Research Departments

Departments of Clinical Studies, Hiroshima and Nagasaki

The Department of Clinical Studies conducts health examination programs of the Adult Health Study (AHS) and the F_1 (A-bomb survivors' children born after May 1, 1946) studies that provide the opportunity for a number of specific studies.

The AHS biennial examinations were initiated in 1958 and continue today. The AHS clinical examination is the only point of regular direct contact with the survivors and provides health benefits to them through early disease detection. It functions as the principal source of biological materials which make possible a wide variety of valuable special studies by various RERF departments and outside investigators. Sera and blood cells have been collected and stored from the AHS participants since 1969 and 1990, respectively. The AHS program has greatly contributed to the mission of RERF 1) to assess noncancer disease risk from radiation, 2) to determine the radiation effects on physiological or biochemical abnormalities, and to correlate this information with other life experiences and modes and patterns of disease, and 3) to epidemiologically elucidate mechanisms of radiation effects on cancer and noncancer diseases using stored biosamples and clinical and epidemiological information obtained through the heath examinations.

The AHS has greatly increased in importance in recent years as a result of the accumulation of an enormous body of data from serial medical examinations. There is an accumulating evidence of the radiation dose-related increase in noncancer disease morbidity, such as cardiovascular disease, hyperparathyroidism, thyroid diseases, uterine myoma, chronic liver disease, and cataracts, plus subclinical risk indicators and conditions such as inflammation.

Research Scientists of Hiroshima Clinical Studies (First row from left) Michiko Yamada, Saeko Fujiwara, Waka Ohishi, (Second row from left) Yoshimi Tatsukawa, Ikuno Takahashi

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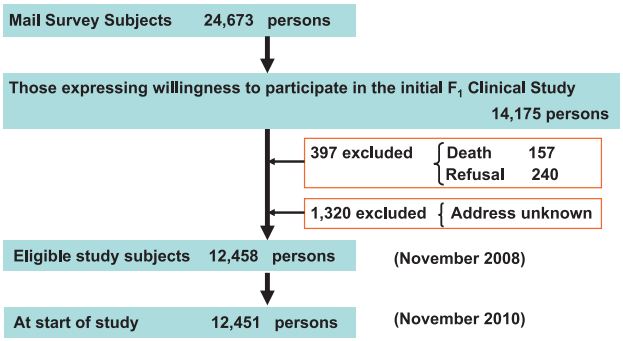
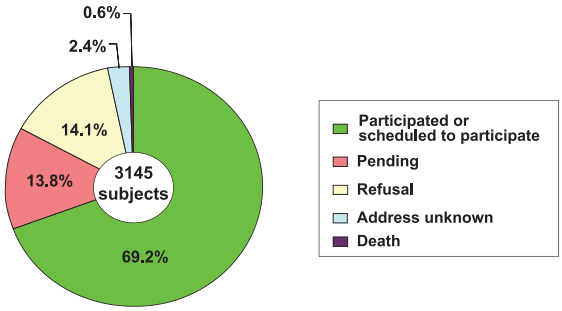
Longitudinal F_1 Clinical Study

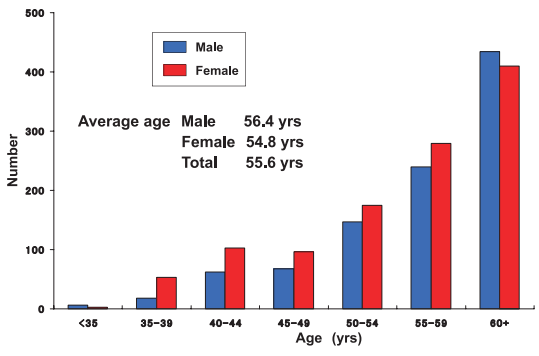
Study Subjects



Status of Participation (Nov. 2010—Nov. 2011)



Age Distribution of Participants (Nov. 2010—Nov. 2011)



The F_1 Clinical Study examinations were conducted on about 12,000 to analyze the potential heritable effect(s) of A-bomb exposure on multifactorial diseases (e.g., diabetes, essential hypertension, coronary heart disease) from 2002 to 2006. However, owing to the young age of the F_1 group (mean age of 48), most of their disease experience is yet ahead, so we are converting the sample to a cohort to follow-up prospectively.

Department of Genetics

In the Cytogenetics Laboratory, genetic effects of radiation were investigated cytogenetically by examining about 16,000 offspring of survivors and the results did not indicate any genetic effects of parental exposure (Awa et al. Obe G, Basle A, eds. Cytogenetics: Basic and Applied Aspects. New York: Springer-Verlarg; 1987, pp 166–83). We then shifted the study to somatic effects of radiation. The frequency of stable-type aberrations (translocations) was examined previously by the solid Giemsa method and is currently being studied using fluorescence *in situ* hybridization (FISH). Another method of biodosimetry being utilized is electron spin resonance (ESR) using tooth enamel. We now know that both FISH and ESR data fit reasonably well to each other. We anticipate that such biodosimetric data will provide information on possible random and systematic dose uncertainties in individual doses calculated by the DS02 dosimetry system, which is valuable for cancer risk estimation. In addition, the laboratory has expanded its scope of research by including genetic studies on breast and skin cancers, developing a green fluorescent protein (GFP) mouse model for quantitative measurement of germ-cell mutations, and finding markers for unrepairable DNA radiation damage.

The Biochemical Genetics Laboratory previously conducted a large-scale protein-level study in which 30 blood proteins of 23,000 offspring (11,000 from the exposed group and 12,000 from the control) were examined (this is equivalent to approximately 0.6-million locus tests for each group) for detecting *de novo* mutants and found five mutations (two in offspring from exposed parents and three in controls). The study was discontinued as the frequency was too low to detect a significant radiation effect. Subsequently, the group has been collecting blood samples and establishing Epstein-Barr virus (EBV)-transformed cell lines from members of survivor families (mother, father, and offspring) for molecular studies. We have also been developing competence in more advanced DNA-based assays. Several small-scale studies were conducted that screened mutations at hyper-variable mini- and micro-

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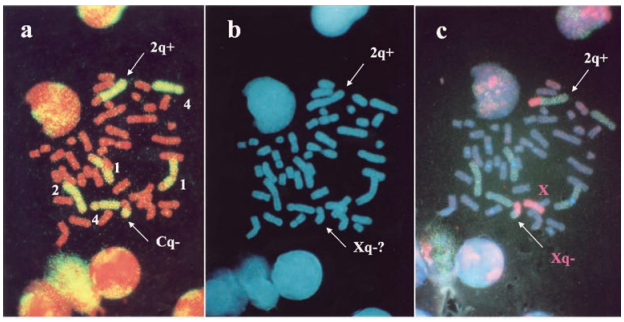
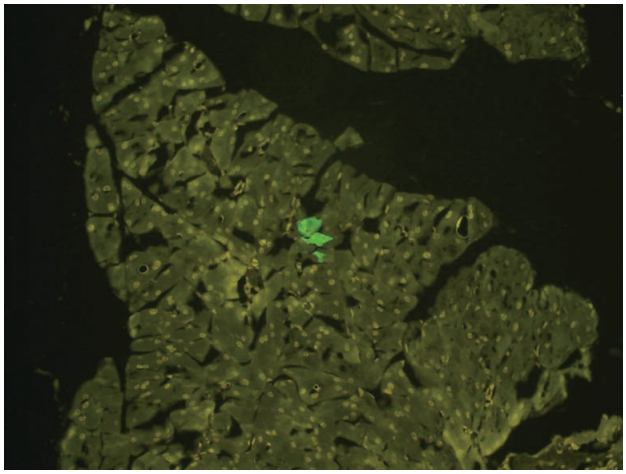
satellite loci, about 1,000 loci by two-dimensional gel electrophoresis, and 2,500 loci by BAC arrays. None of those studies indicated possible genetic effects of parental exposure to radiation. Currently, high-density microarray methods using over one million probes are being introduced to detect deletion/amplification mutations throughout the genome.



Detection of clonal aberrations by sequential FISH-Q staining. In the routine FISH study of A-bomb survivors, confirmations of clonal chromosome aberrations among the translocations detected, and corrections of them are necessary for accurate dose estimation. This figure shows how to confirm the clonal aberrations. After detection of translocations, the abnormal cells were photographed and the metaphase locations were recorded (a). The cells were re-stained for Q-banding to determine the counterpart, unpainted chromosome and the approximate breakpoints of each translocation (b). When the Q-band information failed to identify the unpainted chromosome, additional FISH was carried out using probes specific for the suspected chromosomes (c).



Detection of radiation-induced mutant cells in the pancreas of newly developed HPRTdupGFP mouse. To examine the radiation effects on somatic and germ cell mutation, recombinant mouse systems were developed in which radiation-induced mutants become fluorescent (HPRTdupGFP mice). The photo image shows the pancreatic mutant cells (green) detected in tissues section from 3 Gy irradiated mouse at 3 months. This mouse system could be used for the analyses of radiation-dose effects.

Department of Radiobiology/Molecular Epidemiology

The Department of Radiobiology/Molecular Epidemiology focuses on two research themes, *Immunology* and *Cancer*, and seeks to clarify the mechanisms underlying the epidemiological observations in A-bomb survivors. Immunologic effects are investigated with two approaches, i.e., Immunology studies and Immunogenome studies, which primarily examine immunological phenotypes and genotypes of A-bomb survivors, respectively.

The Immunology studies hypothesize that ionizing radiation accelerates immunosenescence, partially resulting in enhanced risks of aging-related diseases among A-bomb survivors. To strengthen this hypothesis, we are investigating mechanisms of radiation-related immunosenescence related to hematopoietic stem cells (HSCs) and dendritic cells (DCs). On the outcome side, we are evaluating the effects of radiation and aging on the influenza vaccine response, in collaboration with experts outside RERF. We will also develop an integrated scoring system for evaluating immunological and inflammatory status among A-bomb survivors and for providing a robust risk estimation of radiation- and aging-related diseases.

The Immunogenome studies evaluate the genetic basis for interindividual differences in immune functions and the impact of the genetics on susceptibility to radiation-associated diseases. The phenotype-genotype association analyses utilize the A-bomb survivors' stored biological materials and cumulative immunology data. The results obtained from the studies will have the potential to contribute to the individualized prevention of radiation-associated diseases in A-bomb survivors and also other exposed populations.

The Cancer studies aim to clarify mechanistic relationships between radiation exposure and cancer development among A-bomb survivors, based on the hypothesis that A-bomb radiation might affect various carcinogenic pathways in terms of particular genetic and/or epigenetic alterations found in radiation-exposed cancer cases. Toward this end, we elucidate molecular characteris-

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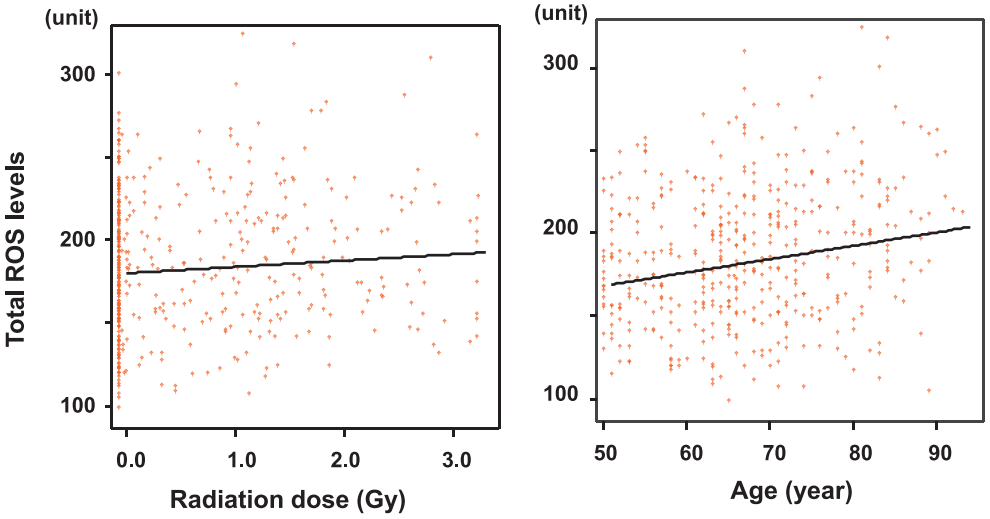
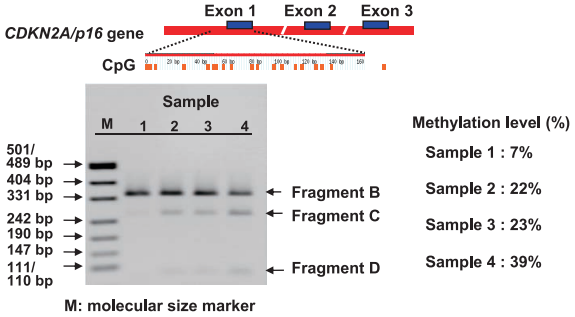
tics in thyroid, colorectal, and lung cancers in members of the Life Span Study. We also evaluate epigenetic alterations in normal cells such as blood cells, to determine whether radiation exposure modulates aging-related epigenetic alterations that may lead to increased risks of aging-related diseases including cancers.

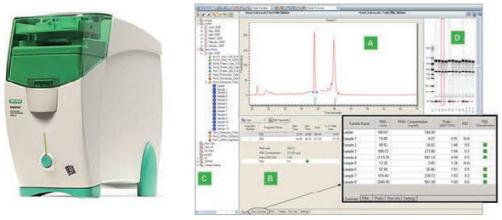


Reactive oxygen species (ROS) generated in biological activities including responses to environmental stresses such as radiation exposure can cause inflammatory diseases. Using the newly developed automated plasma ROS assay system, we observed increased ROS levels with radiation dose (left) or age (right) among 442 AHS participants.

Assessments of DNA methylation levels by COBRA method



Experion automated electrophoresis system



Analyses of epigenetic alterations in peripheral blood were initiated to assess age and radiation effects. Left panel shows an example of COBRA (combined bisulfite restriction analysis) measuring DNA methylation levels in CpG loci of *CDKN2A/p16* gene using blood samples donated from non Adult Health Study (AHS) healthy volunteers. An automatic electrophoresis system, Experion (right panel), in the Cell Biology Laboratory is currently utilized to conduct COBRA for epigenetic assessments.

Departments of Epidemiology, Hiroshima and Nagasaki

A major purpose of RERF is to clarify the late health effects of atomic bomb radiation among the survivors. The follow-up of the Life Span Study (LSS), *in utero*, and children of A-bomb survivors (F_1) cohorts in the Department of Epidemiology is crucial to accomplish the purpose. Follow-up outcomes include deaths and causes of death wherever they may occur in Japan, and cancer incidence in Hiroshima and Nagasaki prefectures. Histological specimens of cancer cases are also collected by the tissue registries and in collaboration with community pathologists. Around 37% of the LSS cohort members were still alive at the end of 2007, including 84% of those who were under 10 years old at the time of the bombing. Also 89% of *in utero* and 90% of the F_1 cohort are still alive. Therefore continued follow-up of these young-age groups for an additional 20 years or more is clearly essential. It is important to establish the consistency of epidemiological evidence with biological mechanisms of radiation effects. It is also essential to epidemiologically evaluate other risk factors for confounding or modification of radiation risks, and to more precisely determine the magnitude of risk for radiosensitive subgroups such as those in early childhood or *in utero* at the time of exposure. International risk assessment groups use the results from these cohorts as the primary basis for radiation risk estimation. The LSS mortality and incidence data have been periodically analyzed and those results were heavily relied upon by the Advisory Committee on the Biological Effects of Ionizing Radiation (BEIR) VII (2005) and the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) (2006), as well as many other radiation risk reports.

High quality cancer incidence data are also essential to evaluate the radiation risks for cancers occurring in those cohorts. For this, RERF has vigorously managed the Hiroshima and Nagasaki tumor/tissue registries. The data have long been included in "Cancer Incidence in Five Continents (CI5)" (by International Agency for Research on Cancer [IARC]/International Association of Cancer Registries [IACR]), a compilation of worldwide cancer incidence data, and are given the highest rating by that consortium. The data for 2003–2007 have been submitted to the latest 10th edition (CI5-10). The data on childhood cancer in 1990–2007 have been also submitted to the "International Incidence of Childhood Cancer, Volume 3 (IICC-3)" (by IARC/IACR). A preservation program of survivors' tissues resected during surgery has begun in collaboration with hospitals in Hiroshima and Nagasaki. These materials, which are utilized by all RERF departments, are important for investigations of cancer pathogenesis associated with radiation exposure.

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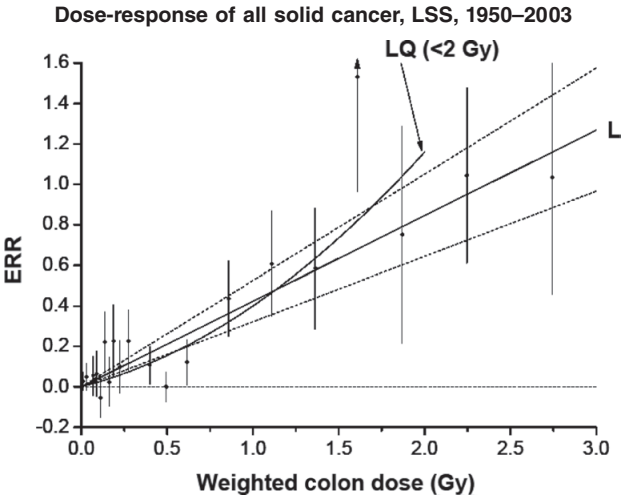


This figure shows the dose relationship of excess relative risk (ERR) for all solid cancer to radiation exposure. The black circles represent ERR and the vertical bars indicate 95% confidence intervals (CIs) for the dose categories, together with trend estimates based on linear (L) with 95% CI (dotted lines) and linear-quadratic (LQ) model for the data restricted to dose <2 Gy.
(Cited from Ozasa et al., *Radiat Res* 2012; 177[3]:229–43. doi:10.1667/RR2629.1)

Risks of radiation for urothelial carcinoma after adjusting for lifestyles, LSS, 1958–2001

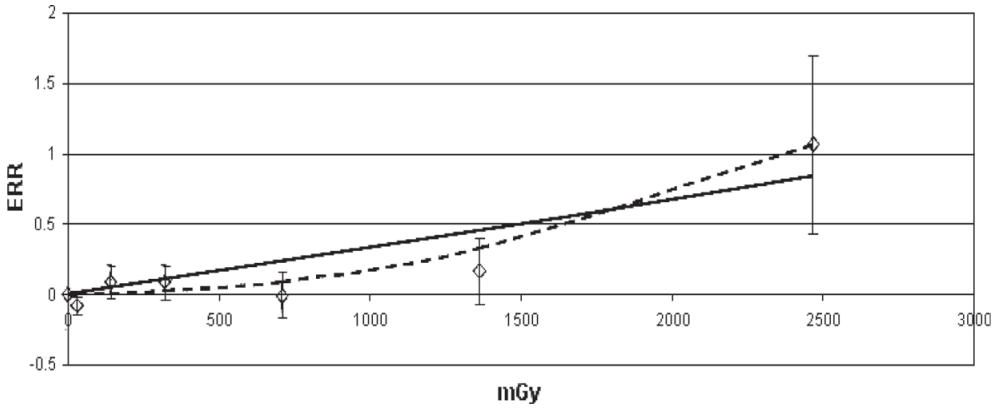
	Background rate (/10,000 person-years)		**Excess relative risk** (Gy)			
	Male	Female	Sex-averaged	Male	Female	F:M ratio
Unadjusted	9.1	2.1	1.00*	0.47	1.5*	3.2
Adjusted for lifestyle factors	5.0	2.6	0.96*	0.44	1.5*	3.4

	Background rate (/10,000 person-years)		**Excess absolute risk** (/10,000 person-year-gray)			
	Male	Female	Sex-averaged	Male	Female	F:M ratio
Unadjusted	9.2	2.2	3.0*	3.6	2.5*	0.70
Adjusted for lifestyle factors	5.0	2.9	2.7*	2.9	2.5*	0.87

* $p < 0.05$

The sex-averaged excess relative risk (ERR) was 1.0 per Gy (100% increase per 1 Gy), lower in males and higher in females. Neither effect modification by age at exposure and attained age, nor interaction between radiation and smoking was significant. Those risk estimates were substantially similar after adjusting for lifestyle factors including education, smoking, alcohol, vegetables and fruits. The sex-averaged excess absolute risk (EAR) was 3.0/10,000 person-year-gray, and rather similar between males and females. The figures were also similar after the adjustment.
(Cited from Grant et al., *Radiat Res* 2012; 177[1]:86–98. doi: 10.1667/RR2841.1)

Excess relative risk of radiation for possible chronic renal failure by dose, LSS, 1950–2003.



The open circles represent ERR and the vertical bars indicate 95% confidence intervals (CIs) for the dose categories. Black line is the linear model; dashed line is the quadratic model for dose relationship. Models adjusted for city*sex, age at exposure, attained age, hypertension, and diabetes. (City*sex is a single combined variable of city and sex with 4 categories. Males in Hiroshima comprise the baseline group.)
(Cited from Adams et al., *Radiat Res* 2012; 177[2]:220–8. doi:10.1667/RR2746.1)

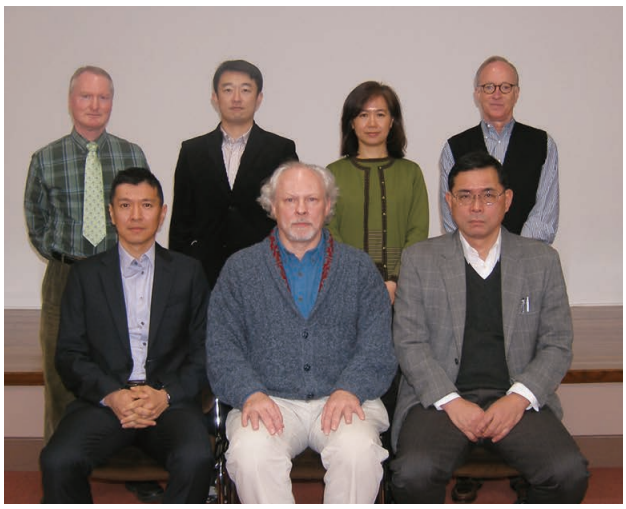
Department of Statistics

The Statistics Department has historically led in the development of analytical methods for major aspects of the RERF research program of risk estimation. This includes the development of tools and methods to handle various problem areas in the longitudinal data of major RERF cohorts, such as dose uncertainty and undocumented out-migration from cancer/tumor registry catchment areas, among many others.

We have evaluated or developed special methods of sub-cohort sampling to increase efficiency and address special issues of effect modifiers or mediators. We also develop many special methods to meet the requirements of RERF's basic science research in genetics, immunology, radiation biology, and molecular epidemiology. Finally, the department implements RERF dosimetry systems and maintains a database of survivor dose estimates, presently using the DS02 dosimetry system provided by a combined external and internal scientific working group, and provides key statistical and dosimetric support to RERF projects in biodosimetry.

Unlike other departments, Statistics provides consulting in addition to research. Given the crucial importance of sound statistical advice in the design, analysis and conduct of studies, the department tries to take a proactive approach to consulting, beginning with early involvement in study design, and devotes a majority of its staff's effort to consulting.

Efforts in dosimetry, including work on dose uncertainty, are by definition central to the RERF mission of evaluating the effect of ionizing radiation on human health, as they provide the denominator for measures of dose response. The department does not undertake dosimetry-related research for its own purposes unless it stands to measurably improve the RERF dosimetry. In other areas, regarding both consulting and research, Statistics provides information on study designs and the statistical power of potential studies. This is critical information in evaluating how well a project will be able to address its proposed scientific question, which in turn impacts its value to the RERF mission.



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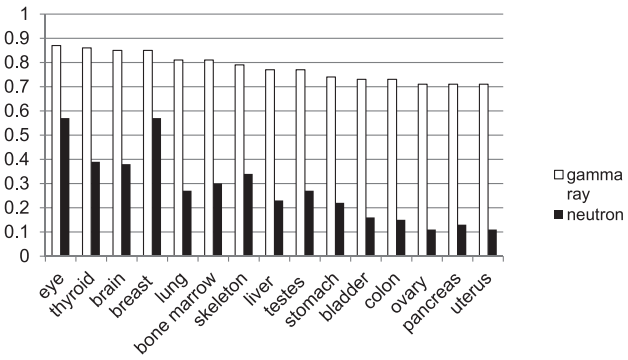

Cohort-average transmission factors for organs calculated by DS02: Hiroshima adults.
(Cullings, *Radiat Prot Dosimetry* 2012; 149[1]:2–14)

Department of Information Technology

Hiroaki Katayama, Research Scientist and Department Chief, ITD

The Department of Information Technology (ITD) consists of the Systems Technology Section and the Library and Archives Section, both of which are engaged in support activities for RERF's research. The Systems Technology Section is responsible for the maintenance of computer/network environments and the protection and management of our extensive and complex set of electronic research databases. The Library and Archives Section is responsible for organizing and providing access to scientific articles and historical documents.

The Systems Technology Section has been engaged in the maintenance of network and hardware environments, including personal computers, the construction of a variety of databases for analysis (epidemiological research database, resource management database, Adult Health Study [AHS] database, etc.) and the development of relevant application software. The databases are managed in a technologically advanced manner that allows RERF's researchers to more effectively and rapidly access and retrieve essential archives for personal and collaborative purposes. With the aim of helping research scientists understand those databases with a complex structure, the section has also been involved in such research-support activities as the creation of a data dictionary and a document management database. Among the section's recent focus are the prevention of illegal attacks into the RERF network and infections with computer viruses, and the addition of new functions for personal information management at RERF in line with implementation of the Personal Information Protection Law.

The Library and Archives Section is composed of the Library and the Archives Units; the former handles the procedures for purchase and storage of scientific journals, management and preservation of books, and handling of requests from RERF's research scientists for copies of papers. The recent rapid growth in demands for such services has moderately impacted ITD's operations. The Archives Unit is responsible for the storage, indexing and digitizing of historical archival materials, and processing the associated invoices and distribution of RERF publications. Loss of personnel represents an area of concern due to retirements that have affected the Library Unit and the Archives Unit. Meanwhile, as the number of orders for ABCC-RERF-related historical materials from outside RERF is on the rise, we are considering how to smoothly proceed with our duties.

ITD participates in a variety of collaborative projects with outside research organizations. For example, we have already embarked on research collaboration with the Bioinformatics, Human Genome Center of the Lawrence Livermore National Laboratory; and we are communicating with the University of Texas School of Public Health concerning exchange of technical expertise. ITD is providing technical expertise to the Osaka Medical Center for Cancer and Cardiovascular Diseases in constructing a comprehensive cancer control system in Osaka prefecture. Among ITD's research and collaborative activities are: participation in the World Health Organization (WHO) Radiation Emergency Medical Preparedness and Assistance Network (REMPAN); cooperative technical expertise for Hiroshima University as one of western Japan's tertiary medical institutes for the radiation exposed; establishment of a nationwide standardized model for a population-based cancer registry database system and introduction thereof throughout Japan as part of the third Ten-year Strategy for Cancer Control conducted by the Ministry of Health, Labour and Welfare; and creation of an epidemiological research database devoted to the Ministry of Education, Culture, Sports, Science and Technology (MEXT) Grant-in-Aid for Scientific Research project on low-dose radiation effects from nuclear tests conducted in Semipalatinsk, Republic of Kazakhstan by the former Soviet Union.