model systems are of limited relevance because they fundamentally alter the proliferation state of endothelial cells. Therefore, no clear picture emerges from the relatively large number of studies published.

4.3 Ongoing European experimental studies

4.3.1 Concept and preliminary results from NOTE (FP6, 09/2006 – 10/2010

NOTE is an integrated project which intends to investigate the mechanisms and biological impact of non-targeted effects after low doses. The project is funded by the European Commission in the 6th Framework Program for Nuclear Research and Training. The NOTE-consortium brings together 20 research organisations from Europe and Canada involved in the discovery, characterisation and mechanistic investigation of non-targeted effects of ionising radiation in cellular, tissue and animal models and is coordinated by Prof. Dr. S Salomaa (STUK Helsinki, Finland).

The consortium includes groups active in microbeam studies, groups advanced in radio-biological approaches and groups involved in biophysical and mathematical modelling of non-targeted effects. The consortium has access to a wide range of advanced radio-biological facilities, including several sources of particle and low LET radiation, two charge particle and one soft X-ray microbeams, low dose rate animal exposure facility, confocal and 3-photon microscopes, equipment for non-invasive 3D deep tissue imaging, broad range of molecular biology methods, gene arrays, proteomics and transcriptomics technologies.

The general objectives of the NOTE project are: (1.) to investigate the mechanisms of non-targeted effects, in particular, bystander effects, genomic instability and adaptive response; (2.) to investigate if and how non-targeted effects modulate the cancer risk in the low dose region, and whether they relate to protective or harmful functions; (3.) to investigate if ionizing radiation can cause non-cancer diseases or beneficial effects at low and intermediate doses; (4.) to investigate individual susceptibility and other factors modifying non-targeted responses; (5.) to assess the relevance of non-targeted effects for radiation protection and to set the scientific basis for a modern, more realistic, radiation safety system; and (6.) to contribute to the conceptualisation of a new paradigm in radiation biology that would cover both the classical direct (DNA-targeted) and non-targeted (indirect) effects.

NOTE research activities are organised in six work packages. Four work packages (WPs 2-5) are problem-oriented, focussing on major questions relevant for the scientific basis of the system of radiation protection. The integration activities provided by WP6 strengthen the collaboration by supporting the access to infrastructures, mobility and training.

WP7 provides dissemination and exploitation activities in the form of workshops and a public website. Managerial activities (WP1) ensure the organisation and structures for decision making, monitoring of progress, knowledge management and efficient flow of information and financing *(Figure 1)*.

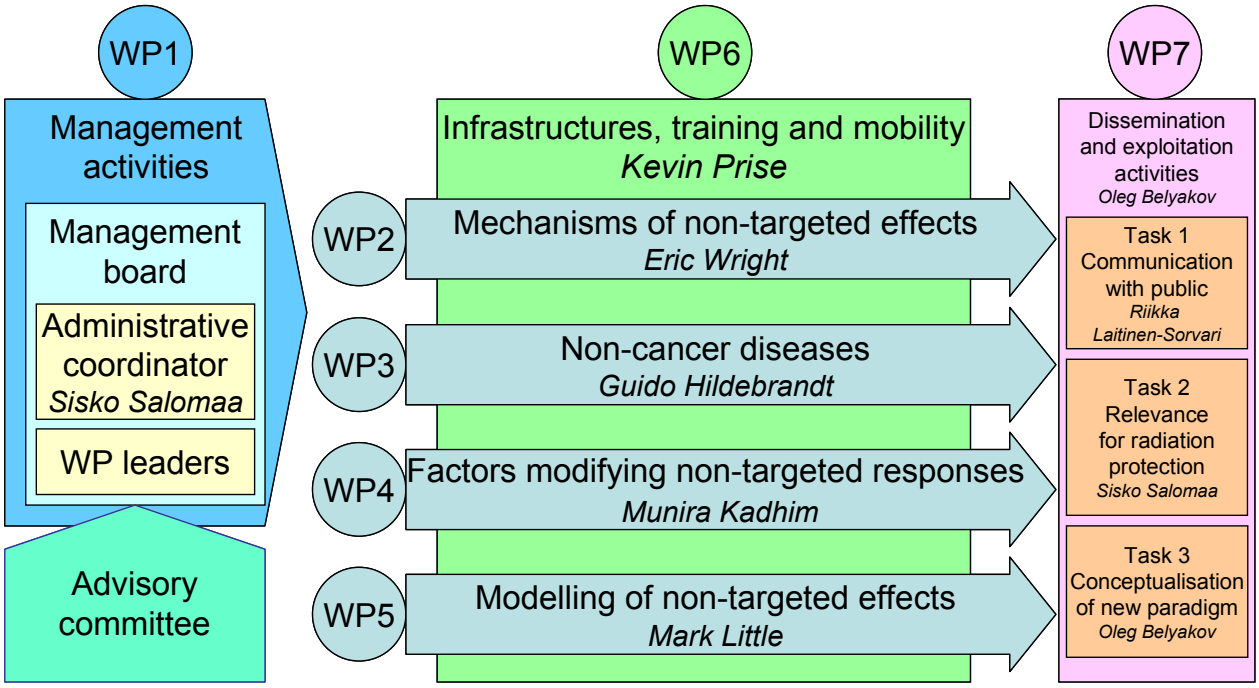

Figure 1: General outline of the NOTE project organisation.

Epidemiological studies could demonstrate that ionising radiation (IR) as a long-term dose-dependent effect may induce an impairment of the immune system as well as a persistent inflammatory profile that could increase the risks of both cancer and non-cancer diseases. Therefore, the main objective of WP3 is to study whether low and intermediate doses of IR can cause non-cancer diseases or beneficial effects in several experimental systems in vitro and in vivo to provide qualitative and quantitative data for mathematical modelling, to better understand low dose risks.

A central component of the experimental work is the investigation of mechanisms of cardiovascular disease induction after low dose exposure in vivo and in vitro.

The main objective of one task of WP3 within the NOTE project is to prove the hypothesis whether ***low dose total body IR*** might modulate atherosclerosis progression depending on (a) genetic background and (b) stage of disease at time of exposure, and furthermore whether this might be mediated by modulation of stress and/or inflammatory responses.

Thus, the experiments are designed to investigate effects of ***low dose total body IR*** on atherosclerosis progression after low dose rate exposure (1 mGy/min, 100 mGy/day, 5 days/week) as compared to high dose rate exposure (0.36 Gy/min) in three genotypes of mice in vivo. Animals are either prone to the diseases (ApoE-/-), normal (ApoE+/+), or knockouts heterozygous for TP53 (ApoE-/- TP53 +/-), and will be exposed to several radiation doses (0, 0.025, 0.05, 0.1, 0.5, 2.0 Gy) either at early stage or late stage disease *(Figure 2)*.

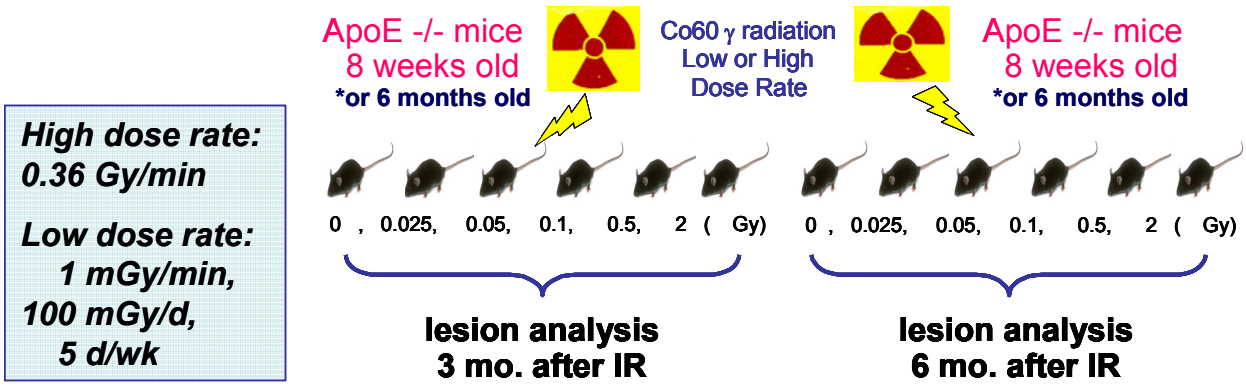

Figure 2: Radiation doses, dose rate, time points of exposure and time points of lesion analysis for the animal experiments. Animals are either prone to atherosclerosis (ApoE-/- TP53+/+), normal (ApoE+/+ TP53+/+) or knockouts heterozygous for TP53 (ApoE-/- TP53+/-).

Since inflammatory and thrombotic changes in endothelial cells have an important impact on the development of atherosclerotic lesions a further objective is to study whether ***low dose total body IR*** at low dose rate or high dose rate induces vascular changes in the hearts of ApoE-/- mice.

Time kinetics of atherosclerosis development and progression will be monitored by morphological (lesion area, number of lesions, and lesion stage) and functional endpoints to establish dose response curves for both high dose rate and low dose rate radiation *(Figure 3)*.

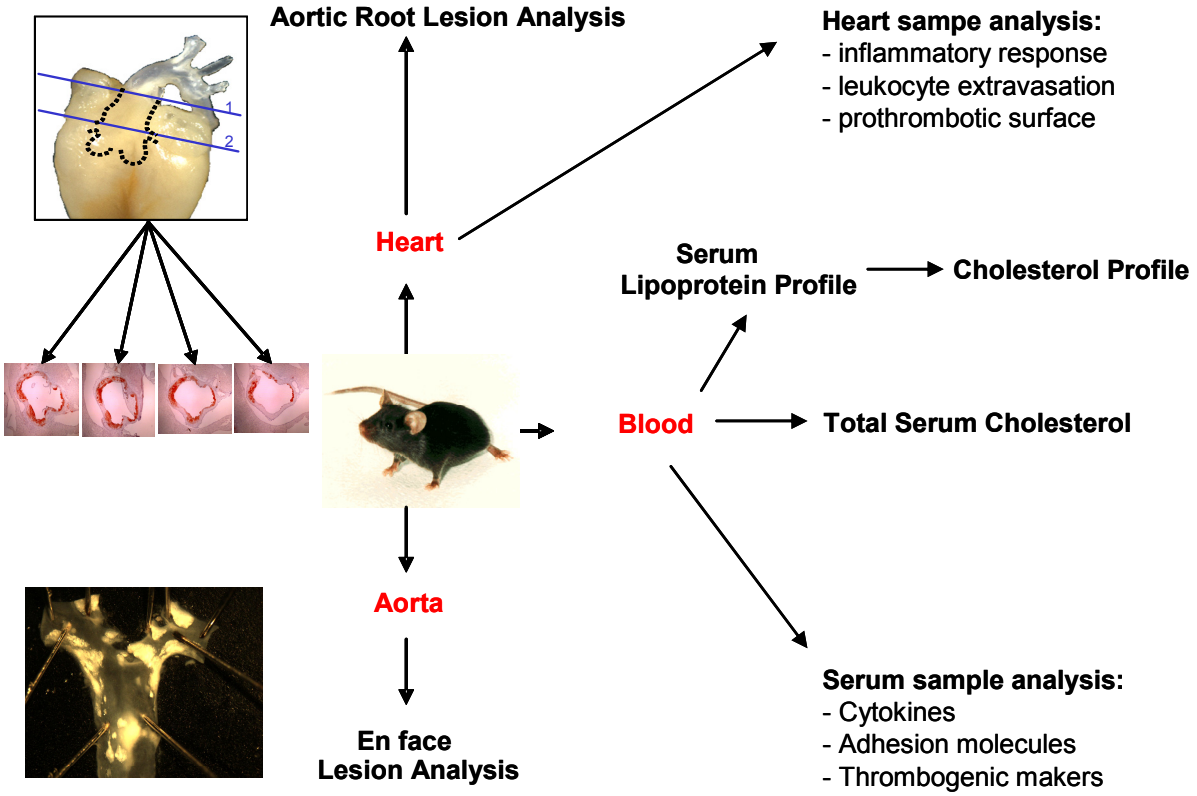

Figure 3: Design and endpoints of the animal experiments.

The preliminary analysis of the experimental data obtained so far within the first 24 months can be summarized as follows:

1. IR at early stage disease of **ApoE-/- Trp53+/+**, **Apo E-/- Trp53+/-,** and **ApoE+/+ Trp53+/+** with doses between 0.025 and 2 Gy at high dose rate or low dose rate either 3 months or 6 months after IR did not affect FPLC serum cholesterol distribution nor serum total cholesterol levels as compared to control.

2. IR at early stage disease of **ApoE-/- Trp53+/+**, **ApoE-/- Trp53+/-**, and **ApoE+/+ Trp53+/+** with doses of radiation between 0.025 and 2Gy at high dose rate or low dose rate either 3 months or 6 months after IR did not affect atherosclerotic lesion development on the luminal surface of the *en face* aorta, compared to mice not exposed to radiation.

3. No atherosclerotic lesions were observed in the aortic root of **ApoE+/+ Trp53+/+** at both low dose rate and high dose rate exposure at either 3 or 6 months after IR.

4. In **ApoE-/- Trp53+/+** exposed at early stage disease to doses of radiation between 0.025 and 2Gy at both low dose rate and high dose rate, there is a trend for no difference in lesion size in the aortic root of mice 3 months after IR, but there is a statistically significant decreased atherosclerotic lesion development in the aortic root of mice 6 months after IR, as compared to mice not exposed to radiation.

5. In **ApoE-/- Trp53+/-** exposed to doses of radiation between 0.025 and 2Gy at low dose rate, there is a statistically significant decrease in atherosclerotic lesion development in the aortic root 3 months after IR. Although this difference is statistically significant, it is important to note that the trend that is observed in these results is for no difference in atherosclerotic lesion development in these p53+/- ApoE-/- mice.

Preliminary statistical analysis of the raw data available so far indicates that there are highly significant ($p<0.00001$) differences between the control (0 Gy) and higher dose groups (0.025-2 Gy) in mean lesion size at 6 months for **ApoE-/- Trp 53+/+**, and also even if less obvious but still significant ($p<0.01$) differences between the control (0 Gy) and higher dose groups (0.025-2 Gy) in mean lesion size at 3 months for **ApoE+/+ Trp 53+/-**.

In addition, further experimental results demonstrate significant differences in peripheral markers of inflammatory response, leukocyte extravasation and prothrombotic surface at both 3 and 6 months after IR in **ApoE-/- Trp 53+/+** which received 0.05 Gy or more. Therefore, it is important to know what happens at earlier times and lower doses so some shorter term samples are needed.

Thus, the morphological data have to be consolidated within the next year, and plasma sample analysis needs to be expanded towards earlier time points after radiation exposure and even lower doses will be included.

4.3.2 Concept of CARDIORISK (FP7, 02/2008 – 01/2011)

CARDIORISK is a collaborative project which specifically intends to investigate the mechanisms of cardiovascular risks after low radiation doses. The project is funded by the European Commission in the 7th Framework Program for Nuclear Research and Training (FP7-Fission-2007-3.1.1). The CARDIORISK-consortium consists of 12 partners across Europe and is coordinated by Prof. Dr. M. Molls (Department of Radiation Oncology of Technische Universität München, Germany).

The aim of this collaborative research project is to elucidate the pathogenesis of early and late alteration in the microcirculation of the heart and of atherosclerotic lesions in arteries after exposure to low radiation doses as compared to high doses. Special emphasis is given on the investigation of early molecular, pro-inflammatory and pro-thrombotic changes as well as perfusion alteration, cardiac cell integrity and immunologic influences.

To achieve the objectives of CARDIORISK, in vivo as well as ex vivo and in vitro studies will be performed. A central component of the work programme consists of the ***local irradiation*** of the heart of experimental animals (ApoE-/- mice) with subsequent isolation of cardio-myocytes and cardiac endothelial cells to provide all participating partners with the same biological material for further studies. Furthermore, structural, morphological and molecular studies will be complemented by functional assays and imaging methods.

Two alternative, but not mutually exclusive, working hypotheses about the pathogenesis of late radiation-induced fatal heart disease after mean radiation exposure of the heart of < 2 Gy single dose averaged over the heart volume have been proposed:

Hypothesis 1: Radiation increases the frequency of myocardial infarction by interacting with one or more steps of the pathogenic pathway of age related coronary artery atherosclerosis.

Hypothesis 2: Radiation increases the lethality of myocardial infarction, which may occur due to pathologies unrelated to radiation, i.e. by reducing the organ tolerance to minor acute infarctions as a result of persistent or progressive reduction of the microcirculation in the irradiated heart.

The experimental program will investigate both hypotheses on the pathogenic mechanisms of radiation-induced cardiovascular risk at low doses, by studying, in parallel, late radiation injury to coronary and peripheral arteries as well as to the capillary network.

CARDIORSIK consists of 8 workpackages *(Figure 4)*.

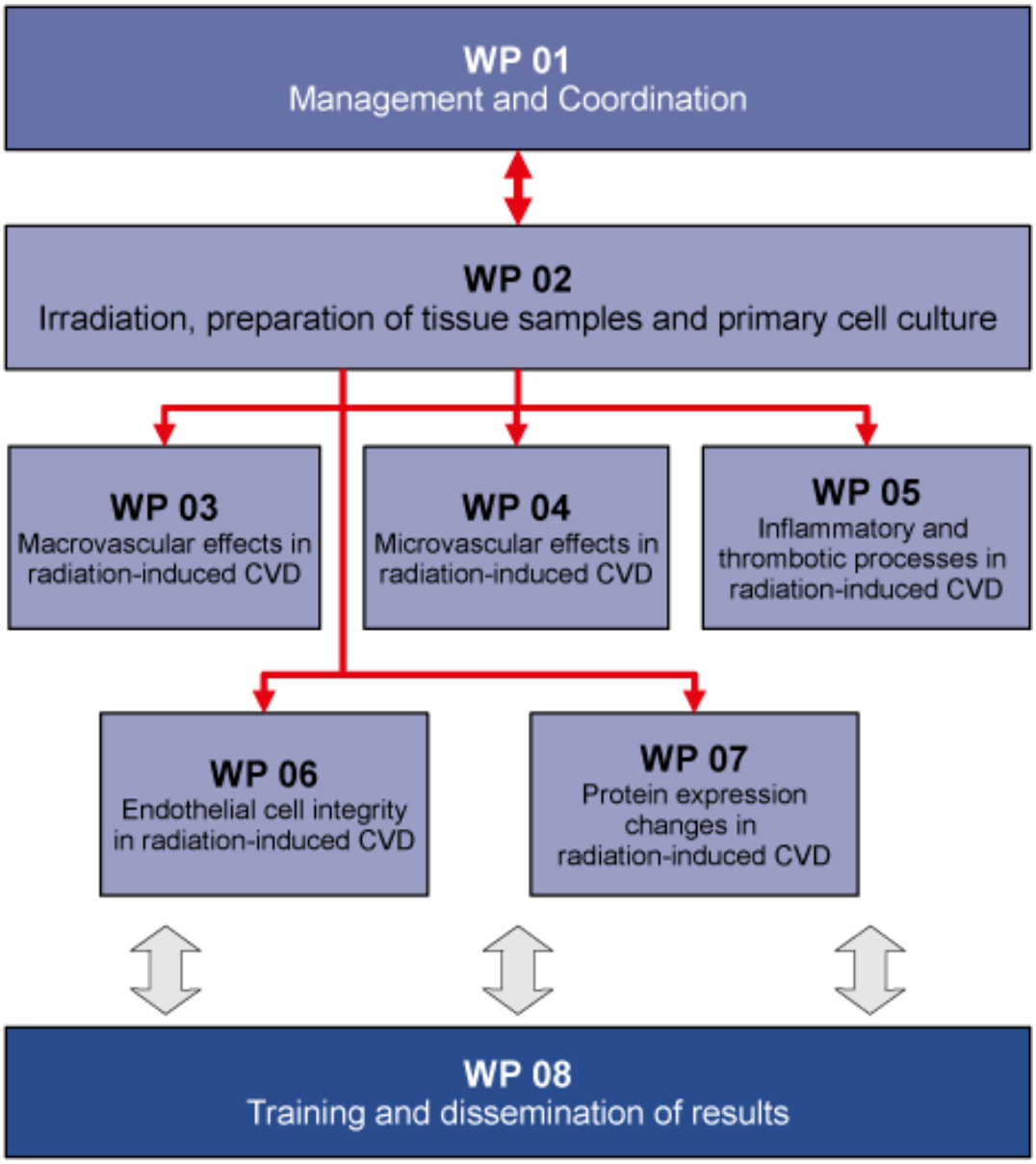

Figure 4: General outline of the CARDIORISK project organisation.

Local irradiation of the hearts and of the two peripheral arteries of different strains of mice and long-term follow-up of the irradiated animals is at the core of this program ***(WP 02)***. The methods of local irradiation of the peripheral arteries of mice are already well established. Using modern imaging and focusing techniques, the established method of local heart irradiation in rats has been adapted to the specific anatomical situation in mice. A range of four to six different radiation doses between 0.01 Gy (as lowest dose) up to a maximal dose of 14 Gy (a. carotis) or 20 Gy (a. saphena) will be given. The heart and/or arteries of these mice will be collected at selected time points up to one year after irradiation. Primary cardiovascular endothelial cells will be harvested for further investigation.

Functional imaging of the microvasculature of the heart and the vascular patency of the irradiated arteries ***(WP 03, WP 04)*** will be performed in a subgroup of locally irradiated animals by micro-SPECT/CT, CT, optical coherence tomography (OCT) and MRI. After low and moderate radiation doses (<2 Gy), investigations will be performed 6, 12 and 18 months, and at shorter intervals after high radiation doses. The aim of these studies is the development of suitable methods to quantify the expected focal and/or overall reduction of blood perfusion in the heart as a result of late micro-vascular radiation damage. This will be validated by morphometric analysis.

Histopathological investigations are planned by several partners ***(WP 03, WP 04)***. The main morphological endpoint in the hearts will be the dose-dependent kinetics, severity and focality of reduction of capillary density in the myocardium and evidence for focal hypoxia in relation to the changes observed by functional imaging.

Additionally, in particular at early times after local irradiation, changes in inflammatory and thrombotic gene expression will be studied by qRT-PCR and changes in endothelial cell function, protein localisation and abundance by immuno-histochemistry ***(WP 05)***. The main morphological endpoint in the arteries will be the nature of potential atherosclerotic plaques (inflammatory and thrombotic features) ***(WP03)***.

Ex vivo investigations of the irradiated arteries ***(WP 06)*** will concentrate on intercellular signaling and, in particular the endothelium-leukocyte interactions and pro-thrombotic alterations of the endothelium. In addition, in frozen sections, the expression of adhesion molecules and thrombomodulin will be studied in close co-operation with the Integrated FP6-project NOTE.

In vitro investigations with primary micro-vascular endothelial cells being isolated at different times after local heart irradiation ***(WP 02)*** are also of central importance. After establishing the primary cultures, cells will be shipped to those partners who will study the different late post-radiation effects in micro-vascular endothelial cells. These effects are considered to play a role in cardiovascular radiation risks after low dose irradiation, in particular stress responses, changes in intercellular communication, changes in three-dimensional remodelling and migration, in particular in co-culture with cardio-myocytes, changes in endothelial permeability and cytoskeleton structure, pro-inflammatory and pro-thrombotic changes, and changes in endothelial cell/cardiomyocyte interactions ***(WP 05, WP 06)***. As a rule, each partner will receive samples from the same primary cell preparation for his/her study, each time.

Furthermore, in vitro investigations of the function and of molecular alterations of the myocardial cells will be performed with special emphasis on the Rho/ROCK pathway in

radiation-induced cardiac remodelling. Specifically, the long-term persistence of phenotypical alterations of endothelial cells will be related to the function of cardiomyocytes isolated from irradiated hearts up to 18 months after irradiation ***(WP 06)***.

Proteomics investigations will identify changes in the protein constitution of irradiated cardiac endothelial cells and heart tissue. The partners involved will subsequently study critical these target proteins from fresh tissue sections of irradiated hearts and in the myocardial cells and endothelial cells isolated from irradiated hearts ***(WP 07)***. The main focus is to map the proteome changes in established and primary endothelial cells after low radiation doses, and to validate key components of the radiation-specific changes in the proteome in the macro- and micro-vascular models of endothelial cell irradiation.

Based on the knowledge gained CARDIORISK intends to provide the impetus necessary to place radiation protection considerations of late cardiovascular effects occurring at low doses in a biological context. Thus, the project might also be of direct benefit in modelling strategies that aim to extrapolate cardiovascular risk from epidemiological studies.

4.4 Conclusions

In recent years, there is growing epidemiological evidence of excess risk of late occurring cardiovascular disease at much lower radiation doses and occurring over much longer intervals after radiation exposure without a clear cut threshold. The resolution of this problem is of considerable importance for radiation protection since it might require a reassessment of the current risk estimation system for so called deterministic radiation effects (i.e. late organ damage).

The examination of currently available experimental data in terms of possible biological mechanisms indicates that the most likely causative effect of radiation is damage to endothelial cells and subsequent induction of an inflammatory response, although it is not clear yet that this would extend to low dose and low dose-rate exposure. In addition, a role for somatic mutation has been proposed that would indicate a stochastic effect. Only full knowledge of the pathogenic pathways is likely to offer a sound scientific basis for solving the question of possible dose threshold for radiation-induced cardiovascular risk at low radiation doses. However, in the absence of a convincing mechanistic explanation of the currently available epidemiological evidence, the existence of a cause-effect-relationship can neither be confirmed nor excluded. Further epidemiological and biological evidence from currently ongoing European research will allow a firmer conclusion to be drawn.

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5 SUMMARY

Working Party "Research Implications on Health and Safety Standards" of the Article 31 Group of Experts[1]

Prepared by Patrick Smeesters, Chairman

5.1 Introduction

This document provides the background, summarizes the presentations and tries to emphasize the potential implications of the Scientific Seminar on Emerging Evidence for Radiation Induced Circulatory Diseases, held in Luxembourg on 25 November 2008. It takes into account the discussions that took place during the Seminar and during the subsequent meeting of the Article 31 Group of Experts on 26 November 2008, although it is not intended to report in an exhaustive manner all the opinions that were expressed. The document has been submitted for comments to the lecturers, as far as their contributions were concerned.

5.2 The Article 31 Group of Experts and the rationale of the RIHSS Seminars

The Article 31 Group of Experts is a group of independent scientific experts referred to in Article 31 of the Euratom Treaty, which assists the European Commission in the preparation of the EU Basic Safety Standards for the protection of the health of workers and the general public against the dangers arising from ionizing radiation. According to the Euratom Treaty and to their Code of Ethics, this group of experts has to give priority to the protection of health, to the safety and to the development of the best available operational radiation protection. For doing so, they have to follow carefully the scientific and technological developments and the new data coming from the world of research, particularly when these could affect the health of the exposed persons.

In this context, a Scientific Seminar is devoted every year to emerging issues in Radiation Protection – generally addressing new research findings with potential policy and/or regulatory implications. On the basis of input from the Directorate General Research of the European Commission and of information provided by individual members of the Article 31 Group of Experts, the RIHSS Working Party proposes relevant themes to the Article 31 Group that could be discussed during a subsequent seminar. After selection of the theme and approval of a draft programme by the Article 31 Group, the RIHSS Working Party deals with the preparation and the follow up of the seminar. Leading scientists are invited to present the status of scientific knowledge in the selected topic. Additional experts, identified

[1]. The following members of the Working Party on Research Implications on Health and Safety Standards of the Article 31 Group of Experts contributed to the preparation of this overview: L. Lebaron-Jacobs, W-U Müller, P. Olko, S. Risica, P. Smeesters (Chairman of the WP), R. Wakeford. They were assisted by external experts (J. Piechowski) and by the following official of the European Commission: S. Mundigl.

by members of the Article 31 Group from their own country, take part in the seminars and act as peer reviewers. The Commission convenes the seminars on the day before a meeting of the Article 31 Group, in order that members of the Group can discuss the potential implications of the combined scientific results. Based on the outcome of the Scientific Seminar, the Group of Experts referred to in Article 31 of the Euratom Treaty may recommend research, regulatory or legislative initiatives. The European Commission takes into account the conclusions of the Experts when setting up its radiation protection programme. The Experts' conclusions are also valuable input to the process of reviewing and potentially revising European radiation protection legislation.

5.3 Background and Purpose of the 2008 Seminar

In recent years, radiation induced blood circulatory system diseases has become a growing concern, particularly in the field of radiotherapy, after irradiation of the heart or of large arteries. There is currently a lot of research in the field, including within the European Research Framework Programmes (FP). Some new challenging data have recently been published or are in the process of being published. Although evidence[2] for radiation induced circulatory disease was reviewed some years ago by the United Nations Scientific Committee on the Effects of Atomic Radiations (UNSCEAR) and published recently in Volume 1 of the UNSCEAR 2006 Report (annex B: Epidemiological evaluation of cardiovascular disease and other non-cancer diseases following radiation exposure), some of these new data were not available at that time for evaluation by UNSCEAR.

Consequently the issue was thought to be ripe for re-evaluation, particularly in the current context of the ongoing revision of the EU and international Basic Safety Standards.

In addition, it is worth mentioning that UNSCEAR is a purely scientific group, which implies that its main concern is to avoid concluding that a causal relationship exists before it is firmly proved ("scientific cautiousness"). The cautiousness expected from the Article 31 Group of Experts, according to his mandate and Code of Ethics, is different: the main concern should be to protect health and to prevent possible health detriment. When there is scientific plausibility of the existence of a risk of serious harm for health, even if there is still uncertainty, they should alert the EU Commission (precautionary principle).

In this perspective, the conclusions of the Article 31 Group of Experts may to a certain extent be different from those drawn by UNSCEAR, even when considering the same set of data, as there could be some policy implications of these data, not only for radiotherapy, but also for radiation protection in general (medical, occupational and emergency exposures). This also justifies the selection of this topic.

[2] In summary, the UNSCEAR Report confirmed that there is an increased risk of cardiovascular disease associated with high radiation doses to the heart, which may be incurred during radiotherapy. For lower doses, the Report concluded that, to date, the evidence for an association between fatal cardiovascular disease and radiation exposure at doses of less than 1-2 Gy comes only from the analysis of the data on the Japanese atomic bombings survivors, which is "not sufficient to establish a causal relationship at these doses".

5.4 Main points arising from the presentations

R. Wakeford and M.P.Little - *Epidemiological evidence for radiation-induced circulatory diseases: A-bomb survivors*

R. Wakeford and M.P. Little explored the evolving evidence for a radiation-associated excess risk of diseases of the blood circulatory system among the Japanese survivors of the atomic bombings of Hiroshima and Nagasaki in August 1945. About 87 000 survivors belong to the Life Span Study (LSS) cohort. This study, investigating mortality and cancer incidence, started in 1950 and is still underway. The Adult Health Study (AHS) is a subset of the LSS that started in 1958, and consists of a cohort of about 20 000 persons followed by biennial health examinations, allowing disease *morbidity* to be investigated for a range of diseases, including circulatory disease. It is worth mentioning that this population was malnourished and composed of a low proportion of men of military age, and that the Japanese included in the LSS had to have survived until the establishment of the cohort in October 1950. It has long been controversial whether there has been selection in this cohort due to a "healthy survivor effect". Possible evidence for this is the negative dose response for non-cancer disease in the early years of follow-up – for which reason most analyses have discarded data before 1968.

First indications of an increased risk of non-cancer disease appeared in the 1970s with evidence of an effect of radiation on the risk of circulatory disease mortality, but only for *women*.

At the beginning of the 1990s, new data were published showing evidence that both the fatal (LSS mortality) and the nonfatal (AHS incidence) forms of coronary heart disease increased with dose (after exposure to high levels of ionizing radiation), particularly for those *less than 40 years of age* at time of bombing – for this sub-group the relative risk of death from myocardial infarction during 1968-86 was 1.57 Gy^{-1} (95% confidence interval: 1.26, 2.76).

Almost 10 years later, new data were published that were consistent with a possible *linear no-threshold* dose-response relationship for the excess relative risk (ERR) of non-cancer mortality and for the ERR for the mortality from heart disease and stroke in the LSS. The 273 radiation-associated non-cancer disease deaths compare with 421 cancer deaths attributable to radiation exposure; 165 of these 273 non-cancer disease deaths are due to heart disease and stroke.

On the basis of current evidence, the speakers concluded that increased risks of circulatory diseases are apparent even below doses of 2 Gy, the data being consistent with a linear no-threshold dose-response relationship but also with a threshold dose of around 0.5 Gy. Under the assumption of an underlying linear no-threshold dose-response relationship, the radiation-associated circulatory disease deaths represent a significant proportion of all radiation-associated deaths, although bias and confounding (which may be negative) cannot yet be reliably discounted as explanations for the association.

T. Azizova and C. Muirhead *Epidemiological evidence for radiation-induced circulatory diseases: occupational exposure*

Circulatory disease risks have been considered among several types of radiation workers. In the first part of their presentation, the speakers summarized the findings from occupational studies published up to mid-2008 (radiologists and radiologic technologists; radon-exposed miners; nuclear industry workers: 14 countries-study; BNFL workers).

The largest cohort studied to date consisted of about 275 000 workers from 14 countries (Vrijheid et al, 2007). The findings for circulatory disease mortality from this analysis were consistent both with no raised risk and with a risk of the size seen in the Life Span Study (LSS) of Japanese atomic bomb survivors. However, the statistical power of this analysis was low, owing to the relatively short follow-up (the average age at end of follow-up was 46 years) and the relatively low mean cumulative external dose (20.7 mSv). In the United Kingdom, McGeoghegan et al (2008) examined circulatory disease mortality among about 42 000 radiation workers at British Nuclear Fuels plc (BNFL). This study, with a longer follow up and higher mean external dose (53 mSv), showed a statistically significantly increasing trend in risk with increasing external dose. It was not possible to adjust for potential confounding factors in the above two analyses.

In addition to the generally low statistical power, the interpretation of all these occupational studies of circulatory diseases is more or less difficult, due to the potential for bias or confounding factors. The “healthy worker effect” complicates comparisons with national mortality rates. There is a potential for misclassification of specific disease types and, last but not least, there is a general lack of information on known risk factors for circulatory diseases (smoking, alcohol consumption …).

Recent important data come from the Southern Urals Radiation Risk Research (SOUL) project, supported by the European Commission’s 6th Framework Programme (Euratom) and the Federal Medical Biological Agency (Russian Federation). In this study risks of morbidity and mortality from circulatory diseases were estimated up to the end of 2000 in the cohort of workers (about 12 000 workers, among which about 3500 women) first employed at the main facilities of Mayak PA in 1948-1958 in relation to external and internal radiation, whilst allowing for age, gender and non-radiation risk factors. The Mayak Production Association (PA) was the first Russian nuclear facility and is located 10 km from the city of Ozyorsk in the Southern Urals. Mayak PA started operation in June 1948 and included all the plants necessary to produce weapon-grade plutonium: reactors, radiochemical plant, plutonium plant and auxiliary plants.

The SOUL study is characterized by a large database on dosimetry and regular medical examinations. In addition, there is very good information on confounding factors (including smoking and alcohol) and quality control checks were conducted on a regular basis. The effects studied were ischemic heart diseases (IHD) and cerebrovascular diseases (CVD).

Individual monitoring of exposures to external gamma doses was conducted from the beginning of operations at Mayak. The average total external gamma dose for the whole employment period was 0.91 Gy (range 0-5.92 Gy) for males and 0.65 Gy (range 0-5.70 Gy) for females. Plutonium body burden was measured (and estimates of internal doses were subsequently derived) only for 30% of workers who were in contact with transuranium

radionuclides. Therefore analyses of internal exposures were restricted to monitored workers. The absorbed dose to liver was used in analyses of internal exposure as surrogate for dose to blood vessels/heart; although these doses would differ, they were considered to be highly correlated. The average absorbed liver dose from internal alpha exposure was 0.40 Gy (range 0 - 17.90 Gy) for males and 0.81 Gy (range 0 - 127.82 Gy) for females.

The risk of morbidity was statistically significantly increased for workers with a total dose from chronic external gamma ray exposure above 1 Gy for IHD, and above 0.5 Gy for CVD, (when compared with doses less than 0.5 Gy). There was a statistically significant increasing trend in IHD and CVD morbidity with increasing total external dose. The risk for IHD morbidity is higher for women than for men (ERR/Gy: 0.71 vs 0.39).

When looking at internal exposures, risks of both mortality and morbidity from IHD and CVD were raised among workers with total absorbed doses to the liver from internal alpha exposure above 0.1 Gy, when compared with workers monitored for such exposures who had lower doses. There were statistically significant increasing trends with increasing total internal alpha dose to the liver in IHD mortality and in CVD morbidity. There was less evidence for a trend with internal dose in IHD mortality after adjusting for external dose, whereas this adjustment had little impact on the findings for CVD morbidity.

Globally, the risk estimates are compatible with those from other large occupational studies and from the A-bomb survivors. In particular, the data are consistent with a linear dose-response relationship from doses as low as 0.5 Gy, but with much fewer limitations in this study regarding statistical power and confounding factors. In addition, this study confirms the acute exposure findings from the LSS data in the context of protracted doses.

K.R. Trott - *Evidence for radiation-induced circulatory diseases among patients treated with radiotherapy*

Radiation-induced thrombosis of the *carotid arteries* and subsequent stroke has been the most commonly reported late circulatory disease after radiotherapy of head and neck cancer. The problem of irradiation of the major blood vessels is bound to become a serious issue in modern radiotherapy. New techniques in clinical radiotherapy, in particular stereotactic radiotherapy, require careful re-consideration of radiation doses to the major blood vessels.

The clinical importance of radiation-induced *heart* disease was recognised later, first based on the follow-up studies in Hodgkin's disease patients. It is only since the early 1990s that the heart has been found to be a critical organ in other areas of radiotherapy and in radiation protection. Breast cancer patients with post-operative radiotherapy were particularly studied. Whereas the risk of death from recurrent breast cancer was the same after left- or right-sided cancer, the risk of death from heart disease was higher by 44% in those women who had cancer of the left breast than in those women who had cancer of the right breast. Another study of breast cancers treated with radiotherapy showed that the clinical benefit relating to death from cancer did not translate into any survival benefit because it was offset by a statistically significant increase of deaths from cardiovascular disease.

The key problem is to know which anatomical structures are important for the risk. Current and planned research on radiation-induced cardiovascular disease in radiotherapy patients, particularly in the RACE project, concentrates on the relationship between local dose and

risk, i.e. the determination of the dose at the site of damage development and thus the identification of the anatomical structures which are the targets that trigger damage development. Closely related is the question how the heart dose is to be reported and limited or constrained in radiotherapy and in radiation protection. Is it the mean heart dose, or the maximum heart dose, or the dose in particular anatomical structures of the heart, such as the left anterior descending coronary artery which in most cases receives the highest radiation dose in radiotherapy of breast cancer?

Some recent studies using SPECT or PET imaging of micro-vascular perfusion demonstrated perfusion defects already within 6 - 12 months after breast cancer radiotherapy**.** More clinical studies are in preparation with the aim of relating those changes in functional imaging and their gradual development to the individual dose distribution.

According to the speaker, the critical open questions are the following:

1. Is there a dose threshold of increased risk? Does the latency to clinical manifestation depend on dose as is suggested by experimental data? In other words, is there a dose dependence of incidence or rather a dose dependence of damage progression rate?

2. What is the clinical nature of cardiovascular disease induced by different radiation doses and dose distributions to the heart? Is the pathology after low radiation doses different, or the same but developing more slowly, compared to that after high radiation doses?

3. In the radiotherapy studies, there are pronounced dose inhomogeneities within the heart. Which part of the heart is most radiosensitive and should be chosen as a reference point for tolerance doses in radiation oncology or for effective dose to be corrected with an organ weighting factor in radiation protection?

G. Hildebrandt and S. Schultz-Hector - *Biological mechanisms of radiation-induced circulatory diseases*

The cardiovascular system has long been regarded as radioresistant because of the extremely low proliferative activity of the endothelial and connective tissue cells and the postmitotic state of cardiac myocytes.

However, as shown during this workshop, there is increasing evidence of an association between radiation and cardiovascular disease, not only after radiotherapy, but also at much lower radiation doses.

In this paper the speakers review the available evidence for a *causal* interpretation of the epidemiological associations between radiation exposure at low and moderate dose and cardiovascular disease, with special emphasis on possible biological mechanisms and taking into account the currently ongoing European experimental studies (NOTE in FP6, CARDIORISK in FP7).

From a review of the published data, the speakers conclude that irradiation of the cardiovascular system is associated with acceleration and de-stabilization of arteriosclerotic lesions and may also cause a decrease in myocardial perfusion. The pathogenesis of both effects needs to be further investigated; pro-inflammatory endothelial cell reactions could play a role in the initiation of both. Observation of perfusion effects are limited to high doses and should be studied at lower doses. The endothelium apparently plays a pivotal role.

In the currently ongoing NOTE study, one of the main objectives is to test the hypothesis whether *low dose total body irradiation* might modulate atherosclerosis progression depending on (a) *genetic background* (including genotypes prone to the disease) and (b) stage of disease at time of exposure, and furthermore whether this might be mediated by modulation of stress and/or inflammatory responses. Various genotypes of mice are studied in vivo. Preliminary statistical analysis of the raw data available so far indicates that there are highly significant differences between the control (0 Gy) and higher dose groups (0.025-2 Gy) in mean lesion size at 6 months for susceptible genotypes. In addition, further experimental results demonstrate significant differences in peripheral markers of inflammatory response, leukocyte extravasation and prothrombotic surface at both 3 and 6 months after IR in susceptible genotypes which received 0.05 Gy or more.

The aim of the CARDIORISK collaborative research project is to elucidate the pathogenesis of early and late alteration in the microcirculation of the heart and of atherosclerotic lesions in arteries after exposure to low radiation doses as compared to high doses. Special emphasis is given on the investigation of early molecular, pro-inflammatory and pro-thrombotic changes as well as perfusion alteration, cardiac cell integrity and immunologic influences. In vivo as well as ex vivo and in vitro studies will be performed. *Local irradiation* of the hearts and of the two peripheral arteries of different strains of mice is at the core of this program. *Functional imaging* of the microvasculature of the heart and the vascular patency of the irradiated arteries will be performed.

The speakers conclude that the examination of currently available experimental data indicates that the most likely causative effect of radiation is damage to *endothelial cells* and subsequent induction of an *inflammatory response*, although it is not clear yet that this would extend to low dose and low dose-rate exposure. In addition, a role for somatic mutation has been proposed that would indicate a stochastic effect. Only full knowledge of the pathogenic pathways is likely to offer a sound scientific basis for solving the question of possible dose threshold for radiation-induced cardiovascular risk at low radiation doses.

5.5 Discussion of the potential implications

Active discussions took place during the Seminar, stimulated by a panel of experts[3], and during the subsequent meeting of the Article 31 Group of Experts on 26 November 2008. As already mentioned, it is not intended to report in an exhaustive manner all the opinions that were expressed. It seems nevertheless important to highlight some of the topics that were discussed.

[3] The members of the panel were the following: Maria Grazia Andreassi (Institute of Clinical Physiology, Massa, Italy), Donal Hollywood (St.Luke's Hospital, Dublin, Ireland), Marc Sapir (ETUI European Trade Union Institute, Brussels, Belgium) and Wolfgang Weiss (BfS, Munich, Germany).

In relation to radiotherapeutic strategies, recent results have been discussed, confirming the radiation-induced cardiovascular risk, but showing an additive role of associated chemotherapy on the risk of heart damage and a greater than additive effect of smoking and radiotherapy on risk of myocardial infarction.

The similarity of the dose response curves observed on the one hand for cancers and on the other hand for circulatory diseases has launched discussions concerning the possible biological mechanisms, the classical deterministic paradigm being challenged by alternative explanations including at least partially stochastic events, opening the door for possible low dose effects.

Regarding biological mechanisms and the currently ongoing research, one of the major difficulties that has been underlined is the fact that laboratory rodents are known to be fairly resistant to arteriosclerosis – possibly due to the absence of any life-style associated risk factors. The disease is absent in wild type rodents, this meaning that good animal models are practically *lacking*. Waiting for convincing or conclusive biological explanation before taking preventive measures could therefore not be a wise decision.

Epidemiological evidences of age and gender differences (higher risk for women and for young age at exposure) were underlined, opening the way for reflection regarding possible policy implications.

It was also suggested to explore with more attention the possible consequences of irradiation in other organs, where damage to blood vessels could also play an important role. Similarly, based on the Mayak data regarding internal exposure, it was suggested to give more attention to possible radiotoxicological effects on blood vessels after internal exposures.

The general flavour of the discussions was that the currently available scientific data are solid enough for imposing us to take this problem seriously into account not only in radiotherapy, but also in the field of radiological protection in general. Particularly underlined was the fact that the absolute number of possible radiation-induced circulatory diseases (despite the relatively low relative risks) makes this topic a public health pertinent issue.

It was agreed that the policy and regulatory implications should be further discussed. Suggestions included:

- the explicit introduction in the BSS of the concept of optimisation and dose constraints for the heart and for the other organs;

- information for the medical community (not only in radiotherapy, as repetitive CT scans in radiology may lead to cumulative exposures that are far from being negligible);

- development and diffusion of radiotherapeutic strategies to minimize unnecessary heart doses (in particular for the treatment of breast cancers);

- stimulation of R&D (including in the field of effects of internal exposures).

6 CONCLUSIONS

Working Party "Research Implications on Health and Safety Standards" of the Article 31 Group of Experts[4]

There was a broad consensus within the Article 31 Group of Experts on the following conclusions:

- Although a lot of confounding factors have to be taken into account, epidemiological evidence is accumulating in favour of an increased risk of circulatory disease for doses higher than 0.5 Gy. This evidence can be observed both after acute exposure and after protracted exposures.

- The radiation-associated circulatory diseases could represent a significant proportion of the radiation-associated mortality, making of this topic a public health pertinent issue at high doses (i.e. radiotherapy, accidental exposures, …).

- The problem of irradiation of the blood vessels and the heart is currently a growing concern in radiotherapy. One of the key problems is to know which anatomical structures are important for the risk (critical targets).

- The possible biological mechanisms are still unknown. Active R&D is ongoing (projects NOTE and CARDIORISK in Europe). A major limitation is however the fact that good animal models are lacking.

- The currently available scientific data are solid enough for imposing us to take this problem seriously into account. Policy and regulatory implications should be further discussed. Suggestions included the explicit introduction in the BSS of the concept of optimisation and dose constraints for the heart and for the other organs, information of public health authorities and of the medical community (not only in radiotherapy, as, for example, repetitive CT scans in radiology may lead to cumulative exposures that are far from being negligible), development and diffusion of radiotherapeutic strategies to minimize unnecessary heart doses (in particular for the treatment of breast cancers) and stimulation of R&D (including in the field of effects of internal exposures).

4 The following members of the Working Party on Research Implications on Health and Safety Standards of the Article 31 Group of Experts.contributed to the preparation of these conclusions: L. Lebaron-Jacobs, W-U Müller, P. Olko, S. Risica, P. Smeesters (Chairman of the WP), R. Wakeford.