# Radiation Research: State of the Science Twenty Years after Chernobyl

Author(s):
Source: Radiation Research, 167(3):338-360.
Published By: Radiation Research Society
https://doi.org/10.1667/RR0890.1
URL: http://www.bioone.org/doi/full/10.1667/RR0890.1

RADIATION RESEARCH **167**, 338–360 (2007)
0033-7587/07 $15.00


# EXTENDED ABSTRACTS

## Radiation Research: State of the Science Twenty Years after Chernobyl

American Statistical Association Conference on Radiation and Health
Pacific Grove, California, June 18–21, 2006

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The 17th ASA Conference on Radiation and Health was held June 18–21, 2006 at the Asilomar Conference Grounds in Pacific Grove, CA. This congenial setting afforded a great opportunity for discussion of the latest research on the health effects of radiation among the 85 attendees representing the fields of statistics, epidemiology, biology, medicine and physics. The aim of the conference was to assist interested scientists currently working in the area of radiation health effects to identify novel quantitative approaches to their research, and to encourage new research opportunities related to radiation health effects across the disciplines. The theme of this year's conference was *Radiation Research: State of the Science Twenty Years after Chernobyl*, and the opening session focused on the most recent scientific findings from the Chernobyl disaster. The conference keynote speaker was Dr. Norman Gentner, past president of the United Nations Scientific Committee on the Effects of Atomic Radiation, who spoke on the topic ''Intersection of Radiation Science and Policy.'' Other sessions of the conference included ''Low-Dose Studies: Evidence from Exposures below ~100 mSv,'' ''Cardiovascular and Cerebrovascular Outcomes and Radiation,'' ''Radiation Studies in Russia and the Former Soviet Union,'' and ''Current Issues in Dosimetry.'' For the first time, the 2006 conference included a poster session with contributions from 14 New Investigator awardees and 16 other attendees. The extended abstracts published here represent presentations by the platform speakers in each of the sessions.

The 2006 Organizing Committee would like to dedicate the publication of these extended abstracts to the memory of Dr. Geoffrey R. Howe, Professor of Epidemiology at the Mailman School of Public Health, Columbia University, who died August 31, 2006. Dr. Howe, our colleague on the 2006 organizing committee,

contributed extensively over many years to the planning of and presentations at the Conference on Radiation and Health. His contributions to the field of radiation epidemiology are legion, beginning with his studies during the 1980s and 1990s of Canadian women exposed to fluoroscopic X rays, his contributions to the BEIR V and VII (Phase I) Committees, recent studies on nuclear workers in the U.S., Canada and the 15-Country IARC study, and his extensive studies of the Chernobyl cohorts. He will be greatly missed as a colleague, collaborator and friend.

## SCIENTIFIC RESEARCH AND LESSONS LEARNED TWENTY YEARS AFTER CHERNOBYL

Ethel Gilbert and Geoffrey Howe, Organizers

Elaine Ron, Chair

Norman Gentner, Discussant

### Scientific Research and Lessons Learned Twenty Years after Chernobyl—Thyroid Cancer

Elisabeth Cardis

*International Agency for Research on Cancer, Lyon, France*

The 26th of April 2006 marked the 20th anniversary of the accident at the Chernobyl nuclear plant in northern Ukraine, the largest nuclear accident in history. As a result of the accident, about 5 million people were exposed to radioactive contamination in Belarus, the Russian Federation and Ukraine (*1*).

The highest organ-specific dose was to the thyroid gland, primarily from ingestion of milk contaminated with radioactive iodines, particularly $^{131}$I. A wide range of thyroid doses were received by the inhabitants of the contaminated areas in the three affected countries. Doses varied with age at the time of the accident, level of ground contamination, and rate and source of milk consumption. Reported individual thyroid doses ranged up to several tens of grays, while average doses ranged from a few tens of milligrays to several grays (*1*). Intake of stable iodine tablets during the first hours after the accident reduced the thyroid dose of the residents of Pripyat by a factor of 6 to 7 on average (*2, 3*).

The main health effect of radiation from the accident observed to date is a dramatic increase in the incidence of thyroid cancer in persons exposed as young people. This increase was observed first in the early 1990s in Belarus and continues in the most contaminated areas of Belarus, Ukraine and the Russian Federation (*4–7*). All together, close to 5,000 cases of thyroid cancer have been diagnosed in Belarus, Ukraine and the four most contaminated regions of Russia during 1986–2002 among those who were children or adolescents at the time of the Chernobyl accident. The majority of cases (around 4,000) were diagnosed among those who were below the age of 15 at the time of the accident. Fifteen cases are known to have been fatal to date (*1, 8*).

At the time of the Chernobyl accident, it was widely held that $^{131}$I was much less carcinogenic than external photon exposure, since little or no experience of the effects of the isotopes of iodine on the child's thyroid was available (*9*). Information on radiation-induced thyroid cancer came from studies of populations exposed to external radiation, mainly the atomic bomb survivors and patients who received therapeutic exposures in childhood and infancy.

A number of epidemiological studies of thyroid cancer after exposure to radioactive iodines from the Chernobyl accident have been reported both in the most contaminated countries and in other European countries (*1, 6, 8*). Most of the published studies have been of the ecological type, where information on dose and health outcomes (and occasionally on potential confounders) is available only at the group or population level. The most up-to-date and comprehensive study is that of Jacob *et al.* (*7*). This type of study, however, can be subject to potential bias, in particular the ecological fallacy (the failure of group level data to properly reflect individual level associations) (*10, 11*). Several analytical studies, in which information is collected at the individual level, in particular case-control and cohort studies, have been published recently, providing important information to evaluate the health risks associated with the Chernobyl accident (*12–15*).

The excess relative risks (ERRs) derived in the case-control and cohort studies are all similar, of the order of 5 to 7 for a dose of 1 Gy, though slightly lower than the estimate from studies of external radiation (7.7 at 1 Gy) (*16*). The risk estimate from the ecological study, on the other hand, is higher but statistically compatible with that from studies of external radiation. The reasons for the difference in risk estimates for the two study designs are not yet clear, although uncertainties in dose estimates may be partly responsible.

There is some indication that iodine deficiency at the time of exposure may increase the risk of developing thyroid cancer among persons exposed to $^{131}$I as children (*14, 17*). Conversely, prolonged stable iodine supplementation in the years after exposure may reduce this risk (*14*). Further studies are needed to confirm these findings.

It is not possible at present to evaluate directly from the Chernobyl experience the future trends in Chernobyl-related thyroid cancers. Based on many decades of follow-up from studies of populations exposed to external radiation (*16*), however, it is expected that these cancers will continue to occur for many more years, although the long-term magnitude of risk cannot be quantified.

Papillary cancer is the primary pathological type of thyroid cancer found in those exposed as children and adolescents to fallout from the Chernobyl accident. The biology of radiation-induced thyroid cancer does not appear to be fundamentally different from that of the thyroid cancers seen in a nonirradiated population, although a slightly greater percentage of radiation-induced thyroid cancers appear to be papillary in nature (*18*). Possible differences in the molecular biology of the tumors, particularly with regard to *RET/PTC* rearrangements and *BRAF* mutations, are unclear at this time (*19, 20*).

While the increased risk of thyroid cancer in those exposed in childhood and adolescence is well demonstrated, the effect of exposure on adults remains unclear. In the only study that has evaluated the risk for adults living in the contaminated areas (*21*), no dose–response relationship was found. No association was observed in studies of Estonian and Russian liquidators (*22, 23*).

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## Leukemia after Radiation Exposure from the Chernobyl Accident

Geoffrey R. Howe and Lydia B. Zablotska

*Mailman School of Public Health, Columbia University, New York, New York*

The accident at the Chernobyl nuclear plant in northern Ukraine in April 1986 led to the contamination of large areas of Belarus, Ukraine and western Russia by radioactive releases. The most obvious long-term health consequence of the accident has been a substantial increase in thyroid cancer among those exposed as children in the three countries, as discussed by Dr. Cardis in her presentation. The other cancer that has received the most scientific attention is leukemia because of its known sensitivity to induction by ionizing radiation and its relatively short latent period. This paper considers the potential impact of exposure from Chernobyl on leukemia risks in the three countries cited above. Before considering the empirical epidemiological evidence, it is first appropriate to consider both the objectives in studying leukemia in this context and the types of approaches that can be used to satisfy those objectives. Broadly speaking, these may be divided into scientific and public health objectives. Scientific objectives may be expressed as determining the existence and magnitude of any dose–response relationship between exposure and disease that can be applied to other similar situations. For example, the studies of thyroid cancer may be considered to have such scientific objectives since there is only limited information from previous experience of the risk of thyroid cancer after exposure to ionizing radiation and, in the case of Chernobyl studies, doses to the thyroid gland are sufficiently high to permit a meaningful evaluation.

Public health objectives include:

1. Resource planning for the long-term consequences of the accident.
2. Providing reassurance to the exposed population that projected risks from Chernobyl exposure are realistic.
3. Directly studying the effects of the world's largest nuclear accident to date.

It should be emphasized that even to achieve public health objectives, appropriately valid scientific methodology has to be used.

Apart from thyroid cancer, the main limitation in studying other diseases resulting from the Chernobyl accident is the problem of statistical power. In general, doses were too low to give studies appropriate power and hence an expectation of obtaining sufficiently narrow confidence intervals around risk estimates. However, it can still be appropriate to study such outcomes for public health purposes since detecting an association or failing to detect an association can be useful in satisfying such objectives.

As to the types of approaches that can be used to ''measure'' the effect of the accident, there are two such approaches. First, there is the risk projection approach in which risks estimated from studies of other populations exposed to much higher doses than Chernobyl are applied to the doses experienced from Chernobyl to project the corresponding risks for a Chernobyl-exposed population. The other approach is to conduct empirical studies in the directly affected population. The advantage of the former approach is increased statistical power, whereas the primary disadvantage is the necessity of extrapolating risks from one population exposed under one set of circumstances to another population exposed under a different set of circumstances. The second approach, on the other hand, has the opposite advantages and disadvantages.

The risk projection approach may be illustrated by a recent publication

(*1*) that applies the BEIR VII (*2*) risk models (largely based on studies of the atomic bomb survivors) to the estimated doses from Chernobyl for countries in Europe, including Belarus, the Russian Federation and Ukraine. This approach predicts 940 leukemia cases due to Chernobyl in Europe up to 2005 and predicts a total of 2400 such cases up to 2065.

The remainder of this paper will be concerned with summarizing the results of empirical epidemiological studies conducted to date in the three most affected republics. These are subdivided in turn by age at exposure since age at exposure is a strong modifier of the relative risk of leukemia in high-dose studies such as the atomic bomb survivors study. Three age-at-exposure groups are considered, mainly *in utero*, childhood and adult exposure.

As a result of this examination, it is concluded that in general such studies have failed to detect any association between Chernobyl exposure and leukemia risk with the possible exception of some studies of clean-up workers.

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## Autoimmune Thyroiditis after the Chernobyl Accident

Alina V. Brenner

*Radiation Epidemiology Branch; Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, DHHS, Bethesda, Maryland*

*Overview of Current Studies*

As a result of the Chernobyl Nuclear Power Plant accident on April 26, 1986, about 5 million residents of Ukraine, Belarus and the Russian Federation were exposed to radioactive fallout with some individuals receiving appreciable exposure (*1*). A strong relationship between such exposure and thyroid cancer has been established (*2*). Much less is known about long-term consequences with respect to benign thyroid diseases such as autoimmune thyroiditis (AIT). Hashimoto's thyroiditis, the most common type of AIT, is characterized microscopically by the infiltration of lymphocytes as well as by fibrosis of the thyroid gland. Clinically, it manifests by the presence of thyroid auto-antibodies, enlarged thyroid, and various degrees of thyroid dysfunction (*3*). Because AIT is common and its prevalence increases markedly with age, even a weak relationship with $^{131}$I exposure could result in a substantial burden among the exposed populations.

There have been about a dozen analytical and ecological studies on this subject (*1, 4*). Studies conducted within 11 years of the accident on children living in contaminated areas in Kaluga, Orel and Tula (Russia), Chernihiv and Kyiv (Ukraine), and Hoiniki (Belarus) found a higher percentage of anti-thyroid positive antibodies (to thyroglobulin, ATG, and/or to thyroid peroxidase, ATPO) relative to children living in uncontaminated control areas. By contrast, the Chernobyl Sasakawa health and medical cooperation project, after examining about 120,000 children from contaminated areas of Belarus, Russia and Ukraine, did not find a significant relationship between prevalence of antimicrosomal antibodies (an earlier term for ATPO) and/or ATG with exposure to radiation on the basis of $^{137}$Cs contamination in the body or soil. Fewer studies examined the relationship with AIT using clinical symptoms other than presence of anti-thyroid antibodies. The Chernobyl Registry of Belarus, which began data collection on non-neoplastic diseases in 1992, reported a temporal increase in prevalence of AIT, including Hashimoto's thyroiditis, among those who were children at the time of the accident. Although cases of disease were verified using medical records, the method of AIT diagnosis was not reported. A study of thyroid pathology in 16,340 thyroid glands removed from 1982 to 1986 and 1987 to 1996 in the Kyiv region found a steady increase in rates of AIT that was attributed to the Chernobyl accident. Finally, based on the results of fine-needle aspiration biopsy in 197 out of 1396 of individuals with ultrasound abnormalities of the thyroid gland, it has been estimated by the Chernobyl Sasakawa health and medical cooperation project that the prevalence of AIT was highest in Gomel and lowest in Mogilev, reflecting the pattern of radioactive fallout.

*Current Problems*

Results to date have been inconsistent, and the studies reported have methodological problems. The majority of studies have not distinguished between the presence of elevated anti-thyroid antibodies and AIT. While elevated levels of anti-thyroid antibodies are a hallmark of AIT, they may also reflect a thyroid autoimmunity phenomenon known to occur in other thyroid diseases and in about 10% of healthy individuals (*3*). Unlike the presence of antibodies, AIT is a clinical outcome that requires increased surveillance and/or treatment. Because there are no internationally accepted classification or criteria for AIT (*3*), comparisons are hampered among the few studies with different AIT outcomes or AIT outcomes that are not clearly defined.

Unlike case ascertainment for cancerous outcomes that may take advantage of cancer registries, case ascertainment for biochemically determined anti-thyroid antibodies and AIT must rely on standardized examination of well-defined source populations. Unfortunately, the origin of individuals eligible for and examined in many of the previous studies is unclear.

Only a few studies estimated the individual thyroid doses retrospectively, and virtually none relied on direct thyroid measurements. The majority of data come from studies using location at the time of the accident as a surrogate for radiation exposure. Additional problems include limited sample size and lack of proper adjustment for basic factors strongly influencing the background rates of AIT and anti-thyroid antibodies, such as sex and age.

*Cohort Study of Thyroid Cancer and Other Thyroid Diseases after the Chernobyl Accident*

Some of the problems cited above are being addressed by the two large cohort studies of thyroid cancer and other thyroid diseases among individuals who were under the age of 18 years on April 26, 1986, had individual thyroid radioactivity measurements made shortly after the accident, and were residents of contaminated territories of Ukraine ($N$ = 13,243) and Belarus ($N$ = 11,970) (*5*). These cohorts were assembled from a common source database of direct thyroid measurements. Since the dose distribution is similar in those individuals who were located and enrolled in the study and all originally eligible participants, there is little potential for selection bias. All study participants are being screened biennially by a team of specialists for a variety of thyroid diseases using standardized procedures; thus a potential confounding of screening by dose is not an issue. In addition to clinical examination, all individuals have their serum tests done making possible evaluation of clinical and subclinical outcomes, including AIT. AIT is defined *a priori* based on various combinations of the following criteria: the degree of elevation of antibodies to thyroid peroxidase (ATPO), thyroid-stimulating hormone (TSH), and the presence of ultrasonographic or palpatory abnormalities suggestive of AIT. $^{131}$I thyroid doses and their uncertainties are estimated from the combination of individual thyroid radioactivity measurements, data on dietary and lifestyle habits, and environmental transfer models.

*Prospects for Future Research*

The Ukrainian and Belorussian arms of the study are conducted according to a similar protocol and have thyroid doses estimated using a similar algorithm from direct thyroid measurements. Therefore, it will be possible to compare the results of dose–response analyses for autoimmune thyroid outcomes. The pooled analysis of both cohorts will certainly increase statistical power and help address effect-modifying issues, such as the potential role of stable iodine intake. Data from subsequent screening cycles in both countries will allow an evaluation of dose–re-

sponse relationships with incident newly diagnosed AIT cases and cases with elevated ATPO, and evaluation of variation in risk over time. It will also be important to compare the behavior of naturally occurring AIT cases with those diagnosed in Chernobyl-exposed populations. Incorporation of uncertainties in risk estimates will be required.

*Conclusion*

There is some epidemiological evidence of an association between low-dose environmental radiation exposure to $^{131}$I from the Chernobyl accident and a higher than expected prevalence of anti-thyroid antibodies that could not be attributed to chance alone. However, until more data from long-term epidemiologically sound studies with individual dose estimates become available, the overall evidence with respect to autoimmune thyroid disease, and AIT in particular, remains inconclusive.

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## Dosimetry in Chernobyl Studies


André Bouville

*National Cancer Institute, Radiation Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Institutes of Health, Bethesda, Maryland*


The Chernobyl accident, which took place in Ukraine on 26 April 1986, resulted in massive releases of radioactive materials into the atmosphere and in the involvement of a large number of workers to decontaminate the site of the nuclear power plant and its surroundings. Regarding the long-term health effects of the accident, the most serious consequence appears to be the incidence of thyroid cancer and other diseases among members of the public who were young children at the time of the accident. Leukemia and cataract among clean-up workers have also drawn attention. The methods used to estimate individual doses in some of the epidemiological studies that have been, or are being, conducted are presented. Estimates of dose also are presented.

*Thyroid Studies*

About five million people reside in the contaminated areas of Belarus, Russia and Ukraine, defined as those with a deposition of $^{137}$Cs per unit area of ground of more than 37 kBq m$^{-2}$ (*1*). The thyroid doses received by the populations of the contaminated areas of Belarus, Russia and Ukraine are mainly due to the atmospheric release of $^{131}$I and are, by far, higher than the doses to any other organ or tissue of the body from any other radionuclide. All epidemiological studies conducted so far (except one) have only considered the intakes of $^{131}$I in the estimation of the thyroid doses. Individual thyroid dose estimates are most accurate when they are based on personal measurements. This is the case for the studies conducted in Belarus and in Ukraine by the National Cancer Institute (NCI) in collaboration with the Ministries of Health of those countries. There are about 13,000 Ukrainian and 12,000 Belarusian subjects in the two cohort studies. The estimation of the $^{131}$I thyroid dose is based on radiation measurements made on all subjects within a few weeks after the accident. The $\gamma$ radiation emitted during radioactive decay of the $^{131}$I present in the thyroids of the subjects was measured by means of radiation detectors placed against the neck. The results of these measurements (called ''direct thyroid measurements''), expressed in terms of exposure rates, are used to derive the $^{131}$I activities in the thyroids at the time of the measurement. The temporal variation of $^{131}$I thyroid activity is estimated using the responses on residential history and on dietary habits obtained during personal interviews and a radioecological model simulating the environmental behavior of $^{131}$I both before and after the time of the direct thyroid measurement (*2*). An age-dependent thyroid dose coefficient is then used to infer the thyroid dose from the time-integrated $^{131}$I thyroid activity. A Monte Carlo procedure is used to obtain the distribution of the probabilities of the dose estimates. The uncertainties in the thyroid dose estimates resulting from $^{131}$I intakes are found to be, as a first approximation, lognormally distributed. The geometric standard deviations associated with these distributions vary from one individual to another and range from 1.6 to 5.0. The medians of the geometric standard deviations are 1.7 for the Ukrainian subjects and 2.1 for the Belarusian subjects. The parameters that account for most of the uncertainty are the thyroid mass and those parameters related to the determination of the activity content of $^{131}$I in the thyroid at the time of the direct thyroid measurement. The geometric means of the thyroid doses are less than 0.3 Gy for 51% of the 24,970 subjects, in the range from 0.3 to 1 Gy for 27% of the subjects, and greater than 1 Gy for 22% of the subjects (*3*).

For most individuals, the thyroid doses are due predominantly to intake of $^{131}$I. The thyroid doses resulting from intake of short-lived radionuclides (mainly $^{133}$I and $^{132}$Te) were delivered within a few days after the accident. The highest contributions of the short-lived radionuclides to the thyroid doses are expected to be found among the residents of Pripyat, which is a city close to the reactor site that was evacuated 36 h after the accident: On the basis of *in vivo* $\gamma$-ray spectrometric measurements on a sample of Pripyat residents, the contribution of short-lived radionuclides to the $^{131}$I thyroid dose was found to be about 20%, on average, for persons who did not use stable iodine prophylaxis and about 50% for persons who took KI pills soon after the accident (*4*). For locations far away from the reactor site, it is estimated that the contribution of the short-lived radionuclides to the thyroid dose is 1% or less. In addition, small contributions, of the order of 1%, to the thyroid dose are due to the intake of long-lived radionuclides, primarily $^{134}$Cs and $^{137}$Cs, and to external irradiation. These doses are delivered at low rates but will extend over several decades after the accident (*5*).

*Leukemia Studies*

The Chernobyl accident caused the deaths of 30 power plant employees and firemen within a few days or weeks. Later on, in 1986 and 1987, about 240,000 clean-up workers (also called ''liquidators'') were called upon to take part in major mitigation activities at the reactor and within the 30-km zone surrounding the reactor. Residual mitigation activities continued until 1990; all together, about 600,000 persons received the special status of ''liquidator.'' The incidence of leukemia and of cataract among the liquidators is the subject of epidemiological studies.

Epidemiological studies of leukemia are conducted by IARC and by NCI with the same method of estimation of individual bone marrow doses for all study subjects. A time-and-motion method of dose reconstruction, called RADRUE, is used for that purpose. The application of this method requires a detailed knowledge of (a) the radiation field, as a function of time after the accident, for multiple locations inside and outside the power plant and (b) the itineraries followed by the clean-up workers while they were exposed to radiation in or near the power plant. The main advantage

of the method is that it can be applied to any subject, whether dead or alive, for any dose level. The central estimates of the individual doses were found to range from 0 to 3200 mGy, with a geometric mean of 12 mGy and an arithmetic mean of 90 mGy. Taking the answers provided by the clean-up workers or their proxies to be correct, the uncertainties in the individual dose estimates are found to be lognormally distributed, with geometric standard deviations ranging from 1.3 to 4.5, with a geometric mean of 1.9 (Chumak *et al.*, manuscript in preparation).

*Cataract Study*

The method of assessment of the doses to the lens of the eye of liquidators is similar to the method used in the leukemia studies. However, in addition to external γ irradiation, the external β-particle irradiation must be taken into account. The problem of β-particle dose assessment to lens was addressed in the framework of the Ukrainian-American Chernobyl Ocular Study, which is a cohort study of cataract among 8607 Ukrainian recovery operation workers. The assessment of the β-particle dose was derived from the γ-ray exposure of the subjects. Gamma-ray to β-particle dose conversion coefficients were calculated using Monte Carlo procedures for a variety of β-particle-emitter spectra and of conditions of exposure. Preliminary findings showed that the distribution of individual β-particle/γ-ray ratios was quite broad, with median value 0.51 and 95% percentile of 1.84.

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## LOW-DOSE STUDIES: EVIDENCE FROM EXPOSURES BELOW ~100 mSv

Amy Kronenberg and Mary Schubauer-Berigan, Organizers

Amy Kronenberg, Chair

Antone Brooks, Discussant

### Evidence for Low-Dose Risks from Epidemiological Studies

Dale Preston

*Hirosoft International, Eureka, California*

Radiation is one of the most carefully studied cancer risk factors in humans. A number of well-designed long-term epidemiological studies involving large populations with good dose estimates are augmented by a vast and growing literature on laboratory and experimental studies of radiation effects. Analyses of these epidemiological studies, particularly the long-term follow-up of survivors of the atomic bombing of Hiroshima and Nagasaki (*1, 2*), are continuing to provide useful quantitative estimates of the long-term effects of radiation exposure on cancer and noncancer risks. Such risk estimates are important both scientifically, for the insights that they provide into the nature of radiation effects on disease risk, and for public policy, where they form the primary basis for international radiation protection standards.

However, despite their many strengths, risk estimates based on the atomic bomb survivor studies have several important limitations, including their inability to provide direct evidence of significant increases in risk at doses of the order of 10–100 mGy or less and uncertainty about how risks based on acute high-dose-rate exposures in a Japanese population should be applied to the populations of interest in radiation protection who often receive low-dose, low-dose-rate exposures and who may have very different baseline cancer or non-cancer disease rates. Resolving these problems requires assumptions about how to extrapolate from doses of 100–200 mGy or more, at which the evidence for radiation dose effects on cancer risks is quite compelling, to doses of a few tens of milligrays or less often received at very low dose rates.

This presentation begins with some comments on the difficulties and limitations of epidemiological studies of the effects of low-dose and low-dose-rate radiation exposures on cancer risks. This is followed by a discussion of recent results on direct inference about low-dose effects and the shape of the solid cancer dose response in cancer mortality and incidence risks in the atomic bomb survivors. The talk continues with a brief review of recent studies that provide direct evidence of low-dose radiation effects in a Russian population exposed as a consequence of environmental contamination resulting from the operation of the Mayak plutonium production complex (*3, 4*) and a pooled analysis of cancer risks in nuclear workers from 15 countries (*5*).

In the recent analysis of 50 years of solid cancer mortality follow-up in the atomic bomb survivors (*1*), there was evidence of a significant increasing trend with dose over the range 0 to 150 mGy and an indication ($P = 0.01$) of upward curvature in the radiation dose response over the 0- to 2-Gy dose range. However, the evidence for this curvature was a largely a reflection of relatively high risks at doses of the order of 1 to 2 Gy and the estimated linear trend over the low dose range was similar to the linear trend over the full dose range. Although based on a shorter follow-up period, new (as yet unpublished) analyses of solid cancer incidence risks among the atomic bomb survivors, which involve considerably more cases than the mortality analyses, provide little evidence for curvature in the dose response ($P = 0.1$). The incidence data are used to examine the effect of how estimates of radiation-associated excess risk per gray and inference about the shape of the dose response in the atomic bomb survivor studies is strongly dependent on the choice of cohort members included in the zero-dose group.

Despite the difficulties in conducting large-scale epidemiological studies, sophisticated analyses of several epidemiological studies (*5, 6*) are providing useful quantitative cancer risk estimates for relatively low-dose, low-dose-rate exposures that complement those based on the atomic bomb survivor data.

The Techa River cohort includes almost 30,000 Russian men and women with a broad range of ages at exposure who received low-dose, low-dose-rate internal and external radiation exposures from radioactive materials released into the river as a consequence of the operation of the Mayak plutonium production complex in the early 1950s. The cohort has 50 years of mortality follow-up with individualized estimates of the dose resulting from the low-dose-rate internal and external exposures (*7*) received by cohort members. Estimated stomach doses (which are taken as representative soft tissue doses) range up to 0.5 Gy, though truly individual dose estimates could be larger, with a mean of 0.03 Gy. The analyses presented in ref. (*6*) reveal that the radiation dose response for solid cancer is statistically significant, with a point estimate of the risk per unit

dose that is considerably larger than that seen for the atomic bomb survivors.

The first analyses of cancer risks in more than 400,000 nuclear industry workers from 15 countries are also providing direct evidence of radiation-associated increases in cancer risks after low-dose and low-dose-rate exposures. At present, the solid cancer risk estimates from the Techa River and nuclear worker studies are larger than the point estimates obtained for atomic bomb survivors. However, confidence intervals for the risk estimates from these studies are quite wide and are statistically compatible with estimates based on the experience of the atomic bomb survivors.

Despite the difficulties in the conduct of large long-term epidemiological studies, various studies are providing compelling evidence of radiation effects at doses of the order of 100–200 mGy and suggesting that small increases in solid cancer risks are likely at lower doses and low dose rates. While these studies will continue to provide important insights on radiation and low-dose cancer risks, they cannot be expected to provide direct evidence of statistically significant effects at very low doses. Thus improved understanding of the biological basis for long-term radiation effects will be increasingly important in the description of low-dose radiation effects on disease rates.

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## Risk of Leukemia at Low Doses: The NIOSH Multi-site Leukemia Case-Control Study


Mary K. Schubauer-Berigan, Robert D. Daniels and Donald A. Fleming

*National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, Cincinnati, Ohio*


Ionizing radiation has been known as a cause of most forms of leukemia for over 50 years (*1*), and quantitative models exist for the association between leukemia and external ionizing radiation [other than chronic lymphocytic leukemia (CLL)], based primarily on studies of populations exposed to relatively high doses (*2–4*). Quantitative estimates of risk at low cumulative doses (e.g., below 100 mGy) are still uncertain, but they have recently been estimated among a combined international study of nuclear workers to be similar to results seen among those exposed to high and moderate radiation doses (*5*). CLL is the only cancer specifically excluded from compensation programs in the U.S., since it is presumed to be a non-radiogenic cancer.

The purpose of this study was to assess quantitatively the association between radiation from all workplace sources and leukemia, controlling for known or suspected confounders of the association. A nested case-control study was conducted among workers at four DOE facilities (Hanford, Los Alamos National Laboratory, including Zia workers, Oak Ridge National Laboratory, Savannah River Site) and the Portsmouth Naval Shipyard, sites thought to have minimal exposure potential to plutonium and other internal emitters. Mortality risks were analyzed separately for CLL and non-CLL leukemia, given the expectation of differential radiogenicity for these two outcomes. Leukemia cases were ascertained by death certificate diagnoses, and leukemia subtype was further identified using available medical records. Forty-three cases of underlying CLL deaths and 172 age-matched controls, and 206 non-CLL cases and 823 age-matched controls were identified with follow-up through 1990 to 1996.

Bone marrow doses were estimated for each worker from both external sources and plutonium (*6*), based on monitoring records. Recorded doses were adjusted for measurement biases from exposure to heterogeneous radiation fields, calibration methods, dosimeter design, dosimeter energy response, and geometry of the critical organ (*7*). Individual external doses from γ- and X-ray exposures below the detection limit were estimated (*7–9*), as were doses from work-related medical X-ray examinations (*10*).

Potentially confounding exposures included cigarette smoking (estimated using workplace medical records) and benzene and carbon tetrachloride exposures. Exposures to the solvents were estimated through a job-exposure matrix developed using workplace records combined with facility process descriptions. Conditional logistic regression was used to estimate the excess relative risk (ERR) per unit of radiation, considering potential confounders such as sex, race and ethnicity, birth cohort, smoking and solvent exposure. Dose lags of 2 years for non-CLL leukemia and 10 years for CLL were employed.

For non-CLL leukemia, the ERR per 10 mSv was 1.44% (95% CI: $<-1.03\%$, 7.59%), adjusting for age, sex and benzene exposure, but it was higher for workers born after 1921 compared to workers born earlier or when excluding leukemias of uncertain subtype. This increased risk was not restricted to high-dose workers: Excluding workers receiving $>100$ mSv, the sex- and benzene-adjusted ERR per 10 mSv was 6.82% (95% CI: −2.87%, 24.1%). The results suggest that overall risks per unit dose among these nuclear workers are comparable to those observed in high-dose populations, although no evidence was observed of a linear-quadratic dose response.

For CLL, the ERR per 10 mSv was −2.0% (95% CI: $<0$, 14%) based on all worker exposures. However, excluding high-dose workers substantially changed these estimates: For workers receiving $<100$ mSv, the ERR per 10 mSv was 15% (−4.7%, 76%). Time window analyses among this group suggested that a latency of 10 years is appropriate. Adjustments for other suspected confounders had little effect on these estimates. Results for non-CLL leukemia analyses are described in detail elsewhere (*11*).

This large study is among the first to specifically evaluate benzene risk in conjunction with ionizing radiation. Since workers with higher benzene exposure scores had higher rates of non-CLL leukemia than unexposed workers, the elevated risks observed among workers with joint exposure to radiation and benzene are also of interest. Studies based on populations with high radiation doses or low background rates of CLL have observed no effect of radiation on CLL risk, leading to the conclusion that CLL is non-radiogenic. However, this study of CLL in a nuclear workforce showed inconsistent evidence of risk associated with low-level exposures: Although confidence intervals are wide, elevations in estimated risk observed below 100 mSv were not seen at higher exposures. Further studies of larger, low- or moderate-dose populations with substantial background risk and lengthy follow-up may be needed to further address the radiogenicity of CLL.

*Disclaimer*

The findings and conclusions in this presentation have not been formally disseminated by NIOSH and should not be construed to represent any agency determination or policy.

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## *In Vitro* Radiation-Induced Neoplastic Transformation: Suppressive Effects at Low Doses


J. Leslie Redpath

*Department of Radiation Oncology, University of California Irvine, Irvine, California*


*Relevance of In Vitro Assays*

The *in vitro* assay of neoplastic transformation has a long history of providing quantitative and mechanistic data that parallel that found *in vivo* (*1*). Such findings relate to the effects of dose, dose rate, radiation quality and chemical modifiers and as such are regarded as being of value to the study of radiation carcinogenesis. This is despite the obvious microenvironmental limitations and lack of an immune response compared to *in vivo*. Another perceived limitation is the refractory nature of primary human cell cultures to radiation-induced transformation to the neoplastic phenotype. Even after immortalization, such cells are difficult to transform neoplastically with radiation, and transformation usually requires repeated high radiation doses over a period of time. Such systems are of value in assessing molecular and cellular characteristics of the radiation-induced tumorigenic cells but are not suitable for quantitative studies (e.g. *2*). The major workhorse assay in the field of quantification of radiation-induced neoplastic transformation *in vitro* has been the C3H 10T½ mouse embryo fibroblast system. Another assay that has recently been gaining wider acceptance is the HeLa × skin fibroblast human hybrid cell assay (*3*). This assay has some advantages, both practical and mechanistic, over the C3H 10T½ system. First, the assay takes 21–24 days to go to completion compared to up to 60 days for the C3H 10T½ assay. Second, the neoplastic phenotype has a cell surface molecular marker that can be used to identify foci of tumorigenic cells, compared to the morphological changes used with C3H 10T½ cells. Third, the mechanism involved in the neoplastic transformation is the loss of putative tumor suppressor located on chromosomes 11 and 14 (*4*). Both the C3H 10T½ and the human hybrid cells should be considered as partway down the pathway from normal to tumorigenic, i.e. preneoplastic. The relevance of use of such cells could also be questioned; however, since most humans have burdens of pre-neoplastic cells in their body, they could be considered to be a particularly relevant target for carcinogens, including radiation.

*Adaptive Response*

The ability of a low dose of radiation, say 1 to 10 cGy, to ameliorate the effect of a subsequent high radiation dose, say several grays, is well described for a variety of *in vitro* and *in vivo* end points. This adaptive response has been linked to radiation-induced protein synthesis, including the up-regulation of DNA repair proteins and cellular antioxidants, as a consequence of the initial low dose [(*5*) and references therein]. It is conceivable that such low-dose responses could affect the shape of dose–response curves at low doses, including for the end point of neoplastic transformation *in vitro*, where there is a certain background incidence, as there is for human cancer.

*Suppressive Effects of Low Doses of Low-LET Radiation for Neoplastic Transformation In Vitro*

Azzam *et al.* (*6*) demonstrated that low doses of 0.1, 1.0 and 10.0 cGy of $^{60}$Co γ radiation suppressed the transformation frequency of C3H 10T½ cells to levels less than that seen spontaneously. This was subsequently verified for a dose of 1 cGy of $^{137}$Cs γ radiation in the human hybrid cell assay (*7*). Full dose–response curves were then developed for a series of low-LET sources including 60 kVp X rays, 28 kVp X rays and 232 MeV protons [(*8, 9*) and references therein]. These dose–response curves were J-shaped and consistently demonstrated suppression at low doses with thresholds between 10 and 20 cGy. Mechanistic studies indicate that multiple mechanisms are likely involved in a dose-dependent fashion. These include the up-regulation of DNA repair and the hyperradiosensitivity to radiation-induced cell death of a transformation-prone subpopulation [(*8, 9*) and references therein]. Bystander effects due to factors excreted into the extracellular medium do not appear to play a role in this assay, although bystander effects as a consequence of gap junction intracellular communication may do so in a way that partially offsets effects due to an adaptive response (*10*). Recent low-dose-rate studies (*11*) have shown that the suppressive effects still exist at low doses and the threshold dose is increased as the dose rate is decreased.

*In Vivo Response at Low Doses*

Dose–response curves consistent with a threshold effect have been found in animal studies (*12*), and low doses have been found to increase the latent period for tumor formation in cancer-prone mice (*13*). Low-dose-rate studies in animals also strongly support the possibility of dose thresholds (*12*).

*Comparison with Epidemiological Findings*

J-shaped dose–response curves cannot be ruled out for radiation-induced leukemia and breast cancer in humans. Relative risks for the high- and low-dose-rate transformation data compare well with those for breast cancer in humans, and while the epidemiological data can be fitted to a linear, no-threshold (LNT) model, the existence of threshold doses cannot be ruled out. The fitting of data to the LNT model is heavily driven by the high-dose data points and tends to obscure what is happening at low doses.

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## γ-H2AX as a Sensitive Indicator of DNA Double-Strand Breaks


Peggy L. Olive

*Medical Biophysics Department, British Columbia Cancer Research Centre, Vancouver, British Columbia, Canada*


Complex DNA lesions such as double-strand breaks are generally accepted to be the most important potentially lethal lesions induced by ionizing radiation. Physical methods used to detect double-strand breaks in individual cells are limited to a sensitivity of about 1–2 Gy, or about 50 double-strand breaks/cell. With the discovery by William Bonner's group of a minor nucleosomal histone, H2AX, that undergoes phosphorylation in response to double-strand breaks (*1*), individual breaks can now be identified.

Phosphorylation of H2AX by kinases like ATM occurs rapidly at large regions surrounding each DNA double-strand break. The reasons for this change are not well understood; however, phosphorylation of H2AX is believed to result in a local change in chromatin structure, retention of repair factors, possible ''tethering'' of DNA ends, and ultimately more accurate repair of the breaks. Our results using the comet assay indicate that H2AX knockout mouse cells, like ATM cells, are able to rejoin breaks at the same rate as repair-proficient cells. However, cells lacking H2AX are more radiosensitive and more susceptible to developing cancer (*2*).

Antibody labeling of the phosphorylated form of H2AX (called γ-H2AX) along with fluorescence microscopy allows identification of individual double-strand breaks. Experiments with radiolabeled iodine incorporated into DNA (*3*) and measurements of γ-H2AX foci in cells with known numbers of radiation-induced breaks (*4*) support the view that one double-strand break produces one γ-H2AX focus. Dose–response curves are linear down to doses of centigrays (*4*) but begin to saturate at doses above 8 Gy. Although foci can be detected microscopically within minutes of irradiation, they continue to enlarge for 30–60 min after irradiation. By this time, most double-strand breaks have rejoined, so there is an apparent discrepancy between γ-H2AX focus formation and double-strand break rejoining. After 30–60 min, foci begin to disappear as a result of dephosphorylation although some foci may remain (or reform?) hours to days later. These residual foci often co-localize with other molecules that form repair complexes including RAD51, BRCA1, 53BP1 and the MRN complex, as well as with foci associated with telomeres (TRF1/2) or chromatin modification (HDAC4, BRCA2). Interestingly, when cells with residual γ-H2AX foci divide, daughter cells show near-identical patterns in terms of focus number and size.

Flow cytometry is a simple way to follow the kinetics of formation and loss of γ-H2AX. The rate of loss of γ-H2AX in the first few hours after irradiation has been associated with greater radiosensitivity in human cell lines (*5*). For most tumor cell lines, the fraction of cells that retain γ-H2AX foci 24 h after X irradiation also correlates with the fraction of cells that survive to form a colony. Exceptions are cell lines that undergo rapid apoptosis and cell lines that exhibit high endogenous levels of γ-H2AX foci. Therefore, analysis of γ-H2AX has the potential to tell us how many double-strand breaks have been produced in a cell, where these breaks are located, and whether the cells containing these breaks are repair proficient.

However, before concluding that a specific γ-H2AX focus is indicative of the presence of a radiation-induced double-strand break, several factors must be considered. As indicated above, a γ-H2AX focus can indicate that a break was present 1 h earlier; however, it may have undergone repair by the time of analysis. γ-H2AX foci are also found at sites of replication blockage, at early apoptosis, during V(D)J recombination, during meiosis, and in senescing cells. Cells replicating their DNA develop small foci that are still present when cells move into $G_2$ phase. Moreover, some tumor cell lines show 50 or more ''cryptogenic'' γ-H2AX foci, most of which do not appear to be associated with double-strand breaks. There is now evidence that bystander cells that have not received direct exposure to radiation can also develop foci (*6*).

The sensitivity for detecting radiation-induced breaks can be influenced by chromatin organization and radiation track structure. Changes in degree of chromatin condensation (mitotic cells or treatment with high salt or histone deacetylase inhibitors) can enhance focus size, which is probably indicative of inability to repair the breaks. Exposure to high-LET particle radiation produces γ-H2AX foci in non-homogeneous clusters along the ion trajectory, providing a way to examine the spatial dynamics of DNA repair molecules but making it difficult to count individual foci (*7*). The ability of γ-H2AX foci to mark individual breaks has stimulated

interest in its application as a biodosimeter. The fact that human lymphocytes show low endogenous expression of γ-H2AX and similar kinetics of focus development and loss for different individuals supports this possibility. However, a concern is that γ-H2AX intensity after irradiation changes continuously over time so that determining the dose received by a cell based on number of foci will require prior knowledge of time of irradiation relative to time of cell fixation.

In summary, immunofluorescence detection of γ-H2AX foci provides a sensitive method for detecting and following the fate of complex DNA lesions produced by ionizing radiation. Although there remain many questions concerning the role of these foci in DNA damage signaling and their ability to unambiguously mark sites of damage or misrepair, γ-H2AX clearly has the potential to greatly improve our understanding of DNA repair dynamics.

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## Radiation-Induced Bystander Effects: Mechanisms and Implication for Low-Dose Radiation Risk Assessment


Tom K. Hei

*Center for Radiological Research, College of Physicians & Surgeons and Department of Environmental Health Sciences, Mailman School of Public Health, Columbia University, New York, New York*


Generations of students in radiation biology have been taught that heritable biological effects require direct damage to DNA. Although evidence suggesting that this simple statement is not strictly true has been around for decades, studies conducted over the past few years have unequivocally shown that both extracellular and extranuclear events play an important role in determining the biological responses to ionizing radiation (*1*). While circumstantial evidence in support of a bystander effect appears to be consistent, direct proof of such extranuclear/extracellular effects are demonstrated most convincingly using charged-particle microbeams. Using the Columbia University microbeam to target an exact fraction of cells in a population and irradiate their nuclei with exactly one α particle each, it has been shown that the frequencies of induced mutations and chromosomal changes in populations where some known fractions of nuclei were hit are consistent with non-hit cells contributing significantly to the response. In fact, irradiation of 10% of a mammalian cell population with a single α particle per nucleus results in a mutant yield similar to that observed when all of the cells in the population are irradiated (*2*).

Evidence for a bystander response based on *in vivo* studies is rather limited. A three-dimensional cell culture model comprised of human-hamster hybrid ($A_L$) and Chinese hamster ovary (CHO) cells in multicellular clusters was used to investigate low-LET radiation-induced bystander genotoxicity (*3*). Using tritiated thymidine that emits short-range β particles to ensure only self-irradiation of the labeled cells, there is evidence that low-LET radiation can induce bystander mutagenesis in a three-dimensional cell culture model. Furthermore, using a three-dimensional Epiderm tissue model and the Columbia University α-particle microbeam, Belyakov *et al.* have recently demonstrated that bystander apoptosis is clearly evident in tissue up to 1 mm from irradiated cells (*4*).

The plethora of data now available concerning the bystander effect fall into two categories: (1) in confluent cultures where physical contacts between irradiated and nonirradiated cells are made and where gap junction communication has been shown to be essential for the process; (2) in sparsely populated cultures where bystander effects may be mediated by damage signals released into the culture medium by the irradiated cells. As a result, incubation of nonirradiated cells with conditioned medium from irradiated cultures may lead to biological effects in these bystander cells. Since the nature of the signaling molecules involved in the two bystander pathways are not known, their mechanisms are not mutually exclusive at this moment. In fact, it is likely that some common initiating or intermediate steps are involved in the two processes. Although p53 is not necessary for the expression of the bystander effect, there is evidence that repair-deficient cells may express a higher bystander response.

Using cDNA microarrays, a number of cellular signaling genes have been shown to be differentially expressed among bystander cells. The transcription level of one gene, cyclooxygenase 2 (COX2), was found to be consistently up-regulated by more than threefold, while the RNA level of insulin growth factor binding protein 3 (IGFBP3) was found to be consistently lower by more than sevenfold in multiple analyses of multiple bystander samples (*5*). Treatment of bystander cells with NS-398, which suppresses COX2 activity, or with exogenously applied IGFBP3 significantly reduced the bystander effect. These results provide evidence that the COX2-related pathway, which is essential in mediating cellular inflammatory responses, is the critical signaling link for the bystander phenomenon.

Radiation-induced bystander effects represent a paradigm shift in our understanding of the radiobiological effects of ionizing radiation. The bystander observations imply that the relevant target for various radiobiological endpoints is larger than an individual cell and suggest a need to reassess the way risk estimates are derived at low doses of radiation exposure. A better understanding of the cellular and molecular mechanisms of the bystander phenomenon together with evidence of their occurrence *in vivo* will allow us to formulate a more accurate model in assessing the health effects for low doses of ionizing radiation.

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## CARDIOVASCULAR AND CEREBROVASCULAR OUTCOMES AND RADIATION

Sarah Darby and Cecile Ronckers, Organizers

Sarah Darby, Chair

Cecile Ronckers, Discussant

### Overview of Studies of Long-term Cardiovascular Effects in Humans

Kiyohiko Mabuchi

*Radiation Epidemiology Branch, National Cancer Institute, National Institutes of Health, Rockville, Maryland*

The risk of cardiovascular disease from high-dose cancer radiation therapy has long been known. Traditionally, the cardiovascular effects of radiation have been considered deterministic, with a threshold dose of about 40 Gy, below which there is assumed to be no clinically significant heart disease. Radiation therapy-related doses have been reduced considerably in recent years, but there is continuing concern regarding the heart disease risk among children treated with modern radiation therapy and for other cancer survivors after modern radiation therapy, and this is amplified by the recent evidence from the atomic bomb survivors of increased risks of non-cancer diseases, including heart disease and stroke, at doses less than 4–5 Gy (*1*).

The purpose of this review is to examine the epidemiological literature on radiation-related cardiovascular disease in radiation-exposed populations, focusing on the risk at doses previously not thought to be harmful for the disease. Historically, the two major sources of data on radiation therapy-related cardiovascular disease risk have been studies of patients irradiated for Hodgkin lymphoma or breast cancer. Early data linked chest doses in excess of 40 Gy to coronary and other heart diseases. The dose to the chest from modern radiotherapy for Hodgkin lymphoma has been lowered and is typically in the range of 30–35 Gy for adults and 15–25 Gy for children. Few data, however, are available for long-term cardiovascular disease risk after radiation therapy as it is currently applied. The heart disease risk from radiation therapy for breast cancer has been estimated from randomized clinical trials and ''laterality studies''. In laterality studies, the radiation-related risk of heart disease is estimated by comparing disease rates for radiation therapy for left-sided and right-sided breast cancer. Results from randomized trials and laterality studies agree that older series of post-mastectomy radiation therapy providing tumor doses of 40–50 Gy results in an increased risk of mortality from cardiovascular disease, which may persist for three to four decades or longer.

Recent randomized trials showed no significantly increased risk of heart disease risk for patients treated with modern radiation therapy. These findings are consistent with a diminished risk of heart disease after modern adjuvant radiation therapy, which typically exposes 0–5% of the left ventricle to about 25 Gy (*2*). However, the follow-up of patients in these studies has not been sufficiently long to allow for the long latency effect, and it is therefore premature to conclude that the radiation therapy-related risk has disappeared. Further follow-up is clearly indicated, since imaging studies indicate the presence of myocardial perfusion defects in breast cancer patients treated with modern radiation therapy. Furthermore, recent data from patients irradiated for peptic ulcer have demonstrated a significantly elevated risk of coronary heart disease mortality linked to lower radiation dose and dose volume (15 Gy to 5% of the heart) (*3*).

The atomic bomb survivor data show significant associations of radiation dose and mortality risks of heart disease, stroke and other non-cancer diseases. The causal nature of these associations is compelling because of the dose–response relationships that cannot be explained by confounding, selection bias or disease misclassification on death certificates. Significant associations are also found for the incidence of coronary heart disease and other related vascular end points (*1*). The shape of the dose response for non-cancer disease is influenced by the possible ''healthy survivor effect'', which is dependent on time and urban-rural residence, resembling the healthy worker effect observed in occupational cohort studies. Adjusted for these effects, the dose response is linear, but there is considerable uncertainty regarding the dose response below about 0.5 Gy. The ERR $Gy^{-1}$ estimates of 0.17 and 0.12 for cardiovascular and cerebrovascular disease mortality, respectively, are lower than that for solid cancer morality, but the modifying effects of age and gender on the risk generally follow the patterns seen for solid cancer risk.

There is a large number of epidemiological studies of radiation-exposed populations worldwide that can provide potentially valuable data for assessing cardiovascular disease risks at a range of low doses. To date, however, published data on cardiovascular diseases from these populations are very limited (*4*). A study of patients irradiated for metropathia hemorrhagica provides suggestive evidence of radiation effects on circulatory disease, but a larger study of irradiated patients with benign gynecological conditions shows no indication of the circulatory effects. Data from the historical UK and U.S. radiologist cohorts provide conflicting results regarding circulatory disease effects, but both of these studies are both ecological in nature and lack radiation dose estimates. In the follow-up study of U.S. radiologic technologists, work history-based exposure estimates indicate an excess risk of circulatory disease (*5*), and further risk data will be forthcoming when dose reconstruction for this cohort is completed. A large number of nuclear worker studies are important sources of low-dose risk information, but the lack of data on smoking and other confounding factors in many of the occupational cohorts severely limits the assessment of the radiation effects on cardiovascular disease. Two major pooled analyses of nuclear worker studies provide conflicting results regarding the dose response for circulatory disease. Currently, the limited published results for cardiovascular disease risk available from low-dose populations vary and are inconclusive. They lack strength either to corroborate or to refute the evidence from the atomic bomb survivor studies. They are difficult to evaluate because of possible publication bias, differences in methods of statistical analysis used, or failure to consider confounders and possible biases. While more studies of cardiovascular diseases risk in other radiation-exposed populations are clearly needed, it will also be helpful to pool and analyze published and unpublished data on cardiovascular disease and radiation from the various populations to increase power for investigating the apparently small disease risk at a low dose.

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## Long-Term Mortality from Heart Disease after Radiotherapy for Early Breast Cancer

Sarah C. Darby, Paul McGale, Carolyn W. Taylor and Richard Peto

*Clinical Trial Service Unit and Epidemiological Studies Unit (CTSU), University of Oxford, Oxford, United Kingdom*

*Background*

Radiotherapy for early breast cancer can decrease breast cancer mortality but increase other mortality, chiefly from heart disease and lung cancer. The average cardiac dose from irradiation of a left-sided tumor can be two or three times that for a right-sided tumor. Particularly during the 1970s, when typical heart and lung exposures were greater than now, the laterality of an irradiated breast cancer could measurably affect cardiac mortality decades later, allowing population hazards to be assessed from routine cancer registry and death certificate data.

*Methods*

A prospective study was established of 308,861 U.S. women with early breast cancer registered in SEER during 1973–2001 and followed for cause-specific mortality until 2002.

*Findings*

Thirty-seven percent of women received radiotherapy. Among those who did not, tumor laterality was of little relevance to subsequent mortality. For women diagnosed during 1973–1982 and irradiated, the cardiac mortality ratio, left versus right tumor laterality, was 1.20 (95% confidence interval 1.04–1.38) <10 years afterward, 1.42 (1.11–1.82) 10–14 years afterward, and 1.58 (1.29–1.95) 15+ years afterward (trend: $2P = 0.03$). For women diagnosed during 1983–1992 and irradiated, it was 1.04 (0.91–1.18) <10 years afterward and 1.27 (0.99–1.63) 10+ years afterward. For women diagnosed during 1993–2001 and irradiated, it was 0.96 (0.82–1.12), with none yet followed for 10 years.

*Interpretation*

U.S. breast cancer radiotherapy regimens of the 1970s and early 1980s appreciably increased heart disease mortality 10–20 years afterward with, as yet, little direct evidence on the hazards 20+ years afterward. Since then, improvements in radiotherapy planning should have reduced such risks, but the long-term hazards in the general populations of various countries still need to be monitored directly.

## Radiation-Induced Heart Disease in Breast Cancer: The "Acute" Effects

Gabor Gyenes

*Division of Cardiology, University of Alberta, Edmonton, Alberta, Canada*

The potential prognostic significance of radiation-induced heart disease of post-mastectomy radiation therapy is now well established. Up to the early 1980s, radiation-induced heart disease appeared to increase mortality in patients with left-sided breast cancer. In a meta-analysis by Cuzick *et al.* published in 1987, an improved overall mortality was found among patients not receiving post-mastectomy radiation therapy (*1*). Both this overview as well as analyses of the individual trials found an excess cardiac mortality and morbidity on the radiation treatment arm (*2–4*). These findings suggested that an excess in radiation-induced cardiac mortality might offset any potential benefit from radiotherapy with regard to breast cancer mortality.

Eventually, these allegations were refuted when two large randomized studies published in 1997 showed that post-mastectomy radiation therapy not only reduced loco-regional recurrence but also significantly improved disease-free and overall mortality rates (*5, 6*). A meta-analysis of 40 studies showed a moderate but statistically significant reduction in the annual death rate from breast cancer, and a follow-up study of Cuzick's first overview also found improved mortality from both cardiac and breast cancer after radiation therapy (*7, 8*).

A series of studies assessed the incidence of radiation-induced heart disease after breast-conserving surgery and adjuvant radiation therapy of the remaining breast when this type of treatment gradually replaced total mastectomy (*9–12*). All of them found very small numbers of complications. Only one (*11*) found an increased incidence of myocardial infarction (2%) in irradiated patients compared with the control patients (1%) who had right-sided disease. However, the absolute myocardial infarction incidence of 1% was quite low in this patient population.

Since it takes at least 10 years on average to develop radiation-induced heart disease, there have been several attempts to predict who is at risk and also to prevent radiation-induced heart disease as much as possible. That is why studies of patients with radiation therapy who are still asymptomatic have gained widespread interest.

Truly acute radiation-induced heart disease occurs only if a large mass adjacent to the heart is irradiated. This is rare and is usually characterized by signs and symptoms of acute pericarditis. If pericardial tamponade ensued secondary to fluid collection in the pericardium, it might need to be drained. Sometimes even pericardial "window" surgery is required. In most of the other cases, what is sometimes called "acute radiation-induced heart disease" is a generally asymptomatic inflammatory response to radiation that may be detected by sensitive imaging methods.

In a prospective study 15 years ago, 54 consecutive breast cancer patients were examined before and after radiation therapy by echocardiography and ECG (*13*). Almost half of the patients received electron therapy after modified total mastectomy and the remaining patients had photon irradiation after breast-conserving surgery. Twenty-three patients were found to have signs of what appeared to be pericardial irritation on either ECG or echocardiography. These findings were more prevalent in photon-irradiated patients (17 compared to 6) and in those with left-sided disease (15 compared to 8). Seven patients developed some of these signs within 1 week after irradiation and once developed these findings disappeared in only a few cases within the follow-up 6 months to 2 years. Some of these patients had further tests (including one who had a coronary angiogram) to rule out coronary artery disease behind the newly developed ECG changes.

In another study (*14*), 17 breast cancer patients who received radiation therapy to the left side of the chest were selected based on a CT-guided radiation treatment plan if it showed that a part of the myocardium would receive at least 65–95% of the total radiation dose. These patients had a preirradiation stress and rest radionuclide perfusion scintigraphy and were enrolled to the study if this test was normal. Five patients were thus excluded or lost to follow-up and 12 patients were followed prospectively with another scintigraphy in an average of 13 months. Eight patients had total mastectomy and four were treated with breast-conserving surgery. Six of the 12 patients had newly developed perfusion defects on the follow-up scintigraphy. All of these defects were nonreversible, i.e. fixed, and were located at the anterior, anteroseptal or apical part of the left ventricle that correlated very well with the irradiated areas of the myocardium. Therefore, the findings could be explained by radiation therapy-induced microvascular damage. Serial echocardiography was also performed to look for changes in left ventricular systolic or diastolic function but none were found.

In a separate trial the approximate percentage of women with early

(Stage I) breast cancer who could be affected by radiation-induced heart disease was also assessed (*15*). Treatment plans for 100 consecutive patients were superimposed on 30 slices of their chest CT images and the expected amount of irradiated myocardium was thus calculated. The results showed that for the full cohort of 100 patients approximately 6% of the mean heart volume received at least 50% of the prescribed dose of 50 Gy. However, about 6% of the patients would receive at least 25 Gy in up to 15–21% of the heart volume, which would translate into an excess cardiac risk of about 9–12%. Another important lesson learned from this study was that no obvious risk factors could be identified to help select patients at high risk for cardiac complications. The most prudent way to predict the risk of cardiac complications is to use 3D radiation treatment planning or to assess the irradiated heart volume by virtual simulation at a level just above the diaphragm where the heart is closest to the chest wall.

It is difficult to know to what extent the above findings would translate into clinically significant coronary artery disease in 10 years. Gagliardi *et al.* attempted to give an answer to this question by using a biological model (*16*). The average excess cardiac risk for Stage I breast cancer patients treated with tangential 6 MV photon beams appeared to be in the range of 2%, which is exactly the rate that was found later in retrospective studies (*9–12*).

In summary, a significant number of breast cancer patients may exhibit signs of early radiation-induced heart disease if tested by sensitive imaging methods. Although these patients are usually asymptomatic, they might be at a higher long-term risk for developing symptomatic late radiation-induced heart disease. Despite the fact that modern radiation therapy affects a smaller proportion of the heart in a smaller proportion of patients than was the case earlier, radiation-induced heart disease still might be an important problem for a few women receiving radiation therapy for breast cancer, and therefore, every possible effort has to be made to save the heart from radiation provided that the treatment of breast cancer is not significantly compromised.

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## Radiation-Induced Cardiovascular and Cerebrovascular Effects: Animal Studies

Fiona A. Stewart, S. Hoving and J. J. Kruse

*The Netherlands Cancer Institute, Division of Experimental Therapy, Amsterdam, The Netherlands*

*Introduction*

Earlier diagnosis and better treatment options have led to improvements in cancer specific survival for most tumor types, but this also results in an increased number of patients at risk for developing treatment-related side effects. At least half of all cancer survivors receive radiotherapy during their treatment, and vascular injury is the major cause of late radiation morbidity. Significant increases in cardiovascular or cerebrovascular disease have been demonstrated after radiotherapy for many types of cancer, including breast, testicular, head and neck, and Hodgkin's disease (*1–4*). The risk of damage increases with time from treatment and is greatest in patients who are irradiated at a young age. Although there is good evidence to identify radiation as an independent risk factor in vascular disease, it is not clear whether this simply represents an accelerated version of age-related atherosclerosis or whether factors specific

to radiation are involved. A better understanding of the mechanisms underlying development and progression of radiation-induced vascular disease would help to identify effective targets for intervention after radiotherapy and suggest management strategies to reduce the risk of treatment related morbidity in long-term cancer survivors.

*Radiation-Induced Atherosclerosis*

Experimental studies in hypercholesterolemic rabbits or mice have demonstrated that irradiation of large arteries results in an accelerated formation of atherosclerotic plaque (*5–8*). The initial event seems to be endothelial cell damage, leading to monocyte adhesion and transmigration into the subendothelial space. In the presence of elevated cholesterol levels, these invading monocytes transform into activated macrophages, which ingest lipids and form fatty streaks in the intima (*5*). Hypercholesterolemia must be present at the time of irradiation for this to occur; wild-type mice fed regular chow after irradiation or transferred to a high fat diet more than 2 weeks after irradiation do not develop fatty streak lesions. The process can be inhibited by the antioxidant CuZn-superoxide dismutase (*6*). This suggests that radiation initiates the formation of atherosclerosis by inducing oxidative damage in the vessels, which enhances oxidation of low-density lipoproteins and allows them to be ingested by macrophages. Smooth muscle cell activation, migration from the media, and proliferation within the lesion occur at a later stage.

Several studies have examined phenotypic differences between radiation-induced and age-related atherosclerotic plaque developing after total-body irradiation followed by bone marrow reconstitution (*9*) or after localized irradiation alone or combined with balloon catheter injury (*10, 11*). These studies showed that lesions developing in the unirradiated arteries of hypercholesterolemic animals were collagenous and had only minimal macrophage infiltration. By contrast, lesions in irradiated arteries were macrophage rich, with high levels of metalloproteinases and other proteolytic enzymes and lipid-filled cores. Our own study (*10*) also revealed persistent endothelial cell damage and intra-plaque hemorrhage, features that were absent from lesions in unirradiated arteries.

These results suggest that radiation not only may induce early endothelial cell damage and fatty streak formation but also may lead to a chronic inflammatory process favoring the development of vulnerable, thrombotic plaque. We are currently investigating whether anti-inflammatory and anti-thrombotic drugs can inhibit both the initiation and the progression of radiation-induced atherosclerosis.

*Radiation-Induced Heart Damage*

Experimental studies in rabbits, rats and dogs have allowed a detailed analysis of the development of radiation-induced injury before the onset of functional and clinical changes [reviewed in (*12*)]. The earliest morphological changes seen in the irradiated heart are reversible changes in the function of capillary endothelial cells, leading to lymphocyte adhesion and extravasation. This is followed by decreases in capillary density, accompanied by loss of the endothelial cell marker alkaline phosphatase (*13, 14*). These early changes in capillary structure and function may be a major factor in the subsequent development of radiation-induced heart disease.

Single doses of 16–20 Gy to the heart induce an exudative pericarditis within 70–100 days. This is followed by focal myocytolysis, with a variable degree of fibrosis, and intimal thickening and perivascular fibrosis in arteries adjacent to myocardial degeneration (*15, 16*). Heart failure occurs as the result of increasing myocardial degeneration, with or without fibrosis. In rats, congestive heart failure has also been seen after recovery from pericarditis (*17*).

Myocardial degeneration, seen from about 10 weeks after irradiation, coincides with the first signs of decreased cardiac function in rats. However, further decreases in function do not occur until shortly before the onset of fatal congestive heart failure, despite progressive degeneration of myocardial mass (*18*). By contrast, both stroke volume and myocardial contractility measured in the denervated heart *ex vivo* showed much earlier deterioration (*19*). This is probably explained by compensatory mechanisms operating *in vivo* to maintain cardiac output.

Distribution of atrial naturetic protein (ANP) is dependent on the severity of the pathological changes after local irradiation of the heart. Immunohistochemical studies demonstrated that whole heart irradiation caused a reduction of atrial ANP due to a substantial loss of ANP producing atrial myocytes and accumulation of collagen (*20*). In the left ventricle, by contrast, a dose-dependent and progressive elevation in ANP expression was observed. This increased ventricular expression is very likely involved in the observed chronic elevation of circulating ANP in the plasma, which accompanies cardiac damage (*21*). Levels of circulating ANP or BNP (brain naturetic protein) in the plasma are useful surrogate markers of cardiac damage, including after irradiation (*22, 23*).

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## RADIATION STUDIES IN RUSSIA AND THE FORMER SOVIET UNION

Ethel Gilbert and Geoffrey Howe, Organizers

Ethel Gilbert, Chair

Dale Preston, Discussant

### Mayak Worker Mortality: Overview and Site-Specific Risks

Natalia Shilnikova,[a] Dale Preston,[b] Pavel Okatenko,[a] Ethel Gilbert,[c] Elaine Ron,[c] Mikhail Sokolnikov,[c] Irina Kuznetsova,[a] Valentin Khokhryakov,[a] Eugeny Vassilenko[d] and Nina Koshurnikova[a]

*[a]Southern Ural Biophysics Institute, Ozyorsk, Russia; [b]Hirosoft International Corporation, Seattle, Washington; [c]National Cancer Institute, National Institute of Health, Rockville, Maryland; and [d]Mayak Production Association, Ozyorsk, Russia*

*Introduction*

Plutonium production in the Soviet Union began in 1948 at the Mayak Production Association. The production facilities included a reactor complex, a radiochemical plant, a plutonium production plant, and various auxiliary departments. During the early period of operation of the Mayak nuclear facility, which is located in the Chelyabinsk region of the Russian Federation, a large number of male and female workers were chronically exposed to external γ radiation and internal α-particle radiation from inhaled plutonium compounds at levels much higher than those considered permissible today. A systematic program of continuing mortality follow-up in a fixed cohort of Mayak works was begun about 20 years ago. Film-badge measurements of external doses are available for most workers with the potential for external radiation exposure. Body burden measurements are available for many of the workers with a potential for plutonium exposure. Thus the cohort of Mayak workers offers a unique opportunity to study health effects of protracted exposure to external γ radiation and internal α-particle radiation. Analyses conducted in various sub-cohorts of these workers demonstrated that plutonium exposure increases mortality risks of cancers in organs of primary plutonium deposition (lung, liver and bone) (*1–4*), while external exposure has been linked to elevated risks of all solid cancers combined and leukemia (*5*). Analyses described here are focused on site-specific cancer risks in the Mayak Worker Cohort.

*Materials and Methods*

The Mayak Worker Cohort currently includes almost 26,000 people hired at the main plants (nuclear reactors, radiochemical plant and plutonium production plant) and selected auxiliary plants (mechanical-repair and water processing plant) between 1948 and 1982. Female workers constitute about 25% of the cohort. Most main plant workers had the potential for external radiation exposure, primarily to γ radiation. Workers at the radiochemical and plutonium production plant could also be exposed to internal α-particle radiation from plutonium (primarily $^{239}$Pu). Workers at the auxiliary plants generally had little or no potential for radiation exposure.

About 80% of the cohort members were monitored for external radiation with film dosimeters and since 1992 with thermoluminescence dosimeters. Average dose of external γ radiation for monitored cohort members is about 0.7 Gy.

Only about 40% of workers who had the potential for internal exposure were monitored for plutonium by determination of its levels in urine. On the basis of these measurements plutonium body burden and doses of internal α-particle radiation to several organs were computed. The average plutonium body burden for the monitored cohort members is 1.63 kBq. Selection for plutonium monitoring was not random: Workers thought to have large exposures were more likely to be selected. Thus the mean plutonium body burden for monitored workers is not a representative value for all workers.

To allow for possible effects of internal exposure when we consider the risk of external exposure, and to make use of follow-up data for workers without plutonium measurements, we have developed a categorical surrogate index, which is defined in terms of basic occupational history data, including work locations, starting dates, the distribution of measured body burden values, and expert knowledge of working conditions at various times in the different facilities (5). Statistical methods used for these analyses are described in detail in refs. (*2*) and (*5*).

*Results*

During the period covered by these analyses (1948–2000) there were about 865,000 person years of follow-up and 2086 solid cancer deaths among cohort members (1656 in men and 430 in women). We analyzed the risks of cancers for which there were more than 20 deaths. These include cancers of the oral cavity (52), esophagus (47), stomach (360), colon (97), rectum (101), liver (74), gallbladder (31), pancreas (97), larynx (49), lung (660), skeleton (36), melanoma (27), breast (72), ovary (27), prostate (57), bladder (45), kidney (63) and brain (54). Cancers at the remaining sites (137) were analyzed as a single group.

As in the previous analyses, the current analyses provide strong evidence that the occupational γ-ray and α-particle exposures increase the risks of all solid cancers, cancers in the organs of primary plutonium deposition, and other solid cancers as a group (*5*). There is a positive association between mortality and dose of external γ radiation for cancers in the oral cavity, esophagus, colon, rectum, gallbladder, pancreas, lung,

melanoma, breast, ovary, bladder, kidney and the remainder group. However, the effect of external radiation dose is statistically significant only for lung cancer ($P < 0.001$) and for the remainder group ($P = 0.04$).

The analyses indicate that internal exposures to α-particle radiation are related to increased risks of lung, liver and skeletal cancers. We also find some evidence for associations between plutonium body burdens or surrogate indices for potential plutonium exposure for a number of sites for which there is little or no direct exposure to plutonium. These sites include stomach cancer, for which mortality in monitored workers is significantly associated with plutonium body burden ($P = 0.025$), and also breast and ovarian cancers, for which the relative risks for unmonitored workers in the highest surrogate index categories are high (though insignificant), and the numbers of estimated excess cases associated with internal exposure are considerably larger than the numbers of excess cases associated with external exposure. Small numbers of cases in this study limit the precision of site-specific cancer risk estimates. These results should be regarded as preliminary, because the estimates of external γ-ray dose and plutonium body burden used in these analyses have a number of limitations. The work on development of improved external and internal dose estimates, including organ-specific doses, has recently been completed, and these estimates will be used in our future analyses.

*Acknowledgments*

Support for this study has been provided by the U.S. National Cancer Institute through NIH Contract Number HHSN261200455004C, ''Epidemiologic Studies of Mayak Cohorts.''

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## Mayak Worker Study: Risks of Lung, Liver and Bone Cancer from Plutonium

E. S. Gilbert,[a] M. E. Sokolnikov,[b] N. S. Shilnikova,[b] D. L. Preston,[c] E. Ron,[a] P. V. Okatenko,[b] V. F. Khokhryakov,[b] E. K. Vassilenko[d] and N. A. Koshurnikova[b]

*[a]National Cancer Institute, National Institute of Health, Rockville, Maryland; [b]Southern Ural Biophysics Institute, Ozyorsk, Russia; [c]Hirosoft International Corporation, Seattle, Washington; and [d]Mayak Production Association, Ozyorsk, Russia*

An epidemiological study of workers at the Mayak nuclear facility in the Chelyabinsk region of the Russian Federation provides a unique opportunity to evaluate the human health effects of plutonium exposure. Although studies of workers exposed in facilities in the United States and United Kingdom show little evidence of increased risks from plutonium exposure, this is likely due to the small number of workers and the low levels of exposure, which greatly limit the potential for detecting and quantifying risks. During the early period of operation (1948–1958), many workers at the Mayak facility were exposed to inhaled plutonium at levels much higher than those experienced by workers in other countries. Over a thousand Mayak workers had estimated body burdens that exceeded 1.5 kBq (40 nCi), a level that served as a guideline for the maximum permissible burden in many countries.

Shilnikova *et al.* (*1*) described the characteristics of the Mayak worker cohort and presented preliminary results of dose–response analyses for several site-specific cancers. The present paper focuses on quantifying the risk of cancers of the lung, liver and bone as a function of internal dose from plutonium to these organs. From both human and experimental animal data, it is known that the lung, bone and liver receive the largest doses from inhaled plutonium.

As a result of an extensive collaborative Russian and U.S. dosimetry program (supported by the U.S. DOE), improved individual Mayak worker external and internal dose estimates have very recently become available. Work related to internal dose from plutonium has been described by Eckerman (personal communication). Dose estimates from plutonium (lung, liver and bone) used in analyses described in this abstract reflect some but not all improvements. For example, the most recent dose estimates take account of smoking histories, whereas those used in this abstract do not.

Lung cancer mortality risks were evaluated by Gilbert *et al.* (*2*). Both the excess relative risk (ERR) and excess absolute risk (EAR) were modeled as a function of external dose and internal dose to the lung from plutonium with detailed attention to the modifying effects of sex, attained age and age at hire. Significant dose–response relationships were demonstrated for both external and internal dose, and risks were adequately described by linear functions. For internal dose, the ERR per gray for females was about four times higher that for males, whereas the EAR for females was less than half that for males. The ERR showed a strong decline with attained age, whereas the EAR increased with attained age until about age 65 and then decreased. For external dose, there was no evidence of modification by sex or attained age, although results were consistent with patterns exhibited for internal dose.

Parallel analyses of lung cancer mortality risks from internal dose in Mayak workers and from external dose in Japanese atomic bomb survivors were also conducted (*2*). These comparisons expressed dose for Mayak workers in sieverts using a quality factor of 20 as recommended by the ICRP for α-particle exposure. Estimates of the ERR per sievert were higher in atomic bomb survivors, but differences might be explained by chance. EAR models indicated different patterns with attained age in the two cohorts, suggesting that until about 65 years of age absolute excess risks were higher in Mayak workers than in atomic bomb survivors but were more comparable at older ages. These results need to be re-evaluated based on the improved dose estimates that have just become available.

Koshurnikova *et al.* (*3*) and Gilbert *et al.* (*4*) established that liver and bone cancer risks were related to plutonium exposure but did not evaluate the dose response due to limitations in dose estimates available at the time. Since then, preliminary dose–response analyses of liver and bone cancer mortality risks indicate that the dose response is non-linear with risks reasonably well described by pure quadratic functions of internal dose. Analyses that evaluate the modifying effects of sex, attained age, time since exposure, and age at hire on ERR and EAR are under way.

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## Radiation Dose and Thyroid Disease Prevalence near the Semipalatinsk Nuclear Test Site in Kazakhstan

C. E. Land,[a] Z. Zhumadilov,[b] B. I. Gusev,[c] M. H. Hartshorne,[d] P. W. Wiest,[e] P. W. Woodward,[f] L. A. Crooks,[d] N. K. Luckyanov,[a] C. M. Fillmore,[a,g] Z. Carr,[a,h] G. Abisheva,[b] A. S. Sigurdson,[b] H. L. Beck,[i] A. Bouville,[a] J. Langer,[j] R. Weinstock,[i] K. I. Gordeev[k] and S. L. Simon[a]

*[a]National Cancer Institute, Division of Cancer Epidemiology and Genetics; [b]Semipalatinsk State Medical Academy; [c]Khazakh Research Institute for Radiation Medicine and Ecology; [d]VA Medical Center, Albuquerque; [e]University of New Mexico Medical School, Department of Radiology; [f]Armed Forces Institute of Pathology; [g]University of Wisconsin Medical School; [h]World Health Organization; [i]Contractor; [j]Research Triangle Institute; and [k]Institute of Biophysics, Moscow*

### *Introduction*

Radioactive fallout from nuclear test explosions in the U.S., the Marshall Islands, the former Soviet Union, Australia, China and elsewhere has affected populations throughout the world to varying extents. Significant fallout is associated with any explosion involving nuclear fission in which the fireball touches the ground. Soil and other particles are drawn up into the fireball, contaminated with radioactive fission products including isotopes of iodine, cesium, strontium and other elements, carried downwind from the explosion site, and deposited on the ground and other surfaces. Radiation exposure is dominated by penetrating γ rays from radionuclides on the ground and less-penetrating β particles from radioiodines ingested through the fallout–pasture–dairy animal–milk pathway. Radioiodines ($^{131}$I and $^{133}$I) are taken up by the thyroid gland and are particularly hazardous to children, who have small, active glands.

Between 1949 and 1962 over 100 nuclear tests were conducted above ground at the Semipalatinsk Nuclear Test Site (SNTS) in northeastern Kazakhstan. Significant fallout deposition occurred at a number of settlements within a few hundred kilometers of the site, most of it from a relatively few tests. In August 1998, in collaboration with the Kazakh Research Institute for Radiation Medicine and Ecology (IRME) and the Semipalatinsk State Medical Academy (SSMA), we carried out a thyroid screening study of some 2000 members of a long-term study cohort of the IRME, formed in the early 1960s and monitored since 1964 (*1, 2*). Our subjects were born between 1930 and 1957 and therefore had been juveniles at some time during the period of testing at the SNTS. Screening was by ultrasound and fine-needle biopsy for prevalent benign and malignant thyroid nodules, finger-stick phlebotomy for thyroid function tests, and questionnaire-guided interviews for residential history, medical history, and recalled childhood consumption of milk and milk products. Our study was designed to use radiation dose response for thyroid nodule prevalence as an indicator of the likely radiation effectiveness of internal radiation dose from $^{131}$I and $^{133}$I compared to external, mainly γ, radiation as risk factors for thyroid cancer. The study depended heavily on reconstructed individual dose estimates based on a joint U.S.-Russian fallout dose reconstruction method (*3, 4*).

### *Results*

Analyses of ultrasound and biopsy data sheets found one or more thyroid nodules in 700 of 1989 screened subjects (35%); by sex, prevalence was 21% among males and 44% among females. Biopsies were performed on 366 subjects who had at least one nodule ≥1 cm in diameter and who consented to the procedure (there were two refusals). Nineteen papillary carcinoma cases were diagnosed in as many subjects (1.0%), including two men (0.25%) and 25 women (1.4%). Nodule prevalence was significantly higher among females compared to males (39% and 18%, respectively) and increased by 3.5% per year of age at screening (range 40–70), but it did not differ between ethnic Europeans and Kazakhs.

A series of workshops was organized with Russian and U.S. experts having intimate knowledge of dose-reconstruction methodology for areas downwind from the SNTS, the Nevada Test Site, and Pacific test sites. These workshops resulted in a joint report by Russian and U.S. investigators providing coefficients, based on Russian measurements and clearly identified assumptions, for estimation of radiation dose to the thyroid from external γ-ray sources and internally deposited $^{131}$I, for each fallout event affecting our screening villages and several other villages where members of our study population reported having been exposed (*3*).

Reconstructed external thyroid doses for individuals were based on residential histories obtained from questionnaire responses and verified using IRME records and were adjusted for estimated time spent outdoors and for shielding by wooden or adobe buildings characteristic of ethnically European and Kazakh villages, respectively (*3, 4*). Thyroid dose from internal irradiation was assumed to be due entirely to the consumption of milk and milk products contaminated with $^{131}$I and $^{133}$I in the time-averaged ratio of 10 to 1. Individual consumption of milk and milk products, by source, were estimated as weighted averages of age-specific general information provided by the IRME for ethnic Kazakh and European villagers during the 1950s, and individually recalled childhood diets from our questionnaire-guided interviews. Iodine uptake and transfer to milk for different dairy animals (cows, goats, horses and sheep) were based on published and experimentally derived information, and human iodine uptake as a function of age was obtained from published sources.

Estimated external dose ranged from 0 to 0.65 Gy with mean 0.05 Gy, and internal dose ranged between 0 and 9 Gy with mean 0.35 Gy. External dose increased with increasing age (more time spent outdoors at older ages), whereas estimated internal dose decreased with age (larger, less active thyroids received smaller doses). The correlation between external and internal dose was 0.46, reflecting the fact that both doses depend on fallout deposition but the internal dose depends additionally on amount and source of milk consumed and on thyroid size, a function of age.

Thyroid nodules were found in 700 screened subjects (35%). Parallel analyses were conducted and internal and external radiation doses were summed (total dose) or given separately, using the model

$$\text{Odds} = \text{Baseline} \times (1 + \text{dose response} + \text{dose-response modifiers});$$

i.e.,

$$\text{Odds}[(X_i), (Y_j), (Z_k)] = \exp\Big(\sum \alpha_{ii} X_i\Big) \times \Big[1 + \sum \beta Y_j \times \exp\Big(\sum \gamma_k Z_k\Big)\Big].$$

Here $\alpha_i$, $\beta_j$ and $\gamma_k$ are unknown parameters corresponding to variables $X_i$, $Y_j$ and $Z_k$, respectively, where $X_1$ and $X_2$ are index variables for male and female sex, respectively, and $X_3$ is the logarithm of age at screening; $Y_1$ and $Y_2$ represent total thyroid dose evaluated separately for males and females, respectively, or, alternatively, $Y_3$ and $Y_4$ represent external and internal dose where sex is treated as a dose–response modifier, $Z_1$; and the dose–response modifier variables $Z_2$, $Z_3$ and $Z_4$ represent age at screening, its square, and total dose (related to radiation-related cell killing at high doses), respectively.

According to both analyses, baseline nodule prevalence was significantly associated with sex and age at screening, with a fivefold greater prevalence odds among women. Nodule prevalence was significantly associated with total radiation dose (2-tailed $P$ value $< 0.001$ for males and $< 0.07$ for females) and significantly and independently associated with external ($P = 0.04$) and internal ($P < 0.001$) dose as separate var-

iables. However, the model fit did not improve significantly ($P = 0.36$) when external and internal doses were given separately, and the estimated REF (i.e., $\beta_4/\beta_4$) for internal compared to external dose (i.e., $\beta_4/\beta_4$) was 0.46 with 95% confidence limits 0.13–6.1; thus the results are consistent both with approximate equivalence in effectiveness and with substantial differences. There was a 12-fold greater linear-model EOR/Gy for men compared to women ($P < 0.001$); given the results for baseline prevalence, this is consistent with an additive interaction between sex and radiation dose as factors influencing nodule prevalence.

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## CURRENT ISSUES IN DOSIMETRY

Irene Jones and Daniel Stram, Organizers

Irene Jones, Chair

Duncan Thomas, Discussant

### Complex Chromosome Aberrations as Biomarkers of Exposure to High-LET Radiation: Plutonium-Worker Pilot Study


Rhona M. Anderson[a], Aleksander V. Sevan'kaev[b] and Dudley T. Goodhead[a]

*[a]MRC Radiation and Genome Stability Unit, Harwell, Didcot, 0X11 ORD, United Kingdom; and [b]MRCC, Obninsk, Kaluga Region, 249020, Russia*


A possible association between exposure to naturally occurring radon gas and its daughters and the development of myeloid leukemias and other cancers has been proposed (*1*). However, epidemiological studies do not support or directly contradict this (*2*), primarily because of the inherent difficulties in the interpretation of such studies due to various confounding factors. With the identification of a specific biomarker of exposure in accessible cells such as peripheral blood lymphocytes (PBL), a more targeted approach in the identification of exposed individuals could enable a causal link between exposure and specific health effects, if present, to be established. Recent cytogenetic studies with this aim have focused on the detection of insertions (transmissible complex aberrations) and subtle intrachromosomal events (*3–6*) principally because these aberrations are mechanistically characteristic of high-LET α-particle exposure, are stable through mitosis and as a consequence, are expected to be capable of long-term persistence. The application of mFISH for the analysis of α-particle-induced chromosome aberrations *in vitro*, however, has changed this view. It is now established that the dominant chromosome exchange types induced in PBL by high-LET α particles are unstable, non-transmissible, complex types (*3*). Importantly, a significant proportion of apparently stable aberrations, detected using a variety of different chromosome painting techniques, can be visualized by mFISH as components of larger unstable complex rearrangements. From a mechanistic perspective, the size of each α-particle-induced complex (based on the number of chromosomes involved) correlates with the number of different chromosome territories in the PBL nucleus predicted to have been intersected by each single α-particle track (*7*). Thus, at low to moderate doses, where each PBL is expected to be traversed by a single particle, the spectrum of cellular damage observed by mFISH and the size range of each complex aberration induced appears specific to the structure of the α-particle track and the geometry of the cell traversed. Accordingly, the complexity of each α-particle-induced complex in each damaged PBL is the principal cytogenetic feature of exposure and could be a useful indicator of high-LET α-particle exposure irrespective of the long-term persistence capability of the cell (*3*). Littlefield *et al.* also proposed that highly complex aberrations could be indicative of exposure to densely ionizing radiation (*8*).

The objective of this study was to assess whether chromosome aberrations of a similar range in complexity and (non-)transmissibility are also induced in PBL of individuals chronically exposed *in vivo* to α-particle radiation. To do this we carried out a limited pilot study of Russian nuclear workers who are known to have large internal deposits of the α-particle emitter plutonium. The estimated doses of the subjects studied were clearly in excess of that which would be received from environmental exposure to radon. The principal aim, therefore, was solely to determine the usefulness of detecting complex chromosome aberrations by mFISH after *in vivo* exposure.

We found a high frequency of complex chromosome aberrations in all of the plutonium-exposed subjects analyzed (2.3–8.5% total complex frequency). Qualitatively, the size range and complexity of aberrations detected were consistent with those previously reported in PBL and that were known to be in their first cell division after *in vitro* exposure to high-LET α particles (*3, 7*). Specifically, unstable, non-transmissible complexes were more common than transmissible types, suggesting that the plutonium-exposed PBL had not progressed through cell division. We surmise that the detection of these complex aberrations in the peripheral blood of these Russian workers chronically exposed to internalized plutonium over a period in excess of 30 years is indicative of either recent/ongoing exposure of PBL to α-particle-emitting plutonium and/or the long-term quiescence of exposed PBL.

In conclusion, complexity and non-transmissibility appear to be the major cytogenetic features of high-LET α-particle-induced chromosomal aberrations in human PBL. As demonstrated in this study (*9*) and elsewhere (*10*), heavily damaged cells appear to be quite long-lived *in vivo* such that significant proportions can be detected after decades of chronic exposure. Recent work assessing the effect of particles with varying incident LET in human hematopoietic stem cells suggests that complex aberrations are a constant feature of α-particle irradiation in spherical cells, albeit in varying proportions. Thus complex chromosome aberrations detected by mFISH (both stable transmissible and unstable non-transmissible types) could be useful qualitative biomarkers of high-LET radiation exposure.

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## Biodosimetry and Dose Uncertainty in the RERF Cohorts: Overview and Innovative Approaches to Analysis

Harry M. Cullings,[a] John B. Cologne[a] and Nori Nakamura[b]

*Departments of [a]Statistics and [b]Genetics, Radiation Effects Research Foundation, Hiroshima, Japan*

Biodosimetry is an important adjunct to dose estimates obtained for atomic bomb survivors by the use of dosimetry systems (T65D, DS86, DS02). Biodosimetric measurements contain individual dose information that does not depend on specification of distance from the bomb or shielding conditions. Although they are available only for limited subsets of survivors, they are of great interest to the evaluation of dose uncertainty, and the evaluation of biodosimetric dose response within these subsets is interesting in its own right. This abstract summarizes existing biodosimetric results on survivors in RERF cohorts, including chromosomal aberrations, electron paramagnetic resonance on teeth, and recorded clinical signs of acute radiation effects, as well as existing information on dose uncertainty from other sources. This will be followed by a discussion of analytical approaches that have been used in the past. These have included evaluations of dose response and dispersion in biodosimetric results based on dosimetry system estimates for individuals, using this to make inference about the uncertainty in the latter, and studies of correlation between different types of biodosimetric results. They have also included innovations such as a combined approach to incorporating data on acute signs of radiation injury and chromosomal aberrations in making inference about uncertainty in dose estimates, and a posterior-means approach to the calibration problem of using the fitted dose response of a biodosimeter to make inference about doses in another population bearing similar data. The abstract concludes with a discussion of current and planned work on biodosimetry and dose estimation, as well as some thoughts on analytical approaches.

RERF data on stable chromosomal aberrations using Giemsa staining were analyzed in 1993 by Stram *et al.* (*1*), $n = 1{,}703$, who found period, city and age-at-exposure effects along with an indication of systematic error in the DS86 dosimetry, and in 2001 by Kodama *et al.* (*2*), $n = 3{,}042$, who found a "highly significant and non-linear dose response" and a city difference as well as a difference among shielding categories, most notably indicating that doses of Nagasaki factory workers might be substantially overestimated by DS86. FISH assays have replaced the previous Giemsa method and have been performed routinely since 1984 in Hiroshima and 1997 in Nagasaki, with totals to date of 1,020 and 555 survivors having been examined in the two cities, respectively. FISH has been compared to solid Giemsa staining on the same individuals, $n = 230$, showing that the latter method detected about 73% of the chromosomal aberrations detected by FISH, as has been suggested from previous studies that compared a Giemsa staining method based on banding (equivalent to FISH in detection efficiency of stable-type chromosomal aberrations) and the solid Giemsa staining data (*3*). A study based on G-banding showed a surprising lack of dose response in survivors exposed *in utero*, $n = 331$, except for a small increase $<1\%$ at doses $<0.1$ Gy (*4*). More than 100 donated teeth were measured using EPR, a physical measurement of $\gamma$-ray dose, and the chromosomal aberrations rate using conventional Giemsa staining was found to be comparable to 70% of the rate determined separately by using FISH with $\gamma$-irradiated cells *in vitro* ($n = 40$ donors) (*5*).

Various other methods have been developed, including assays of mutation such as the erythrocyte glycophorin assay of somatic cell mutation (*6*) and assays related to radiosensitivity such as the micronucleus assay (*7*). In addition, RERF data include information on clinical signs of acute radiation injury such as epilation, oropharyngeal lesions, bleeding and flash burns, for which Gilbert and Ohara (*8*) analyzed the dose response extensively in 1983 using T65DR doses with reduced $\gamma$-ray transmission factors for houses suggested by Marcum[1] and found that there were major differences between cities and shielding categories, suggesting possible differences in either random or systematic errors, which they could not distinguish. Stram and Mizuno (*9*) contrasted the dose response of epilation using dose estimates from DS86 to that using dose estimates from T65D. They found pronounced nonlinearity at low and high doses, a 165% different slope estimate and a different apparent neutron RBE for DS86 compared to T65D, and differences across both city and shielding category.

In the 1960s, Jablon (*10*) examined the available information on dose uncertainty due to uncertainty in survivor location and shielding as inputs to the dosimetry systems, in addition to factors intrinsic to the systems, and concluded that identifiable sources of uncertainty suggested a coefficient of variation of about 30% in random errors of dose estimates. Jablon's estimates of uncertainty in survivor location have continued to be used to the present day, even in the uncertainty analysis of the DS02 report (*11*). In 1989, Fujita and Watanabe[2] reconstructed five neighborhoods in Hiroshima and three in Nagasaki using information from war-era aerial photographs and suggested that survivor location information could be substantially improved by such a method.

As one example of statistical work on the relationship of biodosimetry and dose error, in 1990 Sposto, Stram and Awa (*12*) devised an analysis in which they used two methods to estimate the uncertainty of survivor dose estimates. One method was a regression analysis of the dose response of chromosome aberrations, under a model for epilation dose response and a class of error models, that determined the error consistent with the difference in fitted dose response between survivors with and without epilation, if all of the difference were due to random errors in dose estimates. The other approach was an overdispersion analysis using

[1] J. Marcum, House attenuation factors for radiation at Hiroshima and Nagasaki. Preliminary report prepared for Defense Nuclear Agency. 15 May 1981. Available from R & D Associates, P.O. Box 9695, Marina del Ray, CA, 90291. The recommended factors were 0.55 in Hiroshima and 0.50 in Nagasaki, vs. 0.90 and 0.81, respectively, for T65DR.

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an empirical variance function of dose. Their results generally supported a coefficient of variation of about 50% in dose error. In their 1993 paper Stram *et al.* (*1*) developed a model for the overdispersion of the chromosomal aberration data in addition to evaluating the dose response. In another example, Cologne, Pawel and Preston (*13*) discussed the issues in using a fitted dose response of a biodosimeter to estimate doses in other samples based on the same type of biodosimetric data, particularly the issue of regression bias, and demonstrated the method and advantages of calculation of a posterior mean of true dose conditional on observed biodosimetry, given an assumed prior distribution.

RERF researchers continue to perform assays of chromosomal aberrations and EPR and to research biodosimetry. The use of geographical information systems with maps, aerial photographs and survivor location data is being developed to improve estimates of survivor distance (*14*) and may also contribute to better estimates of prior distributions such as population density. We have formed an intramural working group on dosimetry errors in individual survivors at RERF and plan to continue research in statistical methods such as those described above, along with other approaches such as latent factor analysis. More generally, we plan to investigate new approaches to risk estimation that use individual rather than grouped data, use full-likelihood type (e.g. Bayesian) models to incorporate partial data on related variables including biodosimetric ones among many others, and to pursue a variety of approaches to improving both the quality and uncertainty evaluation of input data and the accuracy, versatility and extensibility to other populations of RERF risk estimates.

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## Molecular Biomarkers (RNA and Proteins) for Early Radiation Dosimetry


William F. Blakely, Marcy B. Grace, Alexandra C. Miller, Natalia I. Ossetrova and Glen L. Manglapus

*Armed Forces Radiobiology Research Institute, Uniformed Services University of the Health Sciences, Bethesda, Maryland*


Identification and validation of early-phase radiation biomarkers are needed to provide enhancement in biological dosimetry capability to assess individuals suspected of exposure to ionizing radiation (*1*). This need is of great importance to provide quantitative indications for early initiation (20 h after radiation exposure) of cytokine therapy in individuals exposed to life-threatening radiation doses as well as to provide effective triage tools for first responders in mass-casualty radiological incidents. Monitoring of radiation exposure by biological dosimetry systems is complementary to physical dosimetry, since they can weigh radiation quality and dose rate according to biological efficacy. Molecular biomarkers are used as diagnostic end points in environmental health and cancer. Hofmann and colleagues (*2*) reported radiation-induced increases of serum amylase in 41 patients after either whole-body irradiation or irradiation of the head and neck region. Our working hypothesis is that gene expression and encoded protein biomarkers detected in biological samples (peripheral blood) can (a) distinguish the concerned public from individuals exposed to radiation and (b) triage exposed individuals by assessing radiation dose and injury. Our research strategy involves use of both *ex vivo* (human) and *in vivo* (mice, non-human primates, radiation therapy patients) radiation model systems. We have employed quantitative methodology to measure multiple gene expression and encoded-protein targets as well as blood serum enzyme activities. Gene expression targets [RAS p21, GADD45A, DDB2, BAX, BCL2, CDKN1A (p21/Waf1/Cip1)] are quantified by real-time reverse-transcriptase polymerase-chain-reaction (RT-PCR) bioassay. Encoded proteins (RAS p21, RAF, GADD45A, BAX, BCL2, p21/Waf1/Cip1, IL6) were detected by the enzyme-linked immunosorbent and microsphere (Luminex™)-based assays (*4, 5*). Blood serum levels of amylase activity were measured using conventional commercial reagents used in blood chemistry analyzers. Results demonstrating radiation-induced up-regulation of gene expression and protein targets derived from varied pathways (proto-oncogenes, DNA damage and repair, cell cycle checkpoints, apoptosis/anti-apoptosis, cytokine, etc.) and radiosensitive tissues (salivary gland) from three radiation model systems have been obtained. These results support the proof-of-concept that use of multiple early-response molecular biomarkers can provide useful diagnostic indices for medical management of radiation casualties.

*Acknowledgments*


AFRRI, the National Institute of Allergy and Infectious Diseases, the National Institutes of Health (Bethesda, MD), supported this research under work units BD-07, and BD-10, research agreement Y1-AI-3823-01, respectively.

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## Analysis of Radiation Dose Response with Tumor Location and Location-Specific Dose in the WECARE Study of Second Breast Cancer

Bryan Langholz,[a] Duncan C. Thomas,[a] Marilyn Stovall,[b] Susan Smith,[b] John D. Boice Jr.,[c] Roy E. Shore,[d] The WECARE Study Group[e] and Jonine L. Bernstein[e]

*[a]Department of Preventive Medicine, University of Southern California, Los Angeles, California; [b]Department of Radiation Physics, The University of Texas, M. D. Anderson Cancer Center, Houston, Texas; [c]International Epidemiology Institute, Rockville, Maryland, and Department of Medicine, Vanderbilt University Medical Center, Nashville, Tennessee; [d]Nelson Institute of Environmental Medicine, New York University Medical Center, New York, New York; and [e]Department of Epidemiology & Biostatistics, Memorial Sloan-Kettering Cancer Center, New York, New York*

*Introduction*

The WECARE (Women's Environment Cancer And Radiation Exposure) Study is a population-based, 1:2 individually (counter-) matched case-control study designed to examine the joint roles of *ATM* gene mutations, radiation exposure and breast cancer. The study design is a nested case-control study from a cohort of unilateral breast cancer patients ascertained by a consortium of five cancer registries, followed for occurrence of second primary breast cancer in the contralateral breast (the cases). For each case, two controls were sampled from the time-since-first-breast-cancer risk set, matched on age at diagnosis of the first cancer, race, and registry, and counter-matched on registry-reported radiotherapy treatment (*1*).

For 609 out of the 708 cases, the *location* in which the second cancer originated was ascertained, as defined by nine regions on the breast. For the remaining cases, the location of the second primary breast cancer could not be assigned. Further, for cases and controls who received radiotherapy for the first breast cancer, radiation dose to the nine locations on the contralateral breast was estimated based on information given in radiation treatment records and reconstruction techniques. Other breast cancer risk factor information, including reproductive history, family cancer history, and treatment of the first breast cancer, was ascertained by interview and from medical records. Thus, for 609 case-control sets, we have location-specific radiation dose for each subject, the location of the case's second primary breast cancer, and subject-level covariate risk factor information. The question we address in this presentation is how to use the location and subject level data in the analysis of radiation dose effects.

*Statistical Methods*

We consider likelihoods based on three dose comparisons. The *case-control-matched-location* approach has the structure of 1:2 case-control data; the dose at the case's second primary breast cancer location is compared to the control doses at the same location. The *case-only* approach has the structure of 1:8 case-control data; the comparison is between the dose at the case's second primary breast cancer location to the doses at the case's healthy locations. The controls are not used in this comparison. Finally, the *case-control-all-location* approach has the structure of a 1:26 case-control data; the comparison is between the dose at the case's second primary breast cancer location to doses at each of the case's healthy locations and all the control locations. Given the structure of these data, it is natural to use conditional logistic regression likelihoods for individually matched case-control data, with each method's comparison units, to estimate radiation (and other factor) relative risks. The likelihoods can be derived formally by characterization of the data as risk set sampling from censored event time data (*2, 3*), in particular, with dose $Z_{i,l}$, the dose for subject $i$ at location $l$ and second primary breast cancer outcome as multivariate counting processes $N_{i,l,\mathbf{r}}(t)$ the (multivariate) counting process that indicates second primary breast cancer occurrence for subject $i$, location $l$ and counter-matched set $r$ up to time $t$. The likelihoods are based on the probability that $dN_{i,l,\mathbf{r}}(t) = 1$, conditional on particular ''summary events.'' The formal analysis validates the use of conditional logistic regression based on the heuristic 1:2, 1:8 and 1:26 case-control data structures for each of the three approaches. Depending on the approach, the information for estimation of the radiation effect parameters is related to the variability of radiation dose over location and/or subjects (within the case-control set). Further, for a given model for radiation effects, all of the approaches are estimating the same radiation rate ratios, but under different modeling assumptions about location effects and subject-level covariates. We summarize the characteristics of the three approaches with respect to model (and design) assumptions as well as sources of statistical information:

*Case-Control-Matched-Location*

Statistical efficiency of this likelihood depends solely on between-subject variability and does not benefit from across-location variability in dose. Because the likelihood supports a model with non-parametric location effects, analysis of radiation effects will be robust to confounding due to (or misspecification of) location effects. On the other hand, modeling of subject-level covariates is required so that the approach is susceptible to subject-level covariate confounding. Finally, since comparisons are case-control, the approach is susceptible to design flaw biases.

*Case-Only*

Efficiency of the case-only approach depends solely on between-locations variability and does not benefit from across-subject variability in dose. Since comparisons are within-subject, this method is not susceptible to subject-level covariate confounding. However, location effects must be modeled, so that case-only analyses are susceptible to residual location confounding due to variation in location-specific second primary breast cancer rates over matching factors such as age and registry or over modeled covariates. As would be expected from a case-series analysis, the case-only likelihood does not depend on the controls at all, so it is not susceptible to design flaw biases.

*Case-Control-All-Location*

Comparisons are both across-subjects and location, so that the likelihood uses ''all the data.'' Thus, in terms of efficiency, the approach benefits from a combination of between-subject and between-location vari-

ability in dose. On the other hand, the method is susceptible to both location and subject-level confounding, as well as design flaw biases.

*Implications for the Analysis of the WECARE Study Data*

To assess the relative efficiency of the three approaches, we empirically computed the statistical information, the appropriate measure of efficiency, under the null of no trend effect as 0.31, 0.06 and 0.34 for case-control-matched-location, case-only, and case-control-all-locations, respectively. Both case-control methods have over six times the information of the case-only method, both because between-subject variability is greater than within-subject variability and because of the counter-matched design. There is little evidence of correlation between radiotherapy treatment and any subject-level factors, but there is a strong gradient in dose with distance from the treated breast. Thus careful modeling of location is needed to ensure that dose effects are not subject to residual location confounding. In conclusion, while in principle the case-control-all-location likelihood offers better efficiency, the case-control-matched-location approach is preferred for the WECARE Study because radiation effects are not susceptible to confounding by location.

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## Dosimetry Based on Radiotherapy Treatment Planning

C. W. Taylor,[a] A. Nisbet,[b] P. McGale[a] and S. C. Darby[a]

*[a]Clinical Trial Service Unit, Oxford University; and [b]Department of Medical Physics and Clinical Engineering, Churchill Hospital, Oxford, United Kingdom*

*Background*

Long-term follow-up of women irradiated for breast cancer has revealed that past breast radiotherapy regimens have increased the risk of death from cardiovascular disease, particularly in the period 10 years or more after irradiation. This risk appears to be dose-related but, at present, available dose–response curves for heart disease after radiotherapy involve few clinical data and are subject to some uncertainty. Further assessment of the relationship between heart dose and death from heart disease requires detailed dosimetry. These measures of radiation exposure of the heart could be combined with studies investigating subsequent heart disease and used to evaluate existing dose–response relationships and to predict the likely cardiac risk from current and future breast radiotherapy regimens.

Modern radiotherapy employs treatment planning systems that make use of data from a computerized tomography (CT) scan of the patient in question together with mathematical algorithms to model the distribution of radiation dose within a patient. The accuracy of such systems has been verified by phantom dosimetry and dose estimates are generally accurate to within $\pm 2\%$ for dose and $\pm 2$ mm for position. Such techniques are now routinely used in the clinic to plan radiotherapy for certain cancer sites. In this study, we have used these modern techniques to reconstruct past breast radiotherapy regimens and to calculate radiation dose received by the heart and the three main coronary arteries in these past regimens.

*Methods*

A large database of internationally used breast radiotherapy techniques from the 1950s onward has been compiled using information from both practicing and retired radiation oncologists from several different countries, from radiotherapy textbooks, and from protocols of randomized trials of radiotherapy for early breast cancer. For each technique, sufficient information was obtained to allow accurate and reproducible reconstruction.

Each breast radiotherapy regimen was reconstructed using ''virtual simulation'' which involved the construction of a virtual three-dimensional patient representation using CT scan data from a representative patient. Radiotherapy beams were designed and applied to this patient representation. After this, the radiation beam and patient information was analyzed by a CT planning system that is able to model the behavior of radiation beams within the patient. Correction was made for tissue inhomogeneities, such as the presence of lung and bone tissue. Using these techniques, around 60 radiotherapy fields of different sizes, orientations, qualities and beam energies were reconstructed. Estimation of dose to a number of different cardiac structures has been performed, including whole heart (including circulating blood volume), left anterior descending coronary artery, right coronary artery, and circumflex coronary artery. A few radiotherapy techniques involved the use of low-energy radiation beams. These are poorly modeled by CT planning, and therefore the distribution of dose within the heart was modeled and calculated using manual planning techniques.

For each technique, dose volume histograms were generated. These describe the graphic relationship between radiation dose and percentage volume of a structure that receives each dose level. They enable estimation of biologically effective dose, mean and maximum dose and percentage volume of each structure irradiated to a number of different dose levels.

Several sources of variability associated with these methods were assessed. The main source of variability in heart dose is likely to be variation in patient anatomy. This was modeled for two commonly used breast radiotherapy techniques: CT scans of five different-sized patients were employed to assess the variation in radiation dose to the heart and coronary arteries caused by anatomical differences.

*Results*

Radiation fields that were used to treat the internal mammary lymph nodes were found to deliver the highest mean heart doses, particularly for left-sided irradiation. Of the cardiac structures considered, the left anterior descending coronary artery generally received the highest mean and maximum doses for all regimens, due to its proximity to the breast.

Reconstruction of the commonly used techniques revealed that radiation dose to the heart has reduced considerably over the past 40 years. For left breast or chest wall radiotherapy, the typical mean heart dose was 13.3 Gy in the 1970s and 2.4 Gy in 2006.

Assessment of the effect of patient anatomy on heart dose showed that, although there was some interpatient variability in dose, there was greater dose variation with tumor laterality and with different regimens.

*Conclusions*

Virtual simulation and CT planning enable the measurement of detailed, accurate estimates of radiation dose to the heart and to structures within the heart, such as the coronary arteries. We plan to combine these dose estimates with information on the risk of heart disease to evaluate existing dose–response relationships and to predict the likely cardiac risk of current and future breast radiotherapy regimens.

## Accounting for Uncertainty in Dose in Dose–Response Analysis: The MCML Method

Terri Kang and Daniel O. Stram

*USC Division of Biostatistics—Research Section, University of Southern California, Los Angeles, California*

### *Introduction*

Most of work on radiation dose errors has centered on relatively simple relationships between true and measured exposures. For example, Pierce *et al.* (*1*) discussed the likely impact of independent lognormal errors in estimation of dose for the A-bomb study. One of the key problems in radiation dose–response analysis is to deal with more complicated error structures than usually considered. For example, Gilbert *et al.* (*2*) addressed the extent to which errors would be correlated from personal dosimeters for different radiation workers because of shared bias factors, related to dosimeter type, which are uncertain. In the U.S. uranium miners cohort, a more complex error structure has been described as being due to interpolation of measurements over time and geographical region (*3*). In perhaps the most elaborate analysis to date, uncertainty in dose was made an integral part of the $^{131}$I estimates for the Hanford Thyroid Disease Study. In that analysis, a Monte Carlo simulation-based dosimetry system provided a set of 100 dose estimates (random samples from the probability distribution of dose) to represent the uncertainty in thyroid dose for the entire cohort. Many uncertainties are ''shared'' in the sense that errors in them will affect many subjects' dose simultaneously. Stram and Kopecky (*4*) termed systems such as that used in the Hanford Thyroid Disease Study (providing multiple realizations of dose for each individual and incorporating both shared and independent error terms) as ''complex'' dosimetry systems and gave several suggestions for how to incorporate multiple realizations of dose explicitly into dose–response analyses.

A primary method advocated by Stram and Kopecky to analyze outcomes, **D** (e.g. ''disease'' for each member of the cohort), using a complex dosimetry system is Monte Carlo maximum likelihood (MCML). This begins with a model $f(\mathbf{D}|\mathbf{X}; \alpha\ \beta)$ for the distribution of disease given true dose **X** for each participant in the study. Here $\alpha$ is an intercept parameter (more generally $\alpha$ may be a set of nuisance parameters) and $\beta$ the dose–response parameter of primary interest. The MCML computes the likelihood of disease given the dosimetry system outputs ($\mathbf{Z}_i$, i = 1, r) as the average

$$f(\mathbf{D}|\ \text{all}\ \mathbf{Z}_i;\ \alpha\ \beta) = 1/r \sum_{i=1}^{n} f(\mathbf{D}|\mathbf{Z}_i\, \alpha,\ \beta) \qquad (1)$$

over all the realizations of dose $Z_i$.

This calculation (Eq. 1) is performed over a grid of values of $\alpha$ and $\beta$ and maximized to find the maximum likelihood estimates (MLE) $\ddot{\alpha}$ and $\ddot{\beta}$. Confidence intervals are estimated by the profile likelihood method in which the 95% confidence interval corresponds those values of $\beta$ for which

$$2 \times [\log f(\mathbf{D}|\alpha_\beta,\ \beta) - \log f(\mathbf{D}|\ddot{\alpha},\ \ddot{\beta})] \leq \chi^2_{1,0.95} = 3.84, \qquad (2)$$

where $\alpha_\beta$ is the value of $\alpha$ which maximizes $f(\mathbf{D}\,|\,\alpha,\ \beta)$ for a given value of $\beta$.

### *Methods*

In the following we evaluate the performance of confidence intervals based on Eq. (2) with the ''naïve'' expectation substitution method. Here the ''naïve'' method simply substitutes the average value, of $\mathbf{Z}_i$ for true **X** in the likelihood $f(\mathbf{D}\,|\,X;\ \alpha\ \beta)$ and computes the MLE, $\ddot{\beta}_{naive}$, $\ddot{\beta}_{naive}$ and confidence limits as usual (from the profile likelihood). Shared error is expected to inflate the variance to be larger than predicted by usual techniques.

Using a model similar to that used by Stayner *et al.* (manuscript submitted for publication), we first simulated disease in risk sets ($n = 223$) that had been computed from a subset of the International Nuclear Workers Study (the ORNL data extracted from the CEDR website). True doses were simulated by modifying each individual's dose assuming that in each 15-year period from 1943–1997 a different dosimeter type was used. For each dosimeter type it was assumed that the a shared bias factor was lognormal with (log scale) mean equal to zero and standard deviation equal to 0.50 (the values used here are chosen for illustrative purposes only).

### *Results*

As expected, the ''naïve'' confidence limits are wider than those from the naïve analysis. To compare the performance of confidence intervals we repeatedly sampled new disease outcomes from the same underlying model, and repeated the MCML and the naïve for each repetition, counting the number of times that the true value for $\beta$ was contained in the interval (*2*). We found that the coverage of 90% confidence intervals were improved by the use of the MCML procedure: The naïve confidence intervals contained the true $\beta$ in only 84% of the simulations, this increased to 88% for the MCML.

### *Discussion*

In this simple simulation experiment the MCML confidence limits appeared to perform reasonably well and were slightly more accurate than the naïve confidence limits. We found in the simulations that the impact of shared errors is more severe on the performance of more extreme upper confidence limits (e.g. 99%) than less extreme (90%) intervals. This phenomenon is expected based on general principles laid out by Stram and Kopecky (*4*).

### *References*

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