5. Research Center for Radiation Protection

Outline of Research Career:
In 1982, Dr. Sakai got a Ph. D. degree in biochemistry from the University of Tokyo. He first worked as a Research Associate in the Department of Radiation Biophysics, Faculty of Medicine, University of Tokyo (1982-1989), and then as a Lecturer in the Department of Radiation Oncology, Graduate School of Medicine, University of Tokyo (1989-1999). The main subjects of his research were radiation induced DNA damage and its repair, and mechanism of radiation induced cell death. From 1983 to 1985 he worked as a research fellow in the Genetics Division, Children's Hospital, Harvard Medical School. At that time, his research subjects were gene amplification and cloning of genes responsible for radiosensitivity. He joined the Central Research Institute of Electric Power Industry in 1999 to research biological effects of low dose radiation. He has been at NIRS since 2006.

Objectives
The Research Center for Radiation Protection was newly established in 2006. The aim of the Center is to provide a scientific basis for radiation protection and safety. Toward this goal, radiation exposure from various sources is measured, the dose-effect relationships for various endpoints are examined, and the mechanisms underlying the effects are investigated. The Research Center disseminates its research results to promote public understanding of radiation effects and to encourage the enactment of more reasonable regulations concerning the use of radiation. The scope of its activity is not limited to Japan. It has been appointed a Collaborating Centre by the International Atomic Energy Agency.

Overview
The Research Center consists of four research groups (Regulatory Sciences Research Group, Experimental Radiobiology for Children's Health Research Group, Radiation Effect Mechanisms Research Group, and Environmental Radiation Effects Research Group), Nakaminato Laboratory for Radioecology, and the Department of Advanced Technologies for Radiation Protection Research.

The activities of the research groups and the Nakaminato Laboratory are described in their respective sections. The Department of Advanced Technologies for Radiation Protection Research consists of four sections. Their activities in FY2007 are summarized below.

The Advanced Analytical Technology Section carried out cooperative projects with other research groups from inside and outside of NIRS to measure trace elements in environmental and biological samples. Also, the research work to clarify the effects of rhenium concentration on environmental Tc-99 analysis was finalized. Two research papers were published on the development of Re and U determination methods.

The Animal Pathology Section provided histopathology technical and diagnostic supports for NIRS intramural research projects.

The Advanced Animal Research Section supported integrated research of molecular and genetic studies with physiological studies in whole animals. Although remarkable progress of radiation biology has been made in genetic, molecular and cellular levels, physiological analysis of whole animal models is necessary for extrapolation to human health. The section supported radiobiological research by application of assisted reproductive technologies (ARTs) in genetically modified laboratory mice, including in vitro fertilization, embryo transfer, micromanipulation of embryos and cryopreservation. Such technologies have also become essential to efficiently conduct large scale animal experiments by providing a large number of animals synchronously.

The Environmental Radioactivity Survey Section initiated three collaborative research projects with three Japanese universities. They involve development of an ultra sensitive radon decay products measuring system, establishment of a calibration procedure for radon and its decay products concentrations and development of a new technique (based on detection of Cherenkov radiation) for radon measurements. In addition to them, other six commissioned works were given to this section using NIRS technologies and facilities.

In the Research Center 58 permanent and 91 temporary members actively conducted their research. They produced 82 original papers; in 70 papers, the researchers were the principal contributors and 12, they were supportive. The Center held a symposium on environmental effects of ionizing radiation. Also, the Center organized an International Workshop on the Biological Effects of Low Dose Radiation.
5.1. Regulatory Sciences Research for Radiation Safety and Protection

Outline of Research Career

Dr. Yonehara received a Ph.D. from Shiga University of Medical Science in 1995 for his study on issues of risk from exposure to residential radon. He joined to NIRS in 1996 and began working on studies related to dose evaluation from environmental radiation. From 2003 to 2006 he worked on development of radiation safety standards as Director for Radiation Protection Policy in Ministry of Education, Culture, Sports, Science and Technology (MEXT). Since returning to NIRS, he has studied dose evaluation from natural radiation sources as well as issues related to radiation safety regulation. Since March 2007, he has been working as Director of the Regulatory Sciences Research Group.

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Objectives

"Regulatory science" can be considered to be an integrated science of uniting views of rationality in science and society. The main objectives of regulatory sciences research for radiation safety and protection are to summarize scientifically based information for radiation safety regulation and to exchange information among different stakeholders to bridge the gap between science and society. The research programs of the Regulatory Science Research Group are focused on four points.

1) Summarizing radiation protection issues

The group aims to summarize achievements of research on radiation protection provided by NIRS, as well as universities and other research institutes in order to contribute to activities of relevant international organizations such as UNSCEAR, IAEA, OECD/NEA, and ICRP. The group also summarizes the information on radiation protection for dissemination to regulatory authorities and the public. To share the information with scientific organizations, regulatory authorities and the public, the group has constructed a research information network.

2) Construction of information databases for radiation risk assessment

The group constructs information databases on risk assessment for people who are exposed to low dose radiation and controllable natural radiation sources. Scientific information on radiological archives of experimental research, and on the exposures and health effects of radiation among different human populations, and on environmental effects of radiation from the epidemiological studies are collected for the databases.

3) Development of mathematical models

Using the results of basic research related to the radiation effects on health and environment, the group develops mathematical models for risk evaluation of health effects due to exposure to controllable natural radiation sources and due to medical exposure. The group also develops models for analysis of radiological effects on the environment.

4) Development of methodology for risk communication

The group collects case examples in which risk information on radiation safety is passed on to the public, and the group analyzes social psychology findings.

Progress of Research

1) Construction of information databases for radiation risk assessment

The scientific achievements related to radiation effects are to be summarized comprehensively in the UNSCEAR report. The group, together with an expert panel in Japan made a great contribution to UNSCEAR activities submitting the relevant data as well as scientific comments on the draft of the UNSCEAR report. The group also took part in summarizing the comments from experts in various fields for drafting the 2007 Recommendations of ICRP. The information on status for exposure due to industrial use of naturally occurring radioactive materials (NORM) was summarized and an integrated database with the information was constructed for workers in affected industries and for consumers of the products as well as researchers. Specific radioactivities of various samples of ores and stones for industrial or building materials were determined experimentally to make up for the lack of relevant data in the database. A method for studying on possible health effects associated with medical exposures during childhood was examined. Experimental results of long term animal exposure experiments were collected and archived in electronic format. Finally, an international workshop was carried out to discuss further research applications within the scope of radiation protection.
2) Study on mathematical models

The group aims to develop two types of mathematical models for regulatory science. The first is a model for simulation of carcinogenesis. The main purpose of studies using this model is to evaluate the radiation risk at low dose exposure. The second type of model is for evaluation of the effects of ionizing radiation on environmental biota and ecosystems, and the effects of other environmental toxicants. In order to study the interactions in a model aquatic microcosm, an individual-based computer simulation model was developed. The microcosm studied consisted of Euglena gracilis as an autotroph algae, Tetrahymena thermophila as a heterotroph protozoa and Escherichia coli as a saprotroph bacteria. There is a strong interaction between Tetrahymena and E. coli as the first is a predator of the second. Ecological toxicity tests were conducted to test the population level impacts of the biological effects of radiation and toxicants on the lethality and mobility factors that influence directly or indirectly influence growth and reproduction. Radiological effects on lethality of E. coli individuals were translated to the reduction of the equilibrium population of Tetrahymena. A synergistic effect was also observed by the simulation at the community level in the case of combined exposure of radiation and a toxicant which reduced the feeding efficiency of Tetrahymena (Fig. 9).

Fig. 9. Population level responses to the combined effects of chronic exposures of gamma radiation and toxicants simulated by SIM-COSM. Fig. (a), (b) and (c) show the combined effects on populations caused by exposures to gamma radiation (100 mGy/h) and toxicants that inhibit 50% of Tetrahymena mobilization, E. coli diffusion and E. coli growth, respectively.

3) Epidemiological study

An epidemiological study on lung cancer associated with residential radon in China is continuing. Recently, several pooled analyses of residential radon studies have indicated an increased risk of lung cancer even at the low radon level. Such consolidated analytical attempts, however, include data from several studies with different measuring methods and devices which are subject to large uncertainties associated with different measurement protocols. One of the most important factors in the measurements for epidemiological studies which should be taken into account is the issue of thoron (220Rn). The results of measurements are likely to be affected by properties of detectors, especially their thoron sensitivity. Thoron concentrations can lead to overestimation of radon concentration and then to underestimation of lung cancer risk if measurement devices with high thoron sensitivity are used without discriminating it. On the other hand, not only radon but also existence of thoron is important itself. Although measurements of thoron gas provide nonessential information for occupant exposure assessment because of its short half-life (55.6 s) which means it cannot become uniform in room air, those of thoron decay products provide important dose information. Unfortunately, no common measurement protocol for thoron decay products or for radon has been established yet. Another important factor related to the measurements is an issue of retrospective radon measurement. Current concentrations of radon do not necessarily represent cumulative exposures to radon in the long term because there are large temporal and spatial variations in radon concentrations. We initiated a case-control study focused on thoron existence and historical radon exposure assessment in a rural area of Gansu Province, China. In our study, newly developed devices are employed for measuring both radon and thoron gases discriminatively, thoron decay products, and cumulative exposures to radon. The survey will be continued until 2009. Before conducting the main study, a pilot study was carried out from October 2006 to April 2007.

4) Study on biosimetry

We studied the chemical induction of premature condensed chromosomes in human peripheral lymphocytes after culturing for 6 h. Many other researchers have attempted this induction without culturing or with short-term culturing, because this technique permits prompt cytogenetic biodosimetry of radiation accidents. Lymphocytes were separated from blood, incubated in the presence of phytohemagglutinin, ATP and p34^cd/cyclin B kinase, and then treated with calyculin A during the last hour. The culture medium was supplemented with a lower concentration of fetal calf serum than conventionally used to minimize its possible interference with the effects of these drugs. We obtained, rarely, a suitable morphology of premature chromosome condensation in short-term cultured lymphocytes for conventional chromosome aberration analysis (Fig. 10).
Fig. 10. Effects of p34cdc2/cyclin B kinase (Cyclin B) and CalyculinA (CA) on the frequency of cells with highly or moderately condensed chromosomes. Lymphocytes were cultured for 6 h in the presence of (2%) phytohemagglutin, 0.1 mM ATP, 0, 50, 100 enzyme unit/ml Cyclin B and 0.05 μg/ml colcemid and treated with 100 or 500 nM CA during the last hour. Frequencies are expressed as the numbers of cells having condensed chromosomes per 1000 cells plated into a culture tube.

5) Development of method for risk communication
We held a “Dialogue seminar” for risk communications among experts for NORM, users of industrial NORM and regulators. Fundamental information on NORM and an introduction to the NORM database developed by our group were illustrated by experts and issues related to NORM were discussed among stakeholders.

Major publications
2) S. Tsuji, R. Kanda: Chemically induced premature chromosome condensation in short-term cultured human peripheral lymphocytes: applications to biodosimetry, Biotechnic & Histochemistry, 82 (1), 29-34, 2007
5.2. Experimental Radiobiology for Children's Health Research Group

Outline of Research Career
Dr. Shimada received a Ph.D. in 1985 from the University of Tokyo. At the Mizuno Biohoronics Project of JST (1985-1987) and at the Tokyo Metropolitan Institute of Gerontology (1987-1989), he worked on innate immunity in carcinogenesis and aging, respectively. Since 1989 at NIRS, he has focused on molecular and cellular mechanisms of T-cell lymphomagenesis and mammary carcinogenesis from the viewpoint of combined effect of environmental carcinogens and the age-at-exposure effect.

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Objectives
With the advent of an era of low birthrate and longevity, concerns about the safety of fetuses and children have been growing. Programs to protect the health of fetuses and children and the safety of the environment are being instituted, particularly in the USA and Europe. These regulations are mainly directed at foodstuffs and chemicals. The Experimental Radiobiology for Children's Health Research Group carries out studies to provide information on the risk of carcinogenesis due to radiation exposure during the fetal and childhood periods, for which there are at present insufficient data. Using animal models, we study the effects of radiation exposure on cancer induction and lifespan shortening. Final goals of this research group are to propose age-weighting factors and relative biological effectiveness (RBE) of neutrons and heavy ions for fetuses and children for radiation protection.

Progress of Research
1) Age dependency of life shortening by irradiation in B6C3F1 mice
Fifty female and male B6C3F1 mice per each group, which have been used in a wide variety of toxicological studies such as the National Toxicology Program (NTP) in USA, were exposed to gamma rays ($^{137}$Cs), carbon ions (13 keV/um) and neutrons (2 MeV) at various ages during fetal to mature adulthood periods. The ages examined were pre-implantation (3 days post-conception (dpc)), major organogenesis (13 dpc), late fetal (17 dpc), neonatal (1 week after birth), prepubertal (3 weeks), post-pubertal (7 weeks) and mature adult stages (15 weeks). The doses ranged between 0.2 and 4 Gy. These mice are now kept under observation. Preliminary observation indicated that female mice appeared more susceptible to radiation-induced lifespan shortening than male mice. Carbon ions were more potent in reducing lifespan than gamma rays when female newborn mice were exposed. Surprisingly, irradiation with gamma rays at late fetal stage had little influence on lifespan shortening, indicating fetuses are rather resistant to gamma-ray-induced lifetime risks compared to infant or adulthood exposure. However, when carbon ions were exposed, fetuses were as susceptible as infants. These results suggest a larger relative biological effectiveness (RBE) of carbon ions for fetus.

2) Age dependency of cancer risks in mammary gland, lung, bone marrow, liver, kidney, brain and intestine
Radiation risks are dependent upon tissues and age at exposure. Among tissues, breast tissue is most susceptible to radiation-induced cancer risk. In order to determine the age effect on mammary tumors, 821 female Sprague-Dawley rats, which have been widely used as a suitable model of human breast carcinogenesis, were irradiated with gamma rays and carbon ions (13keV/um) at doses of 0.2 and 1 Gy. We found an enhancement of tumor incidence after exposure at 3 weeks of age, which was in good agreement with the data of female A-bomb survivors. The tumor incidence after irradiation with carbon ions was almost identical or less than that after gamma-ray irradiation. No increase in mammary tumor incidence was observed in rats exposed prenatally. The lung is one of the important organs for radiological protection of workers and the public because of its high radiation-associated cancer risks. To elucidate the age dependence of its dose-effect relationship, 1, 5 and 15 week-old female Wistar rats (total 760 animals) were irradiated with X-rays at the thoracic region at doses of 0, 1, 3 and 5 Gy. It turned out that the older the rats were at exposure, the higher the incidence of lung tumors was. We also started new research on the age effect on tumor development of kidney, brain and
intestine using mutant and knockout animals such as the Eker rats and $\text{Plc}^-$, $\text{Apc}^-$, and $\text{Mdr}^-$ mice. Kidney was found most susceptible at the perinatal period for radiation tumorigenesis.

3) Combined effect of radiation with chemical carcinogens on lung, uterine and thymic tumorigenesis

The age effect of combined exposures to radiation and a chemical carcinogen has been investigated on pulmonary, uterine and thymic carcinogenesis. Female Wistar rats were irradiated at infant (1 weeks of age), pubertal (5 weeks of age) and adult (15 weeks of age) stages followed by an intraperitoneal injection (1.0 g/kg body weight) of $\text{Nbis}(2$-hydroxypropyl)nitrosamine. The preliminary data indicated a synergism of radiation and chemical exposures when the former was in the pubertal stage. Uterine corpus cancer a typical fatal tumor in women, is increasing in many developed countries. For the age-dependent effects of irradiation on the uterine carcinogenesis, Donryu rats were exposed to gamma-rays at doses of 0.2 and 2 Gy with or without $\text{Nethyl-Nnitro-Nitosoguanidine}$ treatment during juvenile (2 weeks after birth) and adulthood (10 weeks after birth) stages. The rats exposed at prepuberty showed earlier onset of persistent estrus and, interestingly, an increase in uterine cancer development at 10 months of age. $\text{Gpt}$-delta mice were X-irradiated followed by $\text{Nethyl-Nnitrosourea}$ (ENU) treatment to see the mode of mutation induction after combined exposure. It was found that the mode was dose dependent. While combined exposures with a high dose of weekly irradiation (1.0 Gy per fraction for 4 weeks) enhanced lymphoma incidence in a synergistic manner, a small dose (0.2 Gy per fraction) suppressed lymphoma induction. The mutation induction was also suppressed by prior small dose exposure at 0.2 Gy; this finding was in good agreement with lymphoma incidence. These results will provide the information on the relative risk and the age-weighting factor for radiation-induced carcinogenesis.

4) Detrimental effect of uranium on the childhood kidney

Health effects for children in depleted uranium-polluted areas and uranium mining areas are of recent concerns. Uranium and its compounds have the potential to cause nephrotoxicity. Using synchrotron radiation X-ray fluorescence analysis (SR-XRF) with a nano-probe, we demonstrated that uranium accumulated in the epithelium of the proximal tubules, a toxic target site of uranium, followed by an increase in apoptotic cells.

Major publications

5) S. Homma-Takeda, Y. Nishimura, Y. Watanabe, M. Yukawa: Site-specific changes in zinc levels in the epididymis of rats exposed to ionizing radiation, Nuclear Instruments & Methods in Physics Research Section B, 260 (2), 236-239, 2007
5.3. Studies on Radiation Effect Mechanisms

Dr. Nenoi received a Ph.D. from Kyoto University in 1992 for his study on induced accumulation of polyubiquitin gene transcripts after UV-irradiation and TPA-treatment. His research interest is mechanisms of gene transcription after exposure to DNA damaging agents.

Outline of Research Career

Dr. Nenoi received a Ph.D from Kyoto University in 1992 for his study on induced accumulation of polyubiquitin gene transcripts after UV-irradiation and TPA-treatment. His research interest is mechanisms of gene transcription after exposure to DNA damaging agents.

Objectives

Estimation of the low-dose radiation risk has been made using the high-dose data from atomic bomb survivors at Hiroshima and Nagasaki under the assumption that the risk is proportional to the radiation dose without a threshold. However, we do not necessarily have the scientific evidence to support this assumption. We do not have sufficient scientific data on the effects of low-dose radiation on developmental and differentiations anomalies either. Because it is now considered to be difficult to assess the risk of low-dose radiation from animal experiments or in epidemiological data, this research group conducts studies on the mechanism of radiation effects caused by low-dose radiation. The purpose of Radiation Effect Mechanisms Research Group is to derive findings useful in the risk assessment of low-dose radiation which can be used as a basis for the development of appropriate regulatory framework. The following study items are separately investigated by the four teams.

1) Radiation Carcinogenesis Research Team:

Evaluation of indirect effects of low-dose radiation on carcinogenesis (carcinogenesis due to changes in the microenvironment caused by irradiation) and examination of the involvement of DNA repair mechanisms in low-dose radiation-induced carcinogenesis.

2) DNA Repair Gene Research Team:

Clarification of low-dose radiation risk-modifying factors in nonhomologous end-joining DNA-repair and its molecular mechanism.

3) Developmental and Differentiation Anomaly Research Team:

Verification of the validity of radiation regulations relating to developmental and differentiations anomalies by evaluating the effects of low-dose radiation on abnormalities in neural crest cell differentiation.

4) Radioadaptive Response Research Team:

Determination of risk modifying factors specific to low-dose radiation by identifying genes associated with biological responses to low-dose radiation, including radioadaptive responses and signal transduction.

Progress of Research

1) Radiation Carcinogenesis Research Team

It has been thought that radiation-induced cancer is caused by radiation damage induced directly in the target cell. Because radiation causes mutations in the irradiated cells, cancer could occur if cancer-related genes are altered by irradiation. On the other hand, the existence of radiation-induced untargeted carcinogenesis, in which cancers originate from the radiation-induced change in a microenvironment in the irradiated body, has been known for 50 years, and the cause, the mechanism, and its contribution to the risk of the radiation-induced cancer have received attention in recent years. To confirm the existence of radiation-induced untargeted carcinogenesis and to evaluate its contribution to the risk of radiation-induced cancer, we first established an assay system for assessment of the indirect effect of radiation on carcinogenesis using thymus transplantation (Fig. 11). The thymectomized B10 thy1.2 mice were irradiated with 1.6 Gy γ-rays four times at one-week intervals and were transplanted with nonirradiated thymuses of new born B10 thy1.1 mice under the kidney capsule or subcutaneously. The mice were fed under a specific pathogen-free condition for one year and the generation of the T-cell lymphomas in the transplanted thymuses was observed. The origin of the tumors, which were derived from irradiated host cells or from nonirradiated thymic cells, could be determined using the expression of cell surface markers (thy1.1 or thy1.2) in the lymphoma cells. The incidence of thymic lymphomas in mice without thymectomy, which were irradiated four times with 1.6
Gy γ-rays, was 100%, which might include those induced by direct effect of radiation in target cells and those induced by radiation-induced microenvironmental change. The incidence of the transplanted thymus-derived T-cell lymphomas in mice irradiated and then transplanted subcutaneously was 33% (17/51), while that in mice irradiated and then transplanted under the kidney capsule was 50% (14/28). Thus both graft sites were effective in evaluating the indirect effect of radiation on carcinogenesis. There was no induction of T-cell lymphomas in mice transplanted with thymus, but not irradiated; this indicated that the generation of T-cell lymphomas from transplanted thymuses was due to the effect of radiation. These results confirmed the existence of radiation-induced untargeted carcinogenesis for the generation of T-cell lymphomas.

Our chief aim is to clarify the induction-mechanism of mutation by radiation. In particular, the identification of the modulatory factor(s) for a low-dose radiation-risk in non-homologous end-joining (NHEJ) and the elucidation of the molecular mechanism(s) involved with those factor(s) are the focus of our interest. DNA double strand breaks (DSBs) can arise from multiple sources including ionizing radiation (IR), and are the most serious DNA damage. NHEJ, which is a simple mechanism to piece together the broken DNA ends, can function in all phases of the cell cycle and it appears as the major repair pathway in mammalian cells. In the current study, we carried out the generation and characterization of NHEJ-related gene deficient human cell lines to define the biological roles of NHEJ-related genes on DNA damage induced by IR.

Three cell lines having XRCC4, Artemis and MDC1 disrupted, respectively, were established by using a gene targeting technique in a human colon tumor cell line HCT116. Chromosomal aberrations induced by X-ray irradiation were significantly higher in all cell lines deficient in NHEJ-related gene than in parental HCT116. Radio-sensitivities assessed by the survival rate after X-ray exposure were apparently increased in these cell lines in comparison with the parental HCT116. These radioresponsive phenotypes might be due to insufficiency of DNA damage signaling/repair machinery in these deficient cell lines. Discrete foci of 53BP1, ATM (S1981), DNA-PKcs (S2056) and MDC1 formed in response to X-rays mostly co-localized to γ-H2AX foci, a marker of DNA DSBs, in the nucleus of the parental HCT116. The formations of 53BP1 and ATM (S1981) foci, however, were not induced by X-ray exposure in MDC1 deficient cells although positive immunoreactivities with specific antibodies were clearly detected throughout the nuclei of the deficient cells. Furthermore, foci of DNA-PKcs (S2056), relatively smaller than γ-H2AX foci, were formed after X-ray irradiation in MDC1 deficient cells, but those foci did not co-localize to γ-H2AX foci. These results suggest that MDC1 may be associated with the recruitment of DNA damage signaling/repair components, such as ATM, DNA-PKcs and 53BP1, to sites of DNA DSBs induced by IR, and that MDC1 might be the master regulator determining the formation of a specific chromatin microenvironment required for genomic stability.

3) Developmental Anomalies Research Team

To elucidate the mechanism of the effects of low dose high LET radiations on the development of mice as well as neural crest-derived cells, melanocytes at cellular level, pregnant females of C57BL/10J HIR mice at 9 days of gestation were whole-body irradiated with a single acute dose of argon ions. The effect was studied by scoring changes in the postnatal and prenatal development of mice as well as cutaneous coats 22 days after birth and in the melanocyte development in prenatal hair follicles. The percentage of births, the survival to day 22 and the body weight at day 22 were reduced in irradiated mice. By comparing the survival to day 22 for argon ions with that of γ-rays, argon ions were more than twice as effective as γ-rays. The frequency and the size of white spots (white haired skin devoid of melanoblasts and melanocytes) in the mid-
ventrum were increased in irradiated mice. Argon ions were more effective than \( \gamma \)-rays. In 18-day-old embryos, the frequency of abnormalities in the fore and hind legs, tails and eyes as well as of hemorrhage was increased as dose increased and the number of embryos and their body weight were decreased. In 18-day-old embryos, the development of hair follicles was also delayed as dose increased. These results suggest that argon ions seem to have a greater effect on postnatal and prenatal development of mice as well as on the melanocyte development than \( \gamma \)-rays.

4) Radioadaptive response research team

Radioadaptive response is a biodefensive response observed in a variety of mammalian cells and animals where exposure to low dose radiation induces resistance against the subsequent high dose radiation. The radioadaptive response implies that low dose radiation affects cells/individuals in a different manner from high dose radiation. Therefore elucidation of its mechanisms is important for risk estimation of low dose radiation. We investigated the molecular mechanisms for the radioadaptive response in terms of mutation at the HPRT gene locus using the human lymphoblastoid cells AHH-1. First we found that preexposure to the priming dose in the range from 0.02 Gy to 0.2 Gy significantly reduced mutation frequency at the HPRT gene locus after irradiation with 3 Gy of X rays, and that no significant adaptive response was observed with the priming dose of 0.005 Gy. Thus it was shown that the lower limit of the priming dose to induce radioadaptive response may be between 0.005 Gy and 0.02 Gy. Next, we examined the effect of 3-aminobenzamide (3AB), an inhibitor of poly (ADP-ribose) polymerase1, which has been reported to inhibit the radioadaptive response in terms of chromosome aberration. However significant radioadaptive responses in terms of mutation were observed even in the presence of 3AB, suggesting that molecular mechanisms of the radioadaptive response in terms of mutation may be different from that for radioadaptive responses in terms of chromosomal aberration. Alternatively we could not exclude the possibility that the differential effects of 3AB were due to cell type difference. Finally, by performing a comprehensive analysis of alterations in gene expression using HiCEP, we could identify 17 genes whose expressions were significantly altered 6h after irradiation with 0.02 Gy (Fig. 11). We also found 17 and 20 genes, the expressions of which were different with or without priming irradiations of 3 and 18 h, respectively, after challenge irradiation of 3 Gy. By analyzing the gene function, it was found that expression of genes involved in intracellular signaling and redox-regulation is correlatively altered, and therefore can be considered the molecular basis of radioadaptive responses.

Major Publications


Table 1 Number of transcripts whose expression levels were significantly altered in cells treated with priming dose (0.02 Gy) 6h before challenge irradiation.*

<table>
<thead>
<tr>
<th>Time of RNA sampling</th>
<th>Number of transcripts</th>
<th>Identified</th>
<th>Unidentified *</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Single</td>
<td>Multiple</td>
<td></td>
</tr>
<tr>
<td>immediately before challenge irradiation</td>
<td>17</td>
<td>44</td>
<td>3</td>
<td>64</td>
</tr>
<tr>
<td>6h after challenge irradiation</td>
<td>17</td>
<td>56</td>
<td>5</td>
<td>78</td>
</tr>
<tr>
<td>18h after challenge irradiation</td>
<td>20</td>
<td>72</td>
<td>8</td>
<td>100</td>
</tr>
</tbody>
</table>

* Gene expression was considered to be significantly altered when either one of analyses using equally divided RNA samples from 0.02 Gy-irradiated cells showed fold-change larger than 2 or less than 0.5.

** Transcripts, for which the gene was not uniquely identified.
5.4. Studies on Environmental Radiation Effects

Satoshi Yoshida, Ph. D.
Director, Environmental Radiation Effects Research Group

Outline of Research Career:
Education: 1983, Yokohama National University (BE in safety engineering); 1985, Tokyo Institute of Technology (ME in environmental chemistry); 1989, Tokyo Institute of Technology (Ph.D. in environmental chemistry)
Professional Activities: 1989-present, National Institute of Radiological Sciences
Research Interests: Radioecology, environmental chemistry, and ecotoxicology

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Objective
The recent rapid changes in energy production systems and life styles of people worldwide have made environmental radiation research even more important. In order to satisfy the needs for radiation safety and regulations, Environmental Radiation Effects Research Group investigates three subjects related to environmental radiation and radioactivity: 1) effects of radiation on organisms and ecosystems; 2) exposure of public to natural radiation; and 3) marine dynamics of important radionuclides. The group consists of five research teams: Terrestrial Radiation Ecotoxicology Research Team, Aquatic Radiation Ecotoxicology Research Team, Natural Radiation Exposure Research Team, Cosmic Radiation Exposure Research Team, and Marine Radioecology Research Team. The following describes the progress of each of these teams during FY 2007.

Progress of Research
1) Effects on organisms and ecosystems
While the importance of radiological protection of the environment based on scientific principles is increasingly recognized internationally as environmental issues garner more attention, the relevant scientific data are extremely limited. This group conducts studies to evaluate the effects of radiation on representative terrestrial and aquatic organisms as well as studies to estimate radiation dose on those environmental organisms. In addition, the group develops methods to evaluate the ecological effects of radiation using experimental model ecosystems containing various species.

a) Terrestrial Radiation Ecotoxicology Research Team
To understand the impact of radiation on terrestrial ecosystems, plants (particularly cedar tree), fungi, earthworms and springtails were selected and the dose-effect relationships for radiation have been studied. Recently, gene expression as a biomarker has been receiving increased attention in the ecotoxicological research field as it may produce fast, sensitive and diagnostic assays. Therefore, the study to detect radiation responsive genes was started as a new research direction. A novel technology, high-coverage expression profiling (HiCEP), developed by the researchers in NIRS, was applied in the springtail *Folsomia candida* (Collembola) in which the dose-effect relationships for radiation on survival, growth and reproduction were already estimated. A HiCEP analysis showed that several transcript-derived fragments (TDFs) were up-regulated by irradiation in *F. candida*. sequencencing the TDFs revealed that a few of them were similar to genes relating to DNA repair and response to oxidative stress. For the other TDFs, no similarity was found in the gene database, probably because of the limited length of TDFs or limited genome information in springtails. These findings suggest that HiCEP is effective at discovering both known and unknown TDFs, even in non-genomic model organisms such as *F. candida*. HiCEP was also applied to an established cell line derived from a cedar tree.

b) Aquatic Radiation Ecotoxicology Research Team
The studies on the radiation effects of aquatic ecosystems at various end points were carried out by using some selected organisms and ecosystems including experimental model ecosystems.
In order to evaluate ecological effects at the community-level, the multi-species microcosm consisting of eight identified microorganisms and bacteria was acutely irradiated with gamma rays at
100, 500, 1000 and 5000 Gy, and effects on populations were observed. Effects observed in the microcosm were not only direct effects but also indirect effects due to interspecies interactions. The results were analyzed using the ecological effect index (EEI), in which degrees of differences in the population densities between exposed and control microcosms were represented by the Euclidean distance function. A 50% effect dose for the microcosm (ED_{50}), at which the EEI became 50%, was evaluated to be 2000 Gy for gamma rays when the microcosm was exposed in the developing stage. The ED_{50} s evaluated for copper and 2,4,5-T (herbicide) were 0.57 mg/l and 49 mg/l, respectively.

Evaluation of radiation effects on soil bacteria by using conventional methods based on cultivation of isolated bacteria is difficult, since more than 90% of the bacteria existing in soil cannot be cultured on laboratory media. To overcome the drawbacks of these culture-dependent methods, molecular techniques have been widely used in the last decade. In our study, Denaturant Gradient Gel Electrophoresis (DGGE) based on the 16S rRNA gene sequence was applied to estimate the effect of ionizing radiation on soil bacterial community. Our results showed that chronic gamma irradiation at a dose rate of 1.2 Gy/d to a paddy soil system (total dose 6 Gy) changed the structure of bacterial community. Although it is thought that bacteria are less sensitive to radiation exposure, metabolic activities of soil bacteria would be sensitive. Enhancing knowledge of those radiation effects will aid in the development of environmental radiation protection at the ecosystem level.

2) Exposure to natural radiation

Since natural radioactive substances and cosmic radiation at high altitude contribute greatly to the radiation dose received by the general public, it is necessary to quantify the actual level of exposure and to document its features. The group therefore investigates the concentration and exposure doses of radon (\(^{222}\)Rn), thoron (\(^{220}\)Rn), and related radionuclides, mainly in areas with high natural radiation, and analyzes the results together with epidemiological data. The group also aims to collect scientific information on dose and effects of cosmic radiation in aircraft and to provide them in an intelligible way for the general public such as on the Internet.

a) Natural Radiation Exposure Research Team

A preliminary survey on indoor radon/thoron and external gamma ray dose rate was conducted for houses in Gejiu city/Toudaoshui village in Yunnan Province, China. Although several studies have been conducted to investigate the relationship between lung cancer risk and radon exposure for tin miners in this area, previous studies did not take note of the presence of thoron. Thus, discriminative measurements of radon/thoron and their progeny measurements were conducted in the present study. The measurements can be divided into short-term and long-term measurements. For the short-term measurement, a Japan-China joint research team investigated several houses using some active devices such as a pulse-ionization chamber (AlphaGUARD). For the long-term measurement, radon/thoron discriminative monitors (RADOPOT) were placed for about 50 houses. The monitors were retrieved after a few months’ exposure and radon/thoron concentrations were estimated. Deposition rate monitors for measuring thoron progeny concentration were also placed in 30 houses. Similarly, these monitors were retrieved after a few months’ exposure and EETC (Equilibrium Equivalent Thoron Concentration) was estimated.

The measurement results are summarized as follows: (1) radon concentration for 49 houses ranged from 32 to 498 Bq/m\(^3\) with an arithmetic mean of 136 Bq/m\(^3\); (2) thoron concentration for 49 houses ranged from 39 to 7,908 Bq/m\(^3\) with an arithmetic mean of 3,297 Bq/m\(^3\); (3) EETC for 29 houses ranged from 2.0 to 23.9 Bq/m\(^3\) with an arithmetic mean of 10.2 Bq/m\(^3\); (4) Equilibrium Equivalent Radon Concentration (EERC) for 6 houses ranged from 8 to 44 Bq/m\(^3\) with an arithmetic mean of 25 Bq/m\(^3\); and (5) gamma ray dose rate ranged from 0.09 to 0.17 \(\mu\)Sv/h with an arithmetic mean of 0.11 \(\mu\)Sv/h. Very high thoron concentrations were found in many houses in this area. Further dosimetric and epidemiological studies are needed to investigate the possible effects of radon and thoron.

b) Cosmic Radiation Exposure Research Team

More than 16 million Japanese people go abroad every year using aircraft and about 20 thousand people are members of aircraft crews in Japanese airline companies. At high altitude, they are exposed to enhanced cosmic radiation, and additional radiation dose can exceed 1 mSv per year. However, the situation and the health effects of cosmic radiation exposure are still uncertain. The team thus makes efforts to collect scientific information on dose and effects of cosmic radiation and also to provide them in an easy-to-understand way by the general public. Major tasks are (1) calculation of route doses (effective doses received in aircraft) using the most up-to-date method, (2) development of new detectors to verify calculation results, and (3) improvement of dosimetry system for radiological protection of
aircraft crew. Some results obtained by the team are open to the public from the NIRS web site "Japanese Internet System for Calculation of Aviation Route Doses (JISCARD) ". In 2007, an original simulation model which can determine precisely cosmic radiation intensities at aviation altitudes was developed in collaboration with the Japan Atomic Energy Agency. Using this new model, the global map of real-time dose rates at aviation altitudes were provided on the web for the general public. We also developed a neutron irradiation field of $^{241}$Am-Be for calibration of the detector assembly used for validation of the model simulation. We are cooperating with airline companies in Japan, regarding to education and radiation exposure management of aircraft crew members.

3) Marine dynamics of important radionuclides

Because many Japanese nuclear facilities are located in coastal areas facing the Pacific Ocean and the Japan Sea, it is very important to predict the environmental behavior, and thus the fate of radionuclides in marine ecosystems. The group focuses on the development of highly sensitive analytical methods for important radionuclides (e.g., plutonium, americium, iodine etc.) for which data are scarce, and provides data on their activities and isotopic ratios to understand their environmental behavior in marine ecosystems.

a) Marine Radioecology Research Team

A rapid and simple isotope dilution sector-field ICP-MS analytical method was developed in order to obtain precise $^{241}$Am concentration in marine sediment samples. The separation and purification of $^{241}$Am was achieved using a selective CaF$_2$ co-precipitation followed by a TRU extraction chromatography. For the first time, we achieved an extremely low detection limit (0.32 fg/g or 0.041 mBq/g) which is even better than that of alpha spectrometry. The major advantages of our method can be summarized as: rapid sample preparation (1-2 days), less waste generation, high precision, and excellent detection limit.

Surface seawater samples were collected from a site in the vicinity of the nuclear fuel reprocessing facility at Rokkasho, Japan and sites along the Japan Sea coast. $^{239+240}$Pu activities and $^{240}$Pu/$^{239}$Pu atom ratios were determined by $\alpha$-spectrometry and isotope-dilution sector-field ICP-MS. The atom ratios of $^{239}$Pu/$^{240}$Pu in coastal surface seawater, ranging from 0.221±0.019 to 0.235±0.023, were significantly higher than the mean global fallout ratio of 0.18. The contribution of the Pacific Proving Grounds (PPG) close-in fallout Pu was estimated to be 33% using a two end-member model. It was proposed that the oceanic currents accounted for delivery of close-in Pu from the PPG to the studied areas. $^{239}$Pu and $^{240}$Pu derived from the two sources of global fallout and close-in fallout were homogenized in the surface water of the Pacific coast and Japan Sea coast. Data on $^{240}$Pu/$^{239}$Pu atom ratios in seawater samples collected in the vicinity of the Rokkasho nuclear fuel reprocessing plant will provide useful keys for understanding the process controlling plutonium transport and for distinguishing potential sources of Pu.

Major Publications


5.5 Office of Biospheric Assessment for Waste Disposal

Outline of Research Career

Dr. S. Uchida received his doctoral degree from Kyoto University. He has about thirty years' experience in the fields of radioecology and environmental radiochemistry; his special interest is the behaviors of long-lived radionuclides in the environment, e.g., $^{63}\text{Ni}$, $^{79}\text{Se}$, $^{90}\text{Sr}$, $^{99m}\text{Tc}$, $^{131}\text{I}$, $^{137}\text{Cs}$, Th, U, etc. He has improved models and parameters for radionuclides in soil-to-crop systems. He has been proceeding with a project to collect and estimate environmental transfer parameters of radionuclides in relation to radioactive waste management.

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Objectives

The biospheric assessment of radiation dose to human beings related to the releases of long-lived radionuclides from underground nuclear waste disposal sites is very important for the peaceful use of atomic energy. For the assessment, radioecological transfer models and transfer parameters are needed. Environmental conditions, such as climate, vegetation, and soil, affect these parameters. Additionally, agricultural products and food customs in Japan differ from those in Europe and North America. Therefore, we should collect our own practical data in Japan using data from European and North American countries as references.

In this office, environmental transfer parameters, such as soil-to-crop transfer factors (TFs) and soil-soil solution distribution coefficients ($K_s$), have been collected from agricultural fields throughout Japan for 5 years. TF is defined as the ratio of activity concentration in plant to activity concentration in soil, while $K_s$ describes the behavior of radionuclides in the soil in terms of the ratio of concentrations in the soil solid and soil-solution phases. Analyses of stable isotopes and some natural radioisotopes in crops and their associated soils have been carried out in order to obtain TFs under equilibrium conditions, while radiotracer experiments have been applied for $K_s$s in various soils. Since rivers are one of the most important paths of radionuclide transfer from waste disposal sites to agricultural fields, chemical components of major Japanese rivers have also been determined and we published the data book entitled "Elemental Concentrations of Japanese Rivers" (NIRS-M-200). In addition, transfer models for predicting radionuclides' behavior in atmosphere-paddy soil-rice plant systems have been developed.

Progress of Research

1) Soil-Soil Solution Distribution Coefficients For Se, Sr, Sn, Sb, and Cs in Japanese Agricultural Soils

In agriculture, $K_s$ is particularly important for assessing potential crop uptake of various elements. The $K_s$ value depends on which radionuclide is being modeled, the soil group, and soil properties. In order to obtain practical $K_s$ values, it is necessary to obtain $K_s$ for each important radionuclide in many types of soils. In addition, this parameter should be used to determine which soil properties are important factors that determine the variation in $K_s$ for each radionuclide. Many previous studies have used $K_s$ values, but a few studies have collected sufficient $K_s$ data to permit a statistical analysis. In this study, therefore, $K_s$s for five radionuclides (Se-75, Sr-85, Sn-113, Sb-124, and Cs-137) were determined by batch sorption tests in 142 Japanese agricultural soil samples (63 paddy soil and 79 upland soil samples).

The results showed that Se- and Sb-$K_s$s data did not have normal or log-normal distributions, but Sr-, Sn-, and Cs-$K_s$s data had log-normal distributions (Fig. 12). Further, Se-, Sr-, and Cs-$K_d$s values differed between paddy and upland soil samples by t-test ($p < 0.05$). Spearman's rank correlation test was carried out to investigate correlations between $K_s$ values for each radionuclide and soil properties. Combinations of $K_s$ value and soil property having the highest correlation coefficient ($R_s$) for each radionuclide were as follows: Se-$K_s$ - concentration of water soluble P ($R_s = -0.51$); Sr-$K_s$ - concentration of water soluble Ca ($R_s = -0.57$); Sn-$K_s$ - concentration of water soluble Sr ($R_s = 0.57$); and Sb-$K_d$ - concentration of water soluble P ($R_s = -0.67$). Although there were no soil properties which had a good correlation with Cs-$K_s$ values for all soil samples, the best correlated soil property with Cs-$K_s$ values was concentration of water soluble ammonium.
ion (Rs = -0.48) for upland soil samples.

2) Soil-to-crop Transfer Factors of Radium in Japanese Agricultural Fields

Radium-226 ($^{226}\text{Ra}$), an alpha emitter with a half-life of about 1600 y, is a natural decay product of $^{235}\text{U}$. Radium-226 is of special interest because it is an important radionuclide for the assessment of radioactive waste disposal. This radionuclide can reach humans through several transfer paths in the environment. Once Ra is taken into the human body by ingestion of food and water or inhalation, it can distribute into bone where it has a long biological half-life; exposure to Ra can cause cancers and other body disorders. Therefore its long-term management is required and understanding of Ra behavior in the environment is important, especially its soil-to-crop transfer that directly affects the internal radiation dose assessment for the ingestion pathway. Although $^{226}\text{Ra}$ exists in the environment, due to its low concentration in crops, TFs that have been obtained from agricultural fields are limited. In many cases, therefore, TFs used in such models were from the technical report series 364 (TRS-364) compiled by IAEA. These data were obtained in temperate zones mainly from Europe and North America, and thus, the numbers of TFs for rice and crops native to Japan were limited. In this study, we determined the concentrations of $^{226}\text{Ra}$ in upland field crops (leafy vegetables, onion, potato, and so on) and associated soils collected from 45 locations throughout Japan in order to obtain TFs. We also measured alkaline earth metal concentrations to compare their behavior with Ra, which is the last member of this group and whose lighter members, Mg and Ca, are plant nutrients.

The results are summarized in Table 1. Concentrations of $^{226}\text{Ra}$ in the soils collected in southwestern Japan were higher than those in northeastern Japan; however, no correlations between $^{226}\text{Ra}$ concentrations in crops and soils were observed. The TFs ranged from $<1\times10^{-2}$ to $5.8\times10^{-2}$ with a geometric mean of $6.4\times10^{-2}$. These data were within the 95% confidential range of TF-Ra for several crops as reported in the IAEA TRS-364. Among the alkaline earth metals, TF-Ba was similar to TF-Ra.

3) Estimation of Anthropogenic Uranium Amount in 112 Japanese Agricultural Soil Samples due to Application of Phosphatic Fertilizers

Uranium (U) and thorium (Th) behavior in geological environments are relatively close to each other compared to their behaviors and those of other elements. Thus high relationships between their concentrations are usually observed in rocks, non-agricultural field soil samples and river sediments, etc. Indeed, in Japan the concentration ratios of U/Th in these environmental samples, were almost the same, being about 0.20-0.28. However, applications of phosphatic fertilizers to agricultural fields might increase their U concentration since the fertilizers are known to be high in U content (fertilizer U contents are 10-300 times higher than U contents in uncontaminated soils) but low in Th content. Thus the U/Th ratios in phosphatic fertilizers are significantly higher than the natural U/Th ratio.

In order to estimate the excess amount of U ($\text{U}_{\text{ess}}$) in agricultural fields, it is necessary to obtain native U concentrations in those fields. Natural U/Th ratio in non-agricultural fields would be useful to estimate content of Uess in agricultural fields. Concentrations of U and Th in soil are closely related to the original materials of the soil, but U is more mobile than Th is so that the natural U/Th ratio in non-agricultural fields is slightly lower than the parent rock. From the average composition of the Japan upper crust, the U/Th ratio is 0.28, but in non-agricultural fields, 0.22-0.25 ratios were observed.

In this study, we estimated Uess in Japanese agricultural fields due to phosphatic fertilizer application by using inductively coupled plasma mass spectrometry (ICP-MS) to measure concentrations of total U and Th in 112 agricultural soil samples (50 paddy field and 62 upland field soil samples). The samples were collected throughout Japan.

The average concentrations of total Th and U in the paddy field soil samples were 5.7 mg kg$^{-1}$ and 2.8 mg kg$^{-1}$, respectively, while those in the upland field soil samples were 2.6 mg kg$^{-1}$ and 5.5 mg kg$^{-1}$, respectively. These Th and U concentrations showed no differences between paddy field and upland field soil samples. Concentration ratios of U/Th in paddy field and upland field soils were 0.53 and 0.52 on average, respectively, which were much higher than those in Japanese non-agricultural fields (0.23). The results implied that phosphatic fertilizers, which have high U concentrations, increased the total U concentration in the agricultural fields. Thus, using the natural U/Th ratio in non-agricultural areas, we estimated the excess amount of U. About 52% of total U in paddy field soils (ca. 1.5 mg kg$^{-1}$ of U on average) and 50% of total U in upland field soils (ca. 1.3 mg kg$^{-1}$ of U on average) were calculated as excess amounts of U. Thus using of phosphatic fertilizers in agricultural fields makes only a small contribution as external radiation to the general population.

**Major publications**


Table 2: Concentrations of $^{226}$Ra in soil and crop samples on dry weight basis and transfer factors.

<table>
<thead>
<tr>
<th>Crop name</th>
<th>N*</th>
<th>Concentration range (Bq/kg-dry)</th>
<th>Soil Crop TF range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cabbage</td>
<td>5</td>
<td>20.5 - 39.0</td>
<td>0.11 - 0.59 (0.5 - 2.3) x 10^{-2}</td>
</tr>
<tr>
<td>Chinese cabbage</td>
<td>2</td>
<td>13.9 - 36.5</td>
<td>0.47 - 0.59 (1.3 - 4.3) x 10^{-2}</td>
</tr>
<tr>
<td>Lettuce</td>
<td>2</td>
<td>30.9 - 41.7</td>
<td>&lt;D. L. - 0.32 &lt;D. L. - 0.8 x 10^{-2}</td>
</tr>
<tr>
<td>Spinach</td>
<td>1</td>
<td>28.8</td>
<td>0.23</td>
</tr>
<tr>
<td>Carrot (leaves)</td>
<td>1</td>
<td>42.8</td>
<td>1.15</td>
</tr>
<tr>
<td>Japanese radish (leaves)</td>
<td>1</td>
<td>60.3</td>
<td>0.46</td>
</tr>
<tr>
<td>Leak (green part)</td>
<td>8</td>
<td>16.5 - 29.9</td>
<td>&lt;D. L. - 1.72 &lt;D. L. - 5.8 x 10^{-2}</td>
</tr>
<tr>
<td>Onion</td>
<td>3</td>
<td>26.1 - 43.9</td>
<td>&lt;D. L. - 0.15 &lt;D. L. - 0.3 x 10^{-2}</td>
</tr>
<tr>
<td>Japanese radish</td>
<td>3</td>
<td>33.7 - 60.3</td>
<td>0.22 - 0.52 (0.4 - 1.5) x 10^{-2}</td>
</tr>
<tr>
<td>Carrot</td>
<td>1</td>
<td>42.8</td>
<td>1.43</td>
</tr>
<tr>
<td>Potato</td>
<td>5</td>
<td>22.7 - 48.9</td>
<td>0.04 - 0.12 (0.1 - 0.3) x 10^{-2}</td>
</tr>
<tr>
<td>Sweet potato</td>
<td>2</td>
<td>16.0 - 28.5</td>
<td>0.04 - 0.05 (0.2 - 0.3) x 10^{-2}</td>
</tr>
<tr>
<td>Taro</td>
<td>2</td>
<td>39.3 - 45.5</td>
<td>&lt;D. L. - 0.11 &lt;D. L. - 0.2 x 10^{-2}</td>
</tr>
<tr>
<td>Cucumber</td>
<td>1</td>
<td>29.1</td>
<td>0.21</td>
</tr>
<tr>
<td>Sweet pepper</td>
<td>2</td>
<td>30.8 - 33.3</td>
<td>&lt;D. L. - 0.11 &lt;D. L. - 0.3 x 10^{-2}</td>
</tr>
<tr>
<td>Tomato</td>
<td>2</td>
<td>41.9 - 43.5</td>
<td>&lt;D. L. &lt;D. L. &lt;D. L.</td>
</tr>
<tr>
<td>Egg plant</td>
<td>2</td>
<td>19.6 - 41.8</td>
<td>&lt;D. L. - 0.09 &lt;D. L. - 0.2 x 10^{-2}</td>
</tr>
<tr>
<td>Soybean</td>
<td>1</td>
<td>30.0</td>
<td>0.10</td>
</tr>
<tr>
<td>Peanut</td>
<td>1</td>
<td>17.5</td>
<td>0.12</td>
</tr>
<tr>
<td>Wheat</td>
<td>3</td>
<td>24.9 - 41.5</td>
<td>0.06 - 0.17 (0.1 - 0.5) x 10^{-2}</td>
</tr>
<tr>
<td>Barley</td>
<td>2</td>
<td>31.9 - 34.3</td>
<td>&lt;D. L. - 0.05 &lt;D. L. - 0.2 x 10^{-2}</td>
</tr>
</tbody>
</table>

* N: Number of observations