グループ研究「先端科学技術がもたらす環境負荷とその生物影響の認知基準化に関する研究」

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Studies on the Comparative Evaluation on the Environmental Toxicants Released from Advanced Technology and Industry

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1. 概 括

近年の科学技術の発達に伴い、人類は遭遇したことのない種類や量の有害物質にさらされている。さらに、その暴露（被曝）様式は複雑化し、複合的な暴露の問題が顕著化してきている。本グループ研究は、平成５年より放射性核種、放射性物質の暴露、被曝の影響について、多様な観点から検討を行い、現在の放射線被曝者の健康状態を評価するための研究を行っている。

研究の目標は、放射線と放射性物質の影響を評価することである。放射線の影響は、放射線の強度や被ばく時間、被ばく部位によって異なるが、放射性物質の影響は、物質の種類や濃度、被ばく時間、被ばく部位によって異なる。

放射線の影響は、放射線の強度や被ばく時間、被ばく部位によって異なるが、放射性物質の影響は、物質の種類や濃度、被ばく時間、被ばく部位によって異なる。このため、放射線の影響や放射性物質の影響を正確に評価することが重要である。

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1. Summary

We are now continuously exposed to a variety of environmental toxicants that are released from developing technology and industry. The style of exposures become complex and the combined effect of toxicants is concerned. This research group was established in 1996 by researchers gathering from environmental, risk analytical and biological fields, in order to investigate the comparative risk evaluation of radiation and other environmental toxicants.

The main subject of this group was, therefore, "Standardization of the perception of environmental and biological effects of radiation and other environmental toxicants". In the first two years, we concentrated our efforts to develop necessary methods, and then remaining 3 years were used for the comparative studies of radiation (radioactive nuclides) and environmental toxicants (especially heavy metals and rare earth elements). For efficient operation, the group was divided into 4 subgroups: 1) subgroup for mathematical model development, 2) subgroup studying the movement and kinetics of toxicants, 3) subgroup for developing a artificially controlled ecosystem, and 4) subgroup for comparative toxicology. These subgroups maintained tight cooperation, and obtained excellent research outcome described as followings.

The first sub-group, methodology development section, tried to develop the theoretical and mathematical models which could estimate the behavior of environmental toxicants and their biological and environmental impacts. Two kinds of models were developed; the first is a health risk estimation model, and another is computer simulation model of experimental ecosystem. The first one, named HESANS (Health and Environment Safety Analysis Network System) is developed to estimate the health risk of radiation exposure to the population, and is now available through Internet as an interactive risk communication source. The second, named SIM-COSM, is also a computer model, and was developed to simulate the controlled microbial ecosystem (Microcosm), which was developed and used experimentally by the third sub-group. This model adopts the non-linear population dynamics of organisms in Microcosm, and well mimics the growth, foraging, maintenance, reproduction and death of microorganisms in the systems.

The second subgroup developed analytical methods for long-lived radionuclides and trace elements to obtain fundamental data on the levels and behavior of toxic substances in the environment, and established analytical methods using advanced instruments such as ICP-MS, ICP-AES, XRF and Ion-chromatography. This method allowed to analyze quantitatively more than 50 elements in variety of environmental samples (e.g. soil, plants, fungi, water) collected from different places in Japan. This subgroup also studied on the distribution and circulation of radioesium and stable cesium in the forest ecosystem, the $^{240}\text{Pu}/^{239}\text{Pu}$ atom ratios in several soil samples collected in Japan and other countries, and soil-to-plants transfer factors of Cs, Sr, I, Co, Zn, Mn and Ce for several agricultural crops using radiotracer method.

The third sub-group concentrated their effort to construct controlled experimental ecosystems applicable for evaluating the effects and risks of environmental toxicants in comparison with radiation. One of aquatic microbial ecosystems, Microcosm, was introduced and improved for this purpose. The microcosm consisting of flagellate algae as a producer, ciliate protozoa as a consumer, and bacteria as a decomposer, was established and used for evaluating the ecotoxicity of radiation (γ-rays) and some other toxicants (ultraviolet radiation, acidification and metal ions such as Gd$^{3+}$, Cu$^{2+}$, Mn$^{2+}$, Ni$^{2+}$ and Al$^{3+}$). The results indicate that Microcosm used here is a novel, unique and useful method for the comparison of toxicity to ecosystem.

The fourth subgroup carried out the studies on the effects of radiation and environmental toxicants with respect to the comparative toxicology. They found that environmental toxicants such as arsenite (dioxide of arsenic) interfere with the repair of DNA double-strand breaks induced by gamma irradiation. The toxicity of gadolinium (Gd) was also investigated, since this elements began using widely in the advanced industry but its toxicity was not well known. It was demonstrated that cytotoxicity of Gd was remarkably high in mouse alveolar macrophages than in rat alveolar macrophages, and that this species differences may be attributable to the solubility of Gd hydroxide in the phagolysosome. The administration of Gd also affects the metabolism of plutonium (Pu) in rat lung. Since the Gd is widely used as neutron absorber in the reprocessing of nuclear fuel, this finding is very important to estimate the combined effect of alpha radiation (Pu) and Gd.

As described above, this research group obtained a number of important findings with respect to the kinetics and effects of radioactive substances and environmental toxicant.

(Written by Group leader, Dr. Y. Nakamura)
Model for Risk Estimation of Environmental and Biological Impacts of Radiation and Toxic Agents

Yuji Nakamura, Masahiro Doi and Tetsuya Sakashita  (Methodology Development Section)

Abstract

The scope of this study is to develop the theoretical and mathematical models which could estimate the behavior of environmental toxicants and their biological and environmental impacts. To estimate the health risk of radiation exposure to the population, HESANS (Health and Environment Safety Analysis Network System) is developed by featuring updated risk projection models recommended by radiation protection authorities and national vital statistics. HESANS is open for the society through internet as an interactive risk communication source.

To know the mechanism of symbiotic ecology, controlled microbial ecosystem (Microcosm) is reproduced virtually in the computer as a Simulated Model Ecosystem (SIM-COSM). SIM-COSM is a kind of cell automata to regard each microbe as a "particle" wearing demographic parameters, and their living environment is divided by the lattice into the small square "patch". Particles show growth, foraging, maintenance, reproduction and death by changing their living environment and themselves interactivity. The non-linear population dynamics of the particles cause the demographic and environmental stochasticities, which are studied theoretically to know the universal index of ecological hazard to compare the social impacts of environmental toxicants mutually. Outcomes of these theoretical modeling and simulation are returned as a reference to the experimental Microcosm researches.

1. 研究の目的

放射性物質（放射線）による生物・ヒトへの影響評価研究は、線量の概念、定義や線量応答が比較的明瞭であり、低線量から高線量域にわたって多くの知見が蓄積されてきた。これら放射性物質（放射線）の環境生態・生物影響評価を基準として、化学物質等の放射線以外の環境有害物質の環境負荷および生物影響を包括する影響及びリスクを評価する数学モデルを構築、様々なエネルギー生産技術や科学技術のリスク（影響）の比較評価手法を開発することが本研究の目的である。

2. 研究の方法

(b) 基礎情報データベースの整備と環境移行・発がんリスク推定システムの開発

放射性物質（放射線）の環境生態・生物影響評価の基礎となる情報収集・解析として、軽水炉の運用に伴う重要な放射性物質の動態パラメータ、人口動態統計データ、日本人的臓器重量に関する統計データ、等をデータベース化した。

これなら基礎情報データベースは、インターネット接続コンピュータにより操作できる計算機システム（環境・健康影響評価ネットワークシステム）の適用に組み入れた。HESANSでは、わが国の軽水炉立地の安全評価指針に準拠した環境移行評価・住民等への放射線線や食品摂取等の経路での内部放射線被ばくによる線量評価モデルするジュール、国連科学委員会（UNSCEAR）、国際放射線防護委員会（ICRP）、米国原子力規制委員会（NRC）、米国科学アカデミー・電力放射線の健康影響に関する検討委員会（BEIR）等が提案しているリスク推定モデルにより、様々な条件（被ばく線量・線量率、被ばく形態、被ばく時年齢）における健康影響リスク（発がん・遺伝的影響等の確率的影響の誘導リスク）を推定するモジュールを開発した。

放射線被ばくによる健康影響を含むリスクを考慮したデータベース化した。

2-2 屋内ラドンによる肺がんリスク推定モデルの開発

公衆の放射線被ばく源として寄与の大きい屋内ラドンの健康影響については、上述の主要なリスク推定モデルでは、米国等のウラン坑夫の疫学調査（コホート研究）に基づいて屋内ラドン（Ru:222）の肺がん誘発リスクを推定している。しかし、坑道内作業環境と居住家内環境は大きく異なることから、スウェーデン国立放射線防護研究所とカロリスカ医科大学環境医学研究所が共同で実施した「屋内ラドンと肺がんに関する疫学調査（患者対照研究）」結果を基に、スウェーデン等の健康リスクと国内共同研究としてリスク推定モデルを開発した。1991年から1995年までのスウェーデン、日本の人口動態統計データを基礎データとしてデータベース化し、国別、時代別に実環境ラドンによる肺がん生涯リスクを比較解析した。
3. 成果の概要

3-1 健康環境影響評価ネットワーク・システム

健康環境影響評価ネットワーク・システム (Health and Environmental Safety Analysis Network System: HESANS) は、インターネットの web サイトを構築する一連の HTML ページで構成されている。インターネットに接続した端末コンピュータから、ホームページのアドレスを閲覧すると、JAVA によって各種計算条件等の入力を求められ、インタフェースの指示に沿って、設定を進めていくと、web サイトのサーバー側で計算処理が行われ、結果が、端末コンピュータに引き渡され、画面として出力される。

HESANS の環境移行・線量評価モデルは、緊急時被ばく評価、平常時被ばく評価、ライブラリ（核種、農作物、畜産物、家畜、家禽に関する各種パラメータ）のサブモデルで構成されている。平常時被ばく評価・線量評価サブモジュールの初期入力画面を Fig. 1-(a) に示す。放射性物質排出位置の検度数及、計算対象（大気中濃度、地表沈着濃度、内部被ばく線量（呼吸・食物）、外部被ばく線量（大気浮遊性・地表沈着））から選択できる。

気象条件、放射源条件、放射核種等の設定画面を Fig. 1-(b) に示す。放射源の地上高、後部内径、後部速度、近隣の高層建物の影響補正データ（建物高度、床面積）、放出核種の設定、風向、卓越風向頻度、風速、大気安定度、降水率、降水割合、大気混合層高度等、気象データが設定される。

被ばく計算の条件設定画面を Fig. 1-(c) に示す。個人線量又は集団線量の選択、風による地表核種の再浮遊、飼料作物への土壌の付着、食品調理による核種の除去、土壌からの核種の洗出による除去、等、計算サブモジュールを含めるかどうかの選択、ライブラリ（核種、農作物、畜産物、家畜、家禽に関する各種パラメータ）のカスタマイズを行うことができる。

(a) home page : selection of exposure pathway

(b) setting of meteorological parameters and nuclides

(c) setting of demographic parameters

(d) report of effective dose and organ dose

Fig. 1 Environmental transfer and dose estimation modules of HESANS
計算結果は、放射性物質の大気中濃度、地表の濃度について緑図に重ね合わせた分布図として表され、各被ばく経路（内部被ばく線量、外部被ばく線量）の累積線量算定結果については、一覧表として表示される。累積線量の出力例をFig.1-(d)に示す。

HESANSの健康影響評価モデルの初期設定画面をFig.2-(a)に示す。解析するリスクは、死亡（致死がん）リスク、発がんリスク（非致死がんを含む）、遺伝リスク、家屋内ラドン肺がんリスクから選択する。モデル計算のデータベースをここから求め、年齢別死亡率、人口統計（年齢別、癌症別）、肺がん死亡率（年齢別、国別（日本、スウェーデン、米国）、平均寿命（国別（日本、スウェーデン、米国））について、1961年から1992年について整備されている。

致死がんリスクを推定するサブモジュールの設定画面をFig.2-(b)に示す。リスク推定モデルは、(A)放射線影響研究所（相加・相乗）モデル、国連科学委員会1988年報告書（相加・相乗）モデル、国際放射線防護委員会1990年勧告（相加・相乗）モデル、米国原子力規制委員会（NUREG）モデル、国際放射線安全委員会の健康影響に関する検討委員会（BEIR-V）モデルのなかから選択できる。リスクを推定する対象個体の年齢構成（年別、被ばく年齢（被ばく年齢、被ばく期間）、リスク推定期間（生涯リスク、特定期間リスク）の選択、および線量・線量率効果係数(D0REF)が設定できるようにになっている。リスク推定結果は、年齢別過剰死亡率確率として、図と表で示されるようになっている（Fig.2-（c））。

遺伝的リスクは、国連科学委員会1988年報告書において示された倍加線量法により推定・評価することができる。その結果は、遺伝疾患毎に一覧表で表示されるようになっている（Fig.2-（d））。

3-2 屋内ラドンによる肺がんリスク推定モデル
スウェーデン家屋内ラドンと肺癌に関する疫学調査（患者対照研究）結果を基に、スウェーデンとの国際共同研究として肺がんリスク推定モデル（二種類）を開発した。1961年から1992年までのスウェーデン、日本の人口動態統計データから対象とする国と年代を選択し、ラドン濃度、平衡定数（通常は0.4）、居住係数（通常は0.8）を設定して、実環境ラドンによる肺癌がんリスクを推定できる。このモデルから単位ラドン等価線量（WLM）あたりの実効線量換算係数は2.05～13.5 mSvと推定され、従来の疫学的アプローチによる換算係数（3.88 mSv/WLM）と呼気気道モデルによる換算係数（15 mSv/WLM）間の違いを範囲の中に含む結果となった。

HESANSは研究者仕様であり、正確な結果を得るには、放射性物質の環境動態及び放射線健康影響に関する知識が必要であり、今後、一般の利用者を想定したユーザーサイアーティアスの開発が望まれる。
3-3 SIM-COSMによる制御微生物生態系個体群に対する放射線の効果解析

3種微生物で構成される制御微生物生態系（マイクロコズム）を基に、個体ベースモデリング手法を用いて、計算機上で生態系をシミュレーションするコード（SIM-COSM）の概念をFig.3に示す。各微生物個体毎の人口動態学的パラメータ、増殖・拡散モデル、採食行動モデル、各個体毎のエネルギーや取収支モデルより、SIM-COSMにおけるシミュレーション計算結果は、培養初期（50日以内）の各微生物種の個体群動態を追従し、生物群の種内・種間相互作用および生物群と環境との相互作用をあらわすに留るものとなった（Fig.4）。長期培養における生物群の種内・種間相互作用および生物群と環境との相互作用については、参照とする実験データが乏しく、今後の検討課題である。

γ線照射による制御微生物生態系（マイクロコズム）負荷のシミュレーションでは、各生物種の半数致死線量（細胞増殖死をもって寿命の指標とする）から、各生物種個体への直接影響（放射線にヒットした場合の致死）を確率論的な負荷として計算機モデルに与え、個体数の一時的で急激な変化後の個体群動態を推定した。テトラヒメナの半数致死線量4000 Gy、エゴレナ330-170 Gy、大腸菌50 Gy（37℃生存時）であることから、大腸菌の生存曲線で校正した致死的負荷（100 Gy、5000 Gy相当）を培養30日後に付与するシナリオを想定し、SIM-COSMにおいて一時被ばくによる各生物種の個体群動態を観察した。100 Gyと5000 Gyγ線照射マイクロコズム実験と、SIM-COSMによるγ線荷負シミュレーション実験の結果との比較をFig.5-(a)及びFig.5-(b)に示す。Fig.5-(b)では、大腸菌を捕食する従属栄養原生動物であるはずのテトラヒメナが、γ線による大腸菌死滅後にも200日以上生存する実験結果が示されており、SIM-COSMによる予測（被ばく後40日程度で死滅）と大きく異なっている。テトラヒメナが頑

Fig.4 Population dynamics of Microcosm (Fuma et al.) and projection by SIM-COSM

(a) γ-exposure: 100 Gy at 30 days after inoculation

(b) γ-exposure: 500 Gy at 30 days after inoculation

Fig.5 Dose response of simulated microbes by SIM-COSM (○Tetrahymena, ■Euglena, △E.coli) and real microbes in Microcosm by Fuma et. al (○Tetrahymena, □Euglena, ▲E.coli)
境の急激な変化、特に大腸菌個体数の一時的減少 
（Fig. 5(a)）、又は絶滅（Fig. 5(b)）に対応して、何らかの適応をしていることが示唆される。具体的には、従来
は餌として利用しない死骸分解有機物代謝産物等の捕
食や、摂取エネルギー量との取支で代謝エネルギー量
（maintenance cost）を生命維持に必要な最低限まで切り
詰める、等の調整機構の存在が推定される。環境負荷因子
に対する生態系システムとしての適応的なすがおるいとそ
の機構を明らかにするため、様々な計算条件においてシミ
ュレーションを繰り返し、比較解析していくことが、今後
の検討課題である。
放射線照射や他の環境有害物質の照射が、各生物種個
体にとって致命的に作用しない低線量（用量）域の照射であ
っても、生理学的・人口動態学的な因子に影響を及ぼすこ
とも考えられる。特に、この微生物生態系では、唯一の独
立栄養源生物であるユーレナの光合成によって光エ
ネルギーが利用されるため、この光合成の能率が放射線等
の環境有害因子によって阻害（又は亢進）される場合には、
生態系全体のエネルギーの流れ（フロー）に影響を与えるこ
とが予想される。
このような観点から、脂質分析・安定同位体比分析等を
用いて、ユーレナの光合成活性への放射線等環境有害物
質の负荷に関する実験研究を実施し、線量（用量）効果関
係を考察した（液流促進研究、平成 10 年度）。環境
負荷因子が生態系のバランスを乱す因子の一つとし
て、今後、計算モデルへの組み込みを検討している。

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第2サブグループ：微量成分の環境挙動解析に関する研究

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Analysis of the distribution and behavior of toxic and of tracer elements in the environment

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Abstract

In order to assess the levels and behavior of toxic substances in the environment, analytical methods for long-lived radionuclides and trace elements have been developed. Special attention is paid to the elements such as Pu, U, Th, Tc, Cs, Sr, I, Co, Cr, Cu, Mn, Zn and lanthanoid. Analytical methods mainly used were ICP-MS, ICP-AES, XRF and ion-chromatography. Several data for these elements (nuclides) in variety of environmental samples (e.g. soil, plants, fungi, water) have been obtained. For soil, about 50 elements were determined in ca. 100 samples collected from different places in Japan and useful information on the distribution of many elements in Japanese soils were obtained. High iodine concentrations are found in upland soils, particularly in Andosol, while concentrations in lowland soils were considerably lower. Results on the distribution of radium and stable cesium indicate that $^{131}$Cs entered into the forest ecosystem is circulating. New data on the $^{238}$Pu/$^{239}$Pu atom ratios in several soil samples collected in Japan and other countries have been obtained. It was found that the ratios could be used in identifying the source of contamination. Radiotracer techniques were also applied to study the transfer of radionuclides in the environment. Soil-to-plants transfer factors of Cs, Sr, I, Co, Zn, Mn and Ce were investigated for several agricultural crops usually consumed in Japan.

1. 研究目的

第2サブグループにおいては、エネルギー産業等に由来する微量成分である放射性物質、重金属元素、レアメタル等の環境中での分布と挙動並びに人への移行を予測するための生物地球化学的解析を実施した。特に、最新の高感度分析法を用い、環境構成物質（土壤、植物、水、菌類、動物等）中に含まれる種々の放射性及び安定元素の濃度と分布を調べることに重点を置いた。

初期に立てた目標は次の通りである。
(a)環境試料中に含まれる種々の放射性及び安定元素（微量元素）の高感度で精度良い分析法を確立する。
(b)各種の生態系を構成する物質（様々な土壌、植物、水、菌類、動物等）については種々の放射性及び微量元素の分析を行い、各系及びそれによる構成する物質がもつ元素組成の特徴を明らかにする。
(c)環境（主として農耕地並びに森林）における放射性及び安定元素について移行とそのメカニズムを明らかにする。

2. 研究成果

本グループ研究が始まる以前、我々は環境放射生態学研究における放射性核種を対象とした研究を行っていた。放射性核種の分析法については、従来の古典的な方法に頼っている面が多くかたいが、検出感度が悪く、測定時間もかかっていた。また、分析対象は農作物中心を置き、主として放射性核種の移行を調べていた。しかし、本研究グループにおいては、単に放射性核種だけでなく、多くの安定元素（有害元素も含む）も分析し、総合的に研究を進めた。多元素を対象としたのは以下の理由からである。
(1) 放射性核種も元素の一つであり、その環境移行はそれぞれの元素の物理・化学的特性に従っていることから、様々な元素と比較することで環境挙動をより正確に解明できる。
(2) 放射性核種の環境移行は共存する安定元（同位体）の量に左右されるので、安定元素の濃度を把握しておく事は不可欠である。
(3) 放射性核種の環境中での濃度は非常に低く直接分析は難しい為、安定元素を調べる事により、予想していなかった放射性核種が環境中へ放出された場合でもその挙動予測に役立つと考えられる。

本グループ研究を通し、今までは限られた種類の放射性核種だけしかデータが得られなかったが、多元素を対象にする事により、環境挙動を解明する上で科学的な裏付けもでき、研究の幅も広がった。また、ICP-MS（誘導結合プラズマ質量分析法）を積極的に用いる事により、今まで正確な分析が難しかった、$^{238}$Pu/$^{239}$Pu 比、$^{232}$Th/$^{238}$U 比などの分析法（同位体分析も含める）を確立でき、環境中におけるデータを出す事が可能になった。さらに、対象を農耕地だけでなく、我が国の国土の広域を占める森林にも広げられた為、異なる生態系においてデータを比較し、解析する事ができた。特に、日本の各地から採取した代表的な土壌について、約50種類の元素（U, Th など天然放射性元素も含む）の分析を行い、今までに無かったデータをだすことができた。

このように、測定が難しかった核種の分析手法を確立し、
多くの元素も含めた研究を実施したことから、海外の研究者からも注目され、来賓者も多く研究協力も積極的に進めた。また、原子基盤研究や放射能調査研究とすることが部分的に関連をもたせ研究を実施した。更に、1997年に行なった動燃事故の研究においては、クラクフォースのメンバーとして住民への相談や環境のモニタリングとして協力した。そして、1999年のICO事故時には3人の被ばく患者の放射能測定・録音評価や周辺環境のモニタリング等で協力した。

本グループ研究や関連する研究テーマで、第2サブグループの関係した5年間の論文数は、原発論文数で50報報増近くあり（ほぼ全部が欧文）、その他（報文集、解説、著書、報告書など）も数多く出すことができた。

以下に、これらに関連して、本グループ研究を通じて得られた研究成果を述べる。

2.1 安定元素及び放射性物質の高感度分析法の確立

ICP-MS（高周波誘導結合プラズマ質量分析法）は、最近数多く進化した多元素同時分析法と言えるであろう。特に検出感度が優れて1ppb（1pg/ml）以下の測定も可能となった。我々はグループ研究に入ると事前からこの装置（四重極型）を環境試料の分析に本格的に応用はじまった。グループ研究においてはICP-MSの他に、ICP-AES（高周波誘導結合プラズマ発光分析法）、イオンクロマトグラフ分析法、蛍光エッセス線分析法、ファントム線スペクトロメトリー等を用いた分析法を検討した。ここでは、主としてICP-MSに関する分析法に関する研究成果をまとめた。

2.1.1 多元素分析法

ICP-MSは元素半導体産業などで用いられるため、ここではまず環境試料の応用、つまり環境試料中の多元素のICP-MSを用いた分析法を検討した。ICP-MSで測定するためには、水試料を除き、まず試料を溶出しなければならない。また、共存元素の影響を測定時での感度の変化（ドリフトなど）についても、試料の種類ごとに調べる必要がある。

水試料については、0.22 μmのメンプランろ過紙で漉過ごした後、硝酸（高純度硝酸、摩耗化学 AA-100）を加え、3％溶液とした。長期保存するときは5％程度の硝酸溶液にしたほうが目的性の濃度変化は少ない。固体試料については、粉末状態にした試料（約100mg）をテフロン容器に入れ、硝酸、ふっ化水素酸、過塩素酸を加え、マイクロウェーブオーブンで加熱溶解した。酸の量は試料の種類により異なり、例えば土壌試料では、硝酸（7ml）、ふっ化水素酸（3ml）、過塩素酸（0.5ml）である。また、植物試料では、まず硝酸（7ml）とふっ化水素酸（1ml）を加えマイクロウェーブオーブンで加熱溶解した後に過塩素酸（0.5ml）を加えた。それをホットプレート（ホットブロック）上で蒸発乾固した。次に、硝酸に溶かし、さらに希釈した。硝酸の濃度は3％とし、定容した。測定時のドリフトなどを補正するための内部標準としてBi、In、Rh（20ppb）を添加した。

測定には四重極型ICP-MS(Yokogawa PMS-2000)を用い

た。検出下限値は、多くの元素で溶出中の値として1ppb（1pg/ml）以下であった。この値は土壌中のこれらの元素を分析するのに十分な感度である。地質調査所が作成したJB-1、JG-1、JR-2、JA-2等の比較標準物質を使用した分析の質を確かめたところ、保証値と大変良い一致を示した。

2.1.2 U, Thの分析法

自然界に存在するUの同位体は238U（半減期：4.5×10^10年、存在比：99.2745％）、232U（半減期：7.04×10^8年、存在比：0.720％）、234U（半減期：2.45×10^6年、存在比：0.0055％）である。また、Thの自然界に存在する同位体は232Th（半減期：1.405×10^10年、存在比：100％）である。

UとThの分析法には、アルファ線測定法、核磁気のガッケ線状測定方法、放射化分析法、比色法などがある。しかし、前処理法が煩雑であり、検出感度に問題があったので、低濃度の分析は簡単ではない。我々はICP-MS（誘導結合プラズマ質量分析装置）を用いる環境試料の分析法について検討し、感度及び精度に優れた分析手法を確立した。ここでは、分析操作の概要を述べる。

土壌や植物試料については、酸で分解して溶液にして分析する必要がある。そこで、上で述べた安定元素（多元素）分析の方法と同様に、粉末試料をテフロン容器に入れ、硝酸、ふっ化水素酸、過塩素酸を加え、マイクロウェーブオーブンで加熱溶解した。それを、蒸発乾固した後、硝酸に溶かし試料溶液とした（硝酸の濃度は通常2.5％、内部標準としてU、Thとの質量数が近いSiを添加した。）

測定に主として四重極型ICP-MS（Yokogawa PMS-2000、Agilent 7500）を用いたが、重り束型ICP-MS（Finnigan MAT ELEMENT）も使用した。検出下限値はU、Thで溶液中の値として1ppb（1pg/ml）以下であった。この値は土壌や植物中のこれらの元素を分析するのに十分な感度である。地質調査所が作成したJB-1、JG-1、JR-2、JA-2等の比較標準物質を使用した分析の質を確かめたところ、保証値と大変良い一致を示した。

ウランの3つの同位体組成（235U、234U、238U）を正

に測定するためには、上記のように全分解した試料溶液をそのまま測定にかかることのではなく、化学操作を行いUを分離して測定することが不可欠である。通常、その化学分離操作は煩雑で時間かかる。そこで、抽出クロマトグラフ樹脂であるTEVAレジンを用いたウラン分離濃縮法を検討した。試料溶液を6M塩酸で調整した後、TEVAレジン（ミニアカラム充填）に通しUを吸着させた。6M塩酸溶液によりレジンを洗浄して共存多元素を除去した後、Uを0.1M硝酸で溶出させた。溶出として用いた0.1M硝酸溶液30mL中にはほぼ100％のウランが吸収できることがわかった。このとき、ほとんどの共存多元素もTh等は試料通水中及び洗浄液中に流出しておりUのフラクショ

ンに流入しなかった。溶出液中のウラン同位体比はICP-MSで求めた。本実験では、溶出液中のウラン濃度は10-70ppbの範囲であったが、このときUについても十分カウントを得ることができた。また、本文を読むウランの定量及び同位体比の測定結果は、アルファ線スペクトロメトリーの結果とよく一致していた。

10-10
2.1.3 Puの分析法

プルトニウムの同位体は質量数 232～246 まで知られており、その中でも環境汚染を考える上で重要なものは、$^{238}$Pu（半減期：87.7 年）、$^{239}$Pu（2400 年）、$^{240}$Pu（6570 年）と $^{241}$Pu（14.4 年）である。特に $^{239}$Pu と $^{240}$Pu は存在量が多いいため注目されている。

環境中における Pu の濃度と動態を調べることは、原子燃料サイクルに係わる環境安全評価を行う上で大変重要と考えられる。Pu の分析は主として、アルファスペクトロメトリー法により行われているが、環境汚染は非常に低く、分析操作に時間と手間がかかる。また、スペクトロメトリー法では、$^{239}$Pu と Pu ノウルが近いため分離測定は難しい。ICP-MS は通常安定元素の分析に用いられているが、検出感度が富んでいることから、長半減期核種の分析に適していると考えられている。しかし、プルトニウム中の ICP-MS を応用した例は少なく、化学分離法についてもあまり述べられていないので、分離濃縮法と測定法を検討した。また、環境関連の比較標準物質中のプルトニウム同位体に関するデータは殆ど出されていないので、IAEA の比較標準物質中の分析データの $^{239}$Pu の分析も行った。

サンプル量は、プルトニウム濃度が比較的高い試料（$^{239}$Pu 濃度 10Bq/kg 以上）では 1～10g とし、ビーカリーに 8N 硝酸（5 倍以上）を加え、スパイラルして既知量の $^{239}$Pu を添加した。また、プルトニウム濃度が低い試料（一般の環境試料）は 10g～50g と多くのサンプルが必要であったため、予め 500℃で加熱し、有機物を分解した。硝酸による抽出はホットプレート上で 4～6 時間行う。それを温かいうわりガラス濁紙で通過した。この抽出操作を 2 回ほど繰り返し、濁液を一つのビーカーにまとめ、それを蒸発させまるまでホットプレート上で加熱し、その後、硝酸に溶かし分離操作を行った。

プルトニウムの化学分離は、イオン交換樹脂（Dowex 1×8）を用いる方法と、抽出クロマトグラフ樹脂（TEVA）を用いる方法の 2 つを比較した。Dowex 1×8 による分離では蒸発を抽出に R2 の硝酸を加えて溶かした。また、TEVA による分離では 2N の硝酸に溶かした。どちらの樹脂を用いる場合でも Pu の化学形を 4 個にそろえなければならなさ、そこで、NaNO₃、HONH₄Cl 並びに $\text{H}_2\text{O}_2$ の 3 種の試薬を用いて比放射能等を比較検討した。

化学形を 4 個に調整した後、試料溶液を Dowex 1×8 または TEVA に通すことにによる Pu は樹脂に保持される。同濃度の酸を流して洗浄の後、Dowex 1×8 の場合は 5% NH₄I 10M 塩酸溶液、TEVA の場合は 0.1M ヘリドロロン溶液 9M 塩酸溶液を用いて保持された Pu を溶離した。溶離液をホットプレート上で一旦蒸発乾固した後硝酸に溶かし、最終的には 4N 硝酸溶液として、測定に供した。ICP-MS によって測定したピークは $^{239}$Pu、$^{240}$Pu 及び $^{241}$Pu である。また、必要に応じて、Fe などのマトリックス元素の濃度も測った。ICP-MS による測定では、質量数 239 のところに $^{239}$U が妨害を及ぼす能性がある。このため $^{239}$Pu を測定して放射性係数を調べ、内部標準としては Bi を添加したが、最終的な濃度計算は既知量加えた $^{240}$Pu に対する $^{239}$Pu と $^{240}$Pu の比を用いる同位体希釈法で行った。また、測定値の信頼性は Pu の同位体比のスタンダードである NBS-947 を測定し確認した。

なお、Pu は核燃料物質でありたとえば数量を使用する場合でも規制を受ける。そのため、原子燃料の使用許可を取った。

何種類かの試薬（NaNO₃、HONH₄Cl 並びに $\text{H}_2\text{O}_2$）を用いる Pu の化学形を 4 個に調整する条件を検討したところ、NaNO₃ を用いた場合が Dowex 1×8 と TEVA もとても良い結果が得られた。$\text{H}_2\text{O}_2$ を用いた場合が収率が高く化学形を 4 個にするのに十分でないと考えられた。

U の検定係数は Dowex 1×8 でも TEVA を用いた場合でもともに $10^{4}$～$10^{5}$ 程度であった。また、多くのマトリックス元素において検出係数は $10^{4}$～$10^{5}$ 程度であり、ICP-MS の測定に適する範囲まで下げることが可能。今回分析に供した土壌では、U については Dowex 1×8 のほうが良いが、また、希土類元素や鉄の分離には TEVA のほうが良い傾向にあった。

本分析法による $^{239}$Pu の検出下限値、試料溶液中の濃度として、0.05 mBq/ml（または 0.02 ppb 程度）であった。実際の土壌では、試料 20g を用いた場合、$^{239}$Pu では 0.1Bq/kg（乾燥）程度まで測定可能であった。

$^{239}$Pu の検出下限値は、試料溶液中の濃度として 0.17 mBq/ml、20g の試料を用いた場合は 0.35Bq/kg（乾燥）であった。$^{239}$Pu と $^{240}$Pu の検出下限値に差があるのは、$^{239}$Pu の方が半減期が短く放射能濃度が高くなるためである。$^{239}$Pu の測定に及ぼす影響は、溶液中の U 濃度が 1 ppb のとき $^{239}$Pu のピークへの影響は約 0.03 ppb に相当する値であった。これは、検出下限値とはほぼ同程度であり、測定には影響しない。化学分離操作により測定溶液中の U 濃度を 1 ppb 以下にするのは通常の試料ではそれほど難しくはない。

IAEA が作成した種々の比較標準試料を分析した結果、推奨値として示されている $^{240}$Pu の値と良い一致を示した。分析例をあげると（カッコ内は IAEA の推奨値）、IAEA-Sol-6: 1.0 Bq/kg (1.0 Bq/kg)；IAEA-135: 211 Bq/kg (213 Bq/kg)；IAEA-367: 39.5 Bq/kg (38 Bq/kg)；IAEA-368: 29.8 Bq/kg (31 Bq/kg)であった。Pu の同位体比（$^{239}$Pu/$^{240}$Pu 原子比）について得られた値は、アメリカ海の堆積物（IAEA-135: 0.21, アメリカ海の魚（IAEA-134: 0.20, マーシャル諸島の堆積物（IAEA-367）: 0.31, ムルワ環礁の堆積物（IAEA-368）: 0.04、オーストリアの土壌 (IAEA-SOIL-6): 0.19 であった。比較標準試料中の $^{239}$Pu/$^{240}$Pu 比に関する IAEA の推奨値は示されていないことから、これらの値は今後分析を行う上での一つの参考値として役立つと思われる。

今回の研究結果から、環境中の Pu の分析に ICP-MS 法は大変有効と考えられ、今後、環境安全研究やモニタリングへの応用も期待できる。また、$^{239}$Pu $^{240}$Pu 比も測定できることから、Pu の汚染源の同定などにも利用可能である。

2.1.4 Teの分析法

$^{90}$Te（化学的半減期：21 年万）は核分裂生成物の一つであり、環境中には核実験のフォーラムアウトを通じて加わる。また、原子燃料サイクルを考慮した場合、重要な
核種の一つとみなされている。しかし、分析法が難しく、環境でのデータは限られたものしか得られていなかった。そこで、最初に、ICP-MS 法による分析法の開発を行うとともに、ここで、Tc の環境標準物質について検討した。すなわち、Tc の環境標準物質がないことから、Tc の濃度レベルが低い一般の環境試料を定量した場合、その値の妥当性を比較することができなかった。そこで、環境標準物質として利用可能な IAEA 比較標準物質をいくつか選択し、それらの Tc の定量を行い、Tc の環境標準物質としての利用の可能性について検討した。

開発した分析法を Fig. 1 に示す。概要は以下の通りである。試料をビーカーに分離し、トレーサーとして 89Tc（半減期 61 日）を添加した。次に試料を灰化（450℃、24 時）し、4 M 硝酸によって Tc を加熱抽出した（90℃、3 時間）。漏過後、濾液は硝酸濃度で 0.1 M になるように希釈調整し、約 200 mL の溶液とした。この溶液を TEVA・Spec レジンを充填したミクロンカルム（Eichrom 社製）に通し、Tc の分離を行った。このとき、Ru を含むほとんどの共存元素はレジンを通過するが、Tc はレジンに強く吸着する。カラムを 2 M 硝酸溶液で洗浄後、5 mL の 8 M 硝酸溶液により Tc を溶液化した。溶離液は 80℃以下で一時封緘し、さらに ICP-MS（ICP-MS）により Tc の定量を行った。

本分析法による平均回収率は 71±18%であり満足のいくものであった。標準試料の測定結果は(A) IAEA-373（チェルノブイリの牧草地）：0.86±0.07 Bq/kg、(B) IAEA-375（チェルノブイリの土壌）：0.25±0.02 Bq/kg、(C) IAEA-135（アリッシュ海堆積土）：9.6±0.5 Bq/kg であった。これまでに測定した日本の水田土壌中における Tc 濃度は 0.02 - 0.11 Bq/kg であったことから、これらの環境標準物質中の Tc 濃度は一般的な環境中での Tc 濃度レベルよりも高く、チェルノブイリ事故やセラフィールド施設の影響を受けていることが示唆された。以上の結果から、これらの物質が Tc の濃度も充分であり均一性も良いため、Tc の分析のための比較標準物質として使用可能であることが示唆された。

また、本分析法を用いて、日本各地から 10 点の水田土壌採取後、フォーチャート Tc 濃度を測定した。結果、Tc 濃度は 6-1100Bq/kg 乾土であった。この値を核分裂収率が同程度である 137Cs の放射能濃度と比較すると、核分裂収率から得られる放射能濃度比、Tc/137Cs は 2.16×10^-4 であり、今回測定した土壌試料では 10^3 のオーダーであった。137Cs が土壌に吸着されやすい核種であることを考慮すると、得られた結果は、水田に降下・沈着したフォーチャート Tc がほとんど表層土壌に蓄積されていることを示している。

2.2 生態系を構成する様々な物質における元素分布
2.2.1 土壌
土壌は環境の中で大変重要な働きを担っている。植物は土壌から養分を吸収、降水は土壌で浄化され地下へと流れていく。土壌中の元素の濃度に関しては、今まで主として土壌肥学や植物栄養学の観点から研究されてきた。そのため、植物の生長に係わる様々な N, P, K, Fe, Ca, Mn, Zn などが中心となり微量元素に関するデータは限られている。そこで我々は ICP-MS 及び ICP-AES（発光光分析法）を用いた自然的な土壌中の様々な元素を分析し、50 以上の元素についての分析結果が得られた。

Fig. 2 に本研究で得られた約 100 試料の土壌中元素の平均値が原子番号順にプロットした。（主要元素である Si または S はこの方法では測定されないので除いたが、C と N は他の方法で測定した。）この図より、土壌中で 1 % (1000ppm) 程度またはそれ以上存在する元素は、Al, Fe, C, K, Ca, Mg, Na (Si) であった。原子番号が Sequential な要素は、2 位の番号の方が奇数番号よりもも濃度が高い傾向が見られた。土壌の種類によっても濃度に差がある。例えば、U, Th, 腐希土類元素（La, Ce 等）などは西日本に特徴的な黄色土に高い。これは黄色土は花崗岩を母材としており、その化学的特性を反映しているためである。黒土域では V, Cr, Zn, Lu, Yb, Mo, Mn, Cs, Ba, Ti, Th, が低い傾向にある。灰色低地土の元素濃度は多くの元素で全体の平均値とほぼ同じか少し高めであった。

土壌は地域の岩石や堆積物が母材となり、それが風化作用を受け、さらに生物の作用により有機物が加わり生成したものである。そこで、我々が求めた土壌の化学組成を地域の平均的な化学組成と比較してみると、土壌は Na, Mg, K, Ca, Rb, Sr の濃度が低い。これらの元素は風化作用により溶け出していたものと考えられる。一方、Ti, Mn, Zn, Cd, Ta, Pb などは逆に土壌の方が少し高めであり、これらの元素は風化作用を受けてもそれほど減少しないと推測される。

Fig. 1 Analytical method developed in the present study
Table 1. Analytical results on the $^{241}$Pu/$^{239}$Pu atom ratio and $^{239}$-240Pu concentration in Japanese soils

<table>
<thead>
<tr>
<th>Location</th>
<th>Soil type</th>
<th>$^{241}$Pu/$^{239}$Pu (Atom ratio)</th>
<th>$^{239}$-240Pu (Bq/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Hiraizuka (Ibaraki)</td>
<td>Rice paddy</td>
<td>0.171 ± 0.003</td>
<td>0.39</td>
</tr>
<tr>
<td>2. Ohmagari (Akita)</td>
<td>Forest</td>
<td>0.168 ± 0.006</td>
<td>1.4</td>
</tr>
<tr>
<td>3. Rokkashomura (Aomori)</td>
<td>Forest</td>
<td>0.170 ± 0.010</td>
<td>4.2</td>
</tr>
<tr>
<td>4. Rokkashomura (Aomori)</td>
<td>Field</td>
<td>0.168 ± 0.008</td>
<td>0.43</td>
</tr>
<tr>
<td>5. Joetsu (Niigata)</td>
<td>Rice paddy</td>
<td>0.181 ± 0.006</td>
<td>0.64</td>
</tr>
<tr>
<td>6. Ohtoyocho (Kochi)</td>
<td>Forest</td>
<td>0.179 ± 0.005</td>
<td>1.8</td>
</tr>
<tr>
<td>7. Toyotomicho (Hokkaido)</td>
<td>Moor</td>
<td>0.176 ± 0.005</td>
<td>1.5</td>
</tr>
<tr>
<td>Mean value (7)</td>
<td></td>
<td>0.173 ± 0.001</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Fig. 2. Elemental concentrations in Japanese soils (average of ca. 100 samples collected in Japan)

地質と比べて土壌に特異的に濃縮しているのは、C, N, I の 3 元素であり、それらの濃縮率は 100 倍以上である。C と N については植物などの有機物が土壌に加わったためである。

I は、もとより地質を構成する岩石には極微量しか含まれておらず、海水から自動的に大気中に放出されたヨウ素が、乾燥沈着あるいは雨に溶け湿性沈着した結果、土壌中の濃度が高くなったと考えられる。海水からの揮散は海洋微生物の役割により有機ヨウ素（ヨウ化メチル）が生成されることにより起こると考えられている。これが紫外線による分解され無機ヨウ素となり、雨水に溶け土壌入り。I (ヨウ化物イオン) と IO_3^- (ヨウ素酸イオン) は共に土壌に吸着しやすい。特に、亜礫土への吸着は高く、I 濃度も高い。I 程度ではないが、As と Sb も土壌の方が数倍高い値であった、これが水質の影響であるのか分からないと思われる。

次の元素間の相関を見る。U, Th, La, Ce, Yb, Lu についての関係を調べたところ、La と Ce 及び Yb と Lu の間、つまり隣り合った希土類元素の間には非常に高い相関が見られた。しかしこれに希土類元素群（例えば La と Lu）では相関はそれほど明らかではなかった。U と Th の相関はある程度みられた。Th と Ce (La) は比較的良い相関を示した。これも Th と軽希土元素はイオン半径が互いに似ていることにより起因している。

Pu の分析データはある程度出されているものの、まだデータが不十分である。特に、同位体組成（$^{240}$Pu/$^{239}$Pu 比）に関しては極限されたものしかなかった。今回は得られた土壤の Pu 濃度及び $^{239}$Pu/$^{240}$Pu 原子比の分析例を Table 1 に示す。濃度的には、農地に比べて森林土壤の方が高い傾向にあった。我々が測定した日本中部の土壤での $^{239}$Pu/$^{238}$Pu 比は约 0.17 程度（長崎の試料を除く）であり、Krey が報告しているグローバルフォーマットの平均値と良い一致を示している。但し、長崎の西山地区で採取した土壤の $^{239}$Pu/$^{238}$Pu 原子比は約 0.04 と非常に低い、これは長崎に投入された Pu 炸彈の組成を反映していると考えられる。つまり、Pu 炸弾では $^{239}$Pu が濃縮されているため $^{239}$Pu/$^{238}$Pu 比が小さい。

爆心地の近くでは飛び散った爆弾の材料の影響が大きく、そのため低い $^{239}$Pu/$^{238}$Pu 比を示すと考えられる。

日本で採取した試料だけでなく、チェルノブイリやその他の地域の試料（IAEA の標準試料も含む）の分析も行っており、得られたデータを Fig. 3 にまとめた。

チェルノブイリの土壤試料では、約 0.4 程度という高い値が得られた。これは、燃焼度が高い燃料からの汚染を反映していると考えられる。（つまり、原子炉内で、$^{239}$U に中性子があたり, $^{238}$Pu が作られ、それに中性子が与えられ $^{238}$Pu が生成される。そのため、燃焼度が高い方が, $^{239}$Pu/$^{238}$Pu 比が高い傾向にある。）ムルロア環礁の堆積物（IAEA-368）から得られた $^{239}$Pu/$^{238}$Pu 比は約 0.04 であり、これは長崎とチェルノブイリ爆弾の材料を反映していると考えられる。マーシャル諸島の堆積物（IAEA-367）の値は約 0.3 であった。マーシャル諸島では水爆実験も含む様々な核実験を行っており、比較的高い $^{239}$Pu/$^{238}$Pu 比が起こり得るのか現在のところ不明である。

アイリッシュ海の堆積物（IAEA-135）においては、0.21 程度の値が得られた。この値はフォーマットの値よりも少し大きい。セラフィールドの再処理施設で処理された使
用済核燃料中の$^{240}\text{Pu}/^{239}\text{Pu}$比を反映していると考えられる。
原子炉や再処理施設の環境安全評価に関連して、環境中の濃度を測るだけでなくPuの同位体比も調べることは重要と考えられる。同位体（特に$^{240}\text{Pu}/^{239}\text{Pu}$比）を測定することにより、プルトニウムの汚染源の同定も可能になる。また、汚染源周辺でのそれらの比を求めることは、プルトニウムの環境移行を解析する上でも役立つであろう。現在の科学技术庁のPu分析のマニュアルではアルファ線スペクトロメトリー法しか述べられていないが、さらにICP-MS法なども加え、また、分離法にも柔軟性を持たせる必要がある。

2.2.2 植物・菌類
種々の農作物と森林生態系を形成する植物や菌類（キヨコ）についても多元素分析を行っている。ここでは分析データの一例として、木の葉（広葉樹）、シダ、キヨコの分析値を紹介する。植物は主として樹皮から養分を吸収している。そこで、ここで用いたキヨコも菌系は被炭表面に存在している。そこで、植物（キヨコ）と土壌との土壌元素の濃度比を求めた。濃度比を調べることは、各元素の植物（キヨコ）への移行や環境中での循環を評価する上で重要である。求めた濃度比をFig.4に原子番号順に示した。移行比が1より大きい値を示したものは、PとK（木の葉、シダ、キヨコ）、RbとCd（キヨコ、シダ）であった。逆に、濃縮し難いものとしては、Ti、Ga、Zn、U、Th等であった。その他特異なものとしては、シダ類中の希土類元素があげられる。他の植物（木の葉以外も含む）やキヨコに比べて長期間は希土類元素を含むと取り込みやすい。また、Fig.4からキヨコは植物と比べCsとRbを大変吸収しやすいことが分かる。しかし、おおよそアルカリ元素でKの値は3者でそれほど大きな差はない。チェルノブイリ事故の後各国でキヨコ中に高いCs-137濃度が報告されたが、これは、キヨコが安定セシウムを濃縮し易いことに関係している。しかし、キヨコの種類によってはセシウムの濃縮が大きく異なることも明らかになった。また、森林生態系におけるセシウムの分布に関する研究を行ったが、フォールアウトで加わったCs-137は系内では既に安定セシウムと混ざり、

|-----------|------------------------|----------|-----------------------|-----------------------------|--------------------------|----------------|-----------|

![Fig. 3 The $^{240}\text{Pu}/^{239}\text{Pu}$ atom ratio in environmental samples (soil or sediment) from different origins](image)

![Fig. 4 Plant (mushroom) / soil concentration ratios for leaf vegetables, ferns, potatoes and mushrooms (dry weight basis)](image)
Cs-137/Cs 比はほぼ一定で同様な挙動を取っていることが分かった。

2.2.3 天然水
酸性雨や温暖化の問題に関連して天然水の化学組成をその変化に関する研究が重要となっている。森林に酸性雨が降り注ぐと樹冠や土壌から特定の元素が溶出し地下水や川川に入る。また、温暖化によって生態系や土壌のバランスが変化し新たに溶出する元素も出てくる可能性がある。さらに、今後先端技術産業の発展とともに今まで用いられなかった元素（レアーメタルなど）が環境に加わることも予想される。しかし、天然水中の微量元素に関する信頼できるデータを海水を除いては少ないのが現状である。そのため、雨水、川川水、湖沼水、地下水などをについてパックグラウンドデータを取った。水試料の ICP-MS による分析は上記述べた試料試料とは異なる前処理操作が必要であり、主に蒸発と酸等による調整だけで済む。しかし、天然水中の多くの元素は濃度が低いため検出限界が問題になる。また、低濃度の元素を測定する場合は他の元素の分子イオン等の妨害を受けやすいので注意が必要である。

Fig. 5 に雨水の分析結果を示す。試料の採取場所はひたちなか市の海岸（放射線測定放射生物学センターの裏：海岸より 50m）、東海村（海岸より 1km）及び水戸（海岸より 2km）の 3 地点である。それらを ICP-MS 及び ICP 発光法で分析し約 30 元素についてのデータが得られた。海からの距離が近い段階明らかに多い元素は Li, Cl, Na, Mg, Zn, Br, Sr, U であった。また、Rb, I, Ba, La も海岸で採取した降雨中に高い傾向があった。Mn, Fe, Co, Ni, Cu, Mo, Cd, Cs, Pb などは 3 地点ともほぼ同じ値を示した。

ヨウ素は、放射生物学的にも、栄養学的にも重要な元素であるが、今まで分析が難しかったのでデータが不足している。ICP-MS を用いることにより濃縮操作なしにヨウ素を分析でき、今まであまり知られていないが海水中のヨウ素濃度に関するデータが得られた。雨水中のヨウ素濃度は 1～3 ppb 程度であり、春先に高く、夏後に低い傾向にあった。雨水中の濃度は、降水量と共に関わっていると考えられる。つまり、雨の降る量や期間が少ないとの大気中のヨウ素濃度は高くなり、雨水に溶ける（又はウォッシュアウトされる）量も多くなると推定される。事実補正始める雨の濃度は高い傾向にあった。また、化学形態についても ICP-MS にイオンクロマトを接続し形態別分離測定を行い、I と I2 の 2 つが確認できた。

ヨウ素以外の元素の季節変化は La, Ce, Yb, Th については春先に高く夏後に低い傾向が見られた。一方、Ni, Cr, Cd, Mo, Zn などは春先に顕著なピークではなく、上述の元素とは異なった挙動をとると思われる。K, Rb は 11 月に高い値が観測され、大気中に舞い上がった黒気など植物起源の成分からの影響の可能性もある。天然水中の元素の存在量と水を通した環境中での元素の循環に関しては、川川水や地下水などについて得られたデータと合わせて解析中である。

2.3 農耕地並びに森林移行とそのメカニズム
2.3.1 農耕地
農耕地における放射性核種の農作物への移行を、RI トライセ実験により調べた。用いたトライセは、Cs-137, Sr-85, Mn-54, Zn-65, Co-60, I-125 である。我が国の土壌として最も一般的な黒土とを用いた実験に、上記の核種を添加し、それに作物を植え、パイオトロン中で栽培した。収穫後、可食部をサンプリングし、Ge 半導体検出器又是
Nal シンチレーションカウンターを用いて測定した。
得られた値をもとに、土壌－植物移行係数（Transfer Factor）を求めた。尚、移行係数は作物中の放射性核種の濃度を土壌中の濃度で割った値として定義される。

今までの研究を通じて、得られたデータを Table 2 にまとめた。今後は同様な研究を継続的に進め、詳細な検討を行う予定である。

Table 2 には代表的な IAEA がまとめた代表的な移行係数値（IAEA 1982）も示している。農業の研究で用いられる値は、IAEA の値より数倍高い傾向にあった。これは、黒褐色土が放射性セシウムを可溶化する性質を有するため、農作物がこの核種を吸収し易かったと考えられる。一方、ヨウ素の場合、ほとんどの移行係数の値は、IAEA の値より 1 倍低かった。これは、作物が植物吸収し易かったという考えられる。

その後の元で、元素分析の結果から推測して、例えば、U、Th、希土類元素では通常 0.001 以下と非常に小さい値であった。

2.3.2 森林

チェルノブイリ事故の後、放射性 Cs が森林に長期間残留することが明らかになった。事故から 10 年以上たっても森林の有効植物中の濃度はほとんど低下していない。しかし特にキノコは放射性 Cs を蓄積しやすく、被ばく線量評価のため注目されている。森林内での放射性 Cs の長期的な挙動を解明するためには、森林内にともと存在する安定 Cs の関連するアルカリ及びアルカリ土類元素を考慮した総合的な研究が有効と考えられる。そこで、放射性 Cs による汚染の程度が異なる国内外数種の森林で土壌、植物、キノコを採取し、放射性 Cs と関連安定元素を ICP-MS や ICP-AES で定量した。

植物と比較するとキノコは、明らかに Rb と Cs 濃度が高く、Cs と Sr 濃度が低かった。キノコ中の 137Cs, Cs, Rb の濃度は、同じ森林で採取した植物に比べて 1 倍低かった。これは、キノコが本質的に Cs を吸収しやすいことを示している。

1つの森林で採取したキノコ中の 137Cs と安定 Cs との関

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Transfer factors of edible parts of agricultural products by radiotrace experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>agricultural products*</td>
<td>Cs</td>
</tr>
<tr>
<td>cereals</td>
<td>brown rice (dried)</td>
</tr>
<tr>
<td></td>
<td>wheat (dried)</td>
</tr>
<tr>
<td>leaf vegetables</td>
<td>cabbage</td>
</tr>
<tr>
<td></td>
<td>Chinese cabbage</td>
</tr>
<tr>
<td></td>
<td>spinach</td>
</tr>
<tr>
<td></td>
<td>komatsuna</td>
</tr>
<tr>
<td></td>
<td>Japanese parsley</td>
</tr>
<tr>
<td></td>
<td>lettuce</td>
</tr>
<tr>
<td></td>
<td>onion</td>
</tr>
<tr>
<td></td>
<td>sweet pepper</td>
</tr>
<tr>
<td>fruit vegetables</td>
<td>tomato</td>
</tr>
<tr>
<td></td>
<td>radish</td>
</tr>
<tr>
<td>root vegetables</td>
<td>sweet potato</td>
</tr>
<tr>
<td></td>
<td>soy bean (dried)</td>
</tr>
</tbody>
</table>

*TFs were calculated with wet weight of agricultural products except for "dried".
** means the experiment was carried out with other Andosols.
* the data from reference
Fig. 6 Relationships between Cs-137 and stable Cs in mushrooms collected from different forests. (Data for Rokkasho-mura are from Tsukada et al. (1998).)

には、キノコの種類に関わらず正の相関が見られた（Fig.6）。すなわち、放射性 Cs は安定 Cs と共にキノコに取り込まれていることが明らかとなった。両者の比は森林ごとにほぼ一定であった。このことから、核実験のフォールアウトやチェルノブイリ事故によって森林に沈着した$^{137}$Cs は生物的物質循環の中で安定 Cs とほぼ平衡状態になっていることが示唆された。森林ごとの比の違いは、各森林への放射性セシウムの沈着量の違い、土壌中の安定セシウム濃度の違い、森林の植生や土壌の違い等に起因すると考えられる。今後、森林の元素循環モデルを用いてこれらの比を再現できれば、安定 Cs 濃度を用いてキノコ中の放射性 Cs 濃度を予測することが可能と考えられる。

日本の森林土壌中の$^{137}$Cs と$^{87}$Rb 濃度の深度分布を調べたところ、両核種ともほとんどが表層（10cm 以内）に存在し、深さとともに減少した。これらのデータを考慮し、フォールアウトで加わったこれらの核種は、40 年前後経った今も、地表の一部に留まっていることが分かった。

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第3グループ:

制御実験生態系の構築に関する研究

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渡辺嘉人（人間環境研究部）、石井伸昌（科学技術特別研究員）

Studies on Construction of Controlled Experimental Ecosystems for ecotoxicity evaluation,
Hiroshi Takeda, Kiriko Miyamoto, Kei Yanagisawa, Shoichi Fuma, Yoshikazu Inoue, Yoshito Watanabe (Division of Human Radiation Environment), Nobuyoshi Ishii (JSTC, Research Fellow)

The aim of the third sub-group was to construct controlled experimental ecosystems applicable for an ecotoxicity evaluation at the community level and to evaluate the relative ecotoxicity for various toxic agents in comparison with radiation. As a model of aquatic microbial ecosystem, a microcosm was constructed and the stability and reproducibility as an experimental tool were investigated. This microcosm consists of flagellate algae Euglena gracilis Z as a producer, ciliate protozoa Tetrahymena thermophila B as a consumer, and bacteria Escherichia coli DH5α as a decomposer. Three species in the microcosm interact with each other and their populations can be maintained for more than one year. The microcosm was used for evaluating the ecotoxicity of radiation (γ-rays) and some other toxicants (ultraviolet radiation; UV-C, acidification and metals such as Cd²⁺, Cu²⁺, Mn²⁺, Ni²⁺ and Al³⁺). This microcosm test could evaluate not only direct effects but also secondary effects, which should be taken into account for ecological risk assessment. The results indicate that the toxicity of 500 or 1,000 Gy γ-rays is equivalent to 50 kerg/m² UVC, pH4 acidification, 300 μM Cd²⁺, 100 μM Cu²⁺, 10,000 μM Mn²⁺ and 100 μM Ni²⁺, respectively. This suggests that microcosm tests are useful for comparative evaluation of ecotoxicity. By using this experimental model ecosystem, the combined effects from radiation and some other toxicants are now being investigated.

1. 論言

本グループ研究では、人だけでなく生態系を対象として各種有害因子による影響を評価する手法の開発が課題であった。生態系は無機的環境要素と様々な生物種で構成され、その機能は生物種間の相互作用による物質やエネルギーの流れによって支えられている。このような生態系の構造や機能への影響を評価する目的では、自然の生態系はあまりに複雑で解析が困難である。そこで我々は、自然生態系の基本的要素を含んだとしても、室内実験が可能な制御実験生態系を利用することとした。制御実験生態系とは、自然生態系の空間的スケールや構成要素をある程度制御した条件下で操作実験が可能なモデル生態系である。本研究では水圏生態系のモデルであり、フラスコや試験管サイズでの実験が可能なマイクロコラムを用いて、様々な有害因子による負荷実験を行なった。主に用いたマイクロコラムは、水圏微生物生態学の基礎的研究のために最近開発されたものので、ユエラネ、テトラヒメナ、大腸菌の3者微生物で構成されたものである。この3者微生物コラムの生態間相互作用や実験系としての再現性や安定性についての検討は、開発者たちによっても短期的には行なわれていたが、我々の研究目的に必要な長期間実験にも使用可能であるかを検討することから本研究を開始した。なお、この3者微生物で構成されるマイクロコラム以外に、藻類とミジンコで構成されるマイクロコラムも一部本研究に使用した。一方、有害因子としては、放射線 (Co γ線) を始めとし、紫外線（UV-C）、電磁場、酸性化および金属類（鋼; Cu²⁺, ニッケル; Ni²⁺, ガドリニウム; Gd³⁺, アルミニウム; Al³⁺, マンガン; Mn²⁺）を負荷実験に使用した。本研究では、これらの有害因子の相対的な危険度を評価することを一義的な目標としたが、生態系における影響の発現と防御のメカニズムを解析するための基礎的検討の取得にも努めた。このため、マイクロコラムを構成する微生物単離培養系でも同様な負荷実験を行った。

2. 実験および結果

2-1. 3者マイクロコラムの実験系としての有効性の検討

本研究に用いた3者マイクロコラムは愛媛大学の川端らによって開発されたもので、生産者として鞭毛虫ユエラネ（Euglena gracilis Z）、消費者として細毛虫テトラヒメナ（Tetrahymena thermophila B）、分解者として大腸菌（Escherichia coli DH5α）から構成されたものである。これら3者の微生物はいずれも無菌状として分離されたものを使用している。マイクロコラムを培養するための基本培地は、無機栄養塩および金属塩を含んだTaub培地を用い、これに有機物としてプロテアーゼまたはペプトンを0.5mg/mlとなるように添加したものをマイクロコラム培地として使用した。このマイクロコラム培地を、フラスコや小試験管に一定量を入れ、オートクレープ滅菌後、テトラヒメナ、ユエラネ、大腸菌を接種し、マイクロコラムを培養した。なお、培養条件は、温度25℃で、照度2,500lux（暗期12時間）で静置培養し、培養開始後様々な時点で、マイクロコラム構成微生物の個体数密度（Population density）を計測した。ユエラネとテトラヒメナは顕微鏡
観察により個体数を計測し、また大腸菌は PB 培地により混沌平板法で生菌数（コロニー形成能力を持ち菌）を測定した。まずは、この 3 者マイクロコズムの基礎データを得るため、また安定性や再現性を確認するために、以下のよう
にマイクロコズム構成生物種の単独、2 者及び 3 者の長期間培養実験を行った。

両者培養実験

3 種の微生物を組み合わせて培養した場合、比較的安定な個体数密度を維持したのはユーグレナのみで、大腸菌とテトラヒメナはその共存者によって異なる個体数密度とその経時的変化を示した。大腸菌は共存者がユーグレナの場合よりテトラヒメナの場合にその個体数密度は低く抑えられた。また、テトラヒメナはユーグレナとの共存では培養開始後 30 日目以降減死し、テトラヒメナの捕食者（捕）である大腸菌との共存でも培養開始後 30 日目以降次第にその個体数は減少し、300 日目には完全消滅した。このように、マイクロコズム構成生物種のの中で外側からの光エネルギーをを利用して生存できるユーグレナ以外の生物種は、2 者培養においても安定した個体数密度を維持することは出来なかった。なお、ユーグレナもテトラヒメナとの組み合わせでは、培養開始後 200 日目以降はその個体数は減少し、一
年間近くその個体数密度が安定していたのは、大腸菌との
組み合わせの場合のみであった。

3 者マイクロコズム培養実験

3 者の微生物（ユーグレナ、テトラヒメナ、大腸菌）を組み合わせて培養した場合には、各生物種の個体数密度が培養開始 40 日目以降は低下しないで、3 者の生物種が相互
作用し共存関係が成立したものと推定された。このような
結果は、以前川崎研究室で行われた結果と一致することか
ら、この 3 者マイクロコズムが再現性のある実験系であることが確認された。また今回の実験において、生物種間の共存関
係は一年以上維持されることが判明し、長期間の影響評価
実験にも利用できる可能性を示した。Fig.2 に示したように、各生物の個体数は次第に減少する傾向ははあるが、3 者
系統としては 1000 日間維持することが出来た。
3 者マイクロコズムの共存系が成立するメカニズムについて、以下のようなことがこれまでの実験から明らかに
になっている。マイクロコズム培地内にはプロテアーゼペプ
トンが存在し、これを栄養源として、まず大腸菌とユーグ
レナが増殖し、テトラヒメナは卵である大腸菌がある程度
増殖した時点でその個体数を増加させる。プロテアーゼペ
プトンは大腸菌やユーグレナの増殖に伴い比較的短期間で
枯渇するが、葉緑体を持つユーグレナが系外から唯一供給
される光エネルギーを物質エネルギー（有機物）に変換し、
これを系内の栄養源として供給する。ユーグレナによるこ
の光合成過程では、分解者である大腸菌によって供給され
る無機物が利用される。また、各微生物の代謝産物や死骸
が互いに他の微生物の生存を増殖に促進的に働くことを明
らかしている。Fig.2 には、このような 3 者マイクロコ
ズムの生物種間相互作用を示している。

Fig. 1. Variations in the population density of three microbes in the microcosm after inoculation.

Fig. 2. Interactions among three microbes in the microcosm.

2.2. 各種有害因子の負荷による安定期 3 者マイクロコズム
構成生物種の個体数変化

培養開始後各構成生物の個体数密度がほぼ一定となる安
定期の 3 者マイクロコズムに、放射線（γ 線）、紫外線
(υ-V)、酸化および重金属類を負荷し、マイクロコズム
構成生物の個体数変化を調べた。その結果から各有害因子
の比較影響評価を試みた。

放射線

培養開始後 56 日目の安定期における 3 者マイクロコズム
への放射線（60Co-γ 線）の負荷実験を行った。負荷した
γ 線の線量は 0, 50, 100, 500, 1000, 5000 Gy とし、負
荷前後のマイクロコズム構成生物種の個体数変化を調べた。
50 と 100 Gy の照射で、大腸菌の個体数が照射直後に一時的
酸化性
酸化性は1Nの硫酸と硝酸の等量混合液を用い、マイクロコズム培養開始後67日目および128日目の培地をpH4に調製した。培養開始67日目に酸化性を示したマイクロコズムでは、大腸菌の個体数が一時的に減少するが、酸性負荷後10日目以降に対照と同じ個体数に回復した。この回復は共存するユーグレーナによる培地の中性化によりもたらされるものと考えられた。一方、128日目に酸化性を示したマイクロコズムでは、まず大腸菌が死亡し、それに引き続きテトラヒメナも死亡した。このテトラヒメナの死亡は捕食者である大腸菌の死亡による間接的影響であると推測された。
このように、マイクロコズムの酸化性に対する緩衝能力は培養期間によって異なることが判明した。

金属類
銅 (Cu²⁺): 安定期のマイクロコズムにCuSO₄溶液を加え、その構成生物種の個体数に与える影響を調べた。1μM及び10μM Cu²⁺ではどの生物種にも影響が見られなかった。100μMでは、まず大腸菌がすみやかに死亡し、次いでテトラヒメナも死亡したがユーグレーナには影響が見られなかった。ニッケル (Ni²⁺): 安定期のマイクロコズムにNiSO₄溶液を加え、その構成生物種の個体数に与える影響を調べた。1μM及び10μM Ni²⁺では、どの生物種にも影響が見られなかった。100μMでは、まずテトラヒメナがすみやかに死亡し、次いで大腸菌も死亡したがユーグレーナには影響が見られなかった。他の金属では、まず大腸菌が死亡し、次いでテトラヒメナが死亡するとする順序であつたが、Ni²⁺ではこれが逆であった。1000μMでは、テトラヒメナと大腸菌がすみやかに死亡し、次いでユーグレーナも死亡した。

マンガン (Mn²⁺): 安定期のマイクロコズムにMnCl₂溶液を加え、その構成生物種の個体数に与える影響を調べた。100μM Mn²⁺では、大腸菌が一時的に減少したが、ユーグレーナとテトラヒメナには影響が見られなかった。500μMでは、大腸菌とテトラヒメナが一時的に減少したが、ユーグレーナには影響が見られなかった。1000μMでは、大腸菌とテトラヒメナが一時的に減少したが、ユーグレーナは対照よりも個体数が増加した。10000μMでは、大腸菌がすみやかに死亡し、次いでテトラヒメナも死亡したが、ユーグレーナは対照よりも個体数が若干増加した。

ガドリニウム (Gd³⁺): 安定期のマイクロコズムにGdCl₃溶液を加え、その構成生物種の個体数に与える影響を調べた。50μM Gd³⁺では、どの生物種にも影響が見られなかった。100μMでは、大腸菌が一時的に減少したが、その後すみやかに回復し、個体数は対照よりも増加した状態で維持された。ユーグレーナとテトラヒメナには影響が見られなかった。300μMでは大腸菌は、ほぼ死亡し、テトラヒメナは一時的に減少したが、ユーグレーナには影響が見られなかった。1000μMでは、全生物種がすみやかに死亡した。

アルミニウム (Al³⁺): 酸性雨により長期間にわたるアルミニウムが、湖沼などの水準生態系に影響を及ぼす可能性が指摘されている。そこで、マイクロコズムを使って、アルミニウムが水準微生物生態系に及ぼす影響を評価した。

生産者であるユーグレーナ、消費者であるテトラヒメナ、分解者である大腸菌から構成されるマイクロコズムの定常期
Table 1. Effect levels of ionizing radiation and other toxic agents on the microcosm.

<table>
<thead>
<tr>
<th>Effects</th>
<th>γ-ray (Gy)</th>
<th>UV (kerg/mm²)</th>
<th>Electric field</th>
<th>Magnetic field</th>
<th>Acidification</th>
<th>Cu (µM)</th>
<th>Mn (µM)</th>
<th>Gd (µM)</th>
<th>Ni (µM)</th>
<th>Al (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No damage</td>
<td>—</td>
<td>1</td>
<td>1000 V</td>
<td>0.1 T</td>
<td>pH 4.5</td>
<td>10</td>
<td>—</td>
<td>50</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Population decrease</td>
<td>50–100</td>
<td>10</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>100–1000</td>
<td>100</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>Extinction of one or two species</td>
<td>500–1000</td>
<td>50</td>
<td>—</td>
<td>—</td>
<td>pH 3.5</td>
<td>100</td>
<td>1000</td>
<td>300</td>
<td>100</td>
<td>—</td>
</tr>
<tr>
<td>Extinction of all species</td>
<td>5000</td>
<td>100</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
</tr>
</tbody>
</table>

* #: denotes "not examined".

にAlCl₃を加え、各生物種の個体数に与える影響を調べた。10 µMのアルミニウム負荷では影響が見られなかった。100 µMでは、ユーレガレナが負荷51日目以降でコントロールよりも減少したが、他体系では影響が見られず、テトラヒメナでは負荷直後に一時的な増加が認められた。500 µMでは、アルミニウム負荷後で高塩濃度は大きく減少したが、速やかに回復し個体数は一旦コントロールより回復していた。ユーレガレナとテトラヒメナは、負荷直後にそれぞれのコントロールよりも一時的に增加した。1000 µMでは、まず高塩濃度が、次いでアルミニウムが速やかに回復し、その後ユーレガレナが減少した。このように、基本的にはマイクロコズムに対するアルミニウムの影響は負荷直後であるが、一部の構成生物（ユーレガレナ）に着目するとその影響に関しては必ずしも濃度依存性が見られなかった。

2.3.3者マイクロコズム構成生物の個体数変化を指標にした比較影響評価

安定期に1者マイクロコズムへの様々な有害因子（γ線、紫外線、電磁場、酸化性および重金属）負荷実験によって見られた直付けおよび間接的影響を、その程度によって、1: 影響が見られないレベル、2: 構成微生物のいずれかに個体数の平均的に見られるレベル、3: 構成微生物の一種または二種が死滅するレベル、4: 構成微生物のすべてが死滅するレベルの4段階に分けて比較した。例えば、1個または2種の消失が平均的に見られる第3のレベルは放射線で500 Gyか1000 Gyに相当し、このレベルの影響は50 kerg/mm²の紫外線（UV-C）、pH4.0への酸化性、100 µMの鉛（Pb²⁺）あるいはニッケル（Ni²⁺）、300 µMのガドリニウム（Gd³⁺）、1,000 µMのアルミニウム（Al³⁺）、および10,000 µMのマンガン（Mn²⁺）に0.5µMのマンガン（Mn³⁺）に相当することが判明した（Table1）。

このように放射線と他の有害因子による影響の程度は、さらに明確に分類した場合として有害物質の濃度1 µM/Lによる影響の程度を放射線の吸収線葉であるグレイン（Gy）に換算して表すと、重金属Mn²⁺、Al³⁺、Gd³⁺、それぞれ0.05 Gy、0.5Gy、1.7Gy、そしてNi²⁺とCu²⁺は5Gy以上になると試算された。

2.4. 物質動態の解析法およびその変化を指標にした影響評価法の検討

生態系が有する機能への影響を評価するためには、構成生物種への直接的な影響だけでなく、生物種間の物質循環やエネルギー流を解析する必要がある。この物質動態解析のためのモデル実験系として、藻類ミシジンコク・マヒシ－で構築したボトルおよび水槽サイズのマイクロコズムを構築した。この実験系を用いて、炭素の安定同位体1³Cを利用したトレーサー実験を行なった。

1³Cアラビアミドによる淡水プランクトン中の¹³C濃度分析

¹³Cの分析には¹³Cアラビアミド（日本分社 EX-130）を用いた。この装置を試料をケロ化合物で燃焼し生成した燃焼ガスを装置の吸収セルに導入して¹³C¹²Cの外線吸光度を測定し¹³C濃度を計算する。通常吸光度は¹³Cのグラフィンおよび¹³C濃度が99atom%のグラフィンを使用して作成する。その方法は自然試料として炭素量100 µgから10000 µgまで100 µg毎に対応する量のグラフィンをとめて調製し、¹³Cの試料で¹³C濃度が99atom%のグラフィンを炭素量として11 µgから11 µgまで1 µg毎に対を用いたクーパン分析分析の際の¹³C¹²Cの外線吸光信号から作成する。この¹³Cアラビアミドを用いて淡水系に生物を用いた動物性プランクトン（ミシジンコ）への食物連鎖を介した炭素の移行を調べた。

実験に用いたアクリル樹脂製の容器に貯水池から採取し実験室内で繁殖させた藻類の培養液3リットルをとり、¹³C¹²C（99 atom%）350ppmを添加した混合ガスを通した。これを天然ガスの条件下に置きオミシジン（Daphnia magna）を入れて培養し、適時（4-8時間）後に採取した。採取したミシジンコは1個体ずつ実験装置に入れて洗浄乾燥しミシジンコ有機成分中¹³C濃度を求めた。この結果、天然ガスの条件下で¹³Cガスの代謝数次に過疎に¹³Cは取り込まれ、さらに¹³Cはミシジンコに移行していることが確認された。今後この¹³Cトレーサー法を用いて、様々な有害因子を負荷した場合にモデル生態系内の物質循環がどのように影響を受けるかについて調べることとした。

紫外線負荷によるミシジンコへの¹³C取り込みへの影響

¹³Cトレーサー法によるモデル生態系への影響研究として、紫外線負荷の場合の実験を行なった。すなわち、藻類およびミシジンコで構成されるモデル生態系に紫外線（UV-B）を照射し、その照射直後にNaH¹³C3を添加し24時間培養後にミシジンコに取り込み1³C濃度を測定した。なお、藻類の培養は水田土壌1.5kgに乾燥穀物15gを添加して水1リットルを加えて攪拌し温室室内に放置した。約10日間後藻類が増殖し緑色に変化した。溶液中のプランクトンを顕微鏡で観察したところ増殖した藻類はイカダズ、
クロレラおよびムレミカズキモであった。また紫外線照射はUV-Bランプを使用して95μw/cm2で15分、30分、60分間行った。本実験の結果、ミジンコの14Cの取り込みが30分間以上の紫外線照射によって低下することが判明した。これは紫外線の照射によりミジンコの葉類摂食量が低下したためと考えられる。以前より、ミジンコを使用した有害生物の毒性試験法は行なわれなかったが、そのエンドポイントとして実質的には致死率を指標にしている。14Cをトレーサーとした物理移行を影響指標として場合、ミジンコが死亡しない程度における照射の影響を検出することが可能であり、より感度の高い生態系影響指標として利用できる可能性が示唆された。

2-5.3 者マイクロコズムによる放射線と酸化性の複合影響評価

生態系は様々な有害因子に曝されることができがある。したがって、放射線の生態系影響を評価する場合には、放射線とそれ以外の有害因子の複合影響を考慮する必要がある。そこで、放射線と酸化性が細菌微生物生態系（マイクロコズム）に与える複合影響について検討した。放射線負荷は40Coをソースとして、線量率72 Gy/minのγ線を100 Gy照射した。また、酸化性負荷は酸化性を模倣した0.1N硝酸+0.1N硫酸等量混合液をマイクロコズム培地に加えたH4とした。実験は、56日間培養した安定期のマイクロコズムを放射線および酸化性をそれぞれ単独に負荷した場合と放射線負荷直後にH4酸化性負荷した場合、そしていずれの負荷も与えない場合の4つのシリーズで行った。負荷を与えた後、各シリーズのマイクロコズム構成生物の個体数、クロロフィルα量、ATP量、培地のpHとイオン濃度（NO3−、SO4−、NH4+）などが120日間わたり測定した。

その結果、放射線単独負荷では、負荷直後に細菌の個体数がコントロールよりも有意に減少したが、その後はコントロールレベルまで回復した。その他の生物種には影響が見られなかった。酸化性単独負荷では、細菌がコントロールよりも減少する傾向が見られ、テトラヒメナは有意に減少し、ユーガラトはわずかに増加した。これに対し、放射線と酸化性の複合負荷では、負荷直後に細菌がコントロールよりも有意に減少した。その後は増加回復したが、コントロールよりも少々の傾向を示し、テトラヒメナは有意に減少し、ユーガラトはわずかに増加した。以上の結果より、放射線と酸化性による複合負荷はマイクロコズム構成生物の個体数変化に対し相加的な影響を与えることが判明した。

3.考察

放射線とその様々な有害因子による生態系への影響を比較評価する目的で我々が用いたモデル生態系（3者マイクロコズム）は、非常にシンプルであるが、生産者、消費者、分解者という生態系の基本的な構造と機能の一部を含むモデル系である。この実験系は、期間に制限はあるが構成している生物種を単独培養あるいは2者培養することが可能である。本研究では、3者マイクロコズムで見られた影響が発現するメカニズムを解析するため、必要に応じて単独培養への負荷実験を行った。その結果、3者マイクロコズムへの負荷実験では、生物間あるいは生物と有機因子間での相互作用による間接影響や二次的影響の評価も可能であることを明らかにした。

現在、生態系への影響評価については、放射線影響研究の分野でも重要な課題であり、IAEAやICRPとも取り上げている。マイクロコズムは生物間相互作用による放射性物質の動態や放射線による生態系影響発現機構の解析ツールとして放射環境安全研究に有効に利用できると期待される。

本研究での成果
1. 生態系影響研究のモデル実験として3者マイクロコズムを構築
2. 物質動態解析研究のモデル実験として異種・ミジン コープピディーで構築したボトルおよび水槽サイズの マイクロコズムを構築
3. 資源生態解析のための14Cトレーサー法を確立
4. 3者マイクロコズムでの放射線（γ線）、紫外線（UV-C）、電磁場、酸化性、金属類の影響解析
5. 3者マイクロコズムでの各種有害因子の比較影響評価

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-32-
第4サブグループ：

生体内挙動・影響の解析および新しい毒性評価法に関する研究

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Metabolism and Effect of Environmental Toxicants and New Risk Assessment
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The effect of arsenite(As) or nickel(Ni) on the repair of DNA double-strand breaks (dsbs) was studied in γ-irradiated Chinese hamster ovary cells using pulse-field gel electrophoresis. Both As and Ni inhibited repair of DNA dsbs in a concentration-dependent manner; 0.08 mM As significantly inhibited the rejoining of dsbs, while 76 mM Ni was necessary to observe a clear inhibition. The mean lethal concentrations for the As and Ni treatments were approximately 0.12 and 13 mM, respectively. This indicates that the inhibition of repair by As occurred at a concentration at which appreciable cell survival occurred, but that Ni inhibited repair only at cytotoxic concentrations at which the cells lost their proliferative ability.

The cytotoxicity of gadolinium (Gd) was investigated in alveolar macrophages (AM) cultured in vitro. The viability of rat AM was decreased by exposure to gadolinium at doses more than 3 μM, while mouse AM appeared to be resistant even up to 1000 μM Gd exposure. The phagocytic activity of mouse AM was higher than that of AM and mouse AM took up a larger amount of Gd than rat AM. Therefore, the marked difference in the cytotoxic response to gadolinium between mouse and rat alveolar macrophages could not be attributed to the phagocytic activities for the colloidal form of Gd. The cytotoxic sensitivity of AM to Gd present in non-colloidal form was almost the same between mouse and rat AM. Therefore, it is suggested that the extent to which Gd-colloid phagocytes is dissolved in the phagolysosome or the subsequent process to exhibit the cytotoxicity may be different between mouse and rat AM.

The effect of Gd on the lung retention, excretion, and translocation of plutonium (Pu) was also studied in rats instilled intratracheally with Pu-hydroxide with and without Gd. The lung retention of Pu was higher and the focal excretion was lower in the rats administered Pu-hydroxide containing a high concentration of Gd (Pu-GdH) than those administered pure Pu-hydroxide colloid. The administration of Pu-GdH induced the inflammatory reactions in the lung. The delayed alveolar clearance of Pu in the rats administered Pu-GdH may be attributable to the change in physicochemical characteristics of colloid and the inflammation induced in the lung by Gd.

科学研究の発展に伴って種々の化学物質が環境中に放出されるようになり、その影響に対する問題が懸念されている。本研究の目的は、重金属や希土類元素などの有害物質の毒性を放射線の生体影響を基盤として明らかにするとともに、放射線との複合影響についても検討し、これら環境中で発生する放射線の人体への影響を相互に比較できる形で提示することを目的としている。新しい毒性評価法に関する研究として、二酸化マンガンの非毒性由来の細胞（CHO, Chinese hamster ovary）を、吸入毒性（呼吸器毒性）を評価するために培養肺胞マクロファージ（AM, Alveolar Macrophage）を使用した細胞毒性試験法を開発してきた。これらの細胞毒性試験法を使用し、環境毒性物質として重金属や希土類元素の毒性を評価した。幾つかの重金属は環境毒性物質として歴史的に有名で、今なお影響は未だに精に毒性評価試験法ができていない。近年、重金属のいくつかについてはX線に誘発されたDNA損傷の修復過程や及びその影響を指標とした毒性評価が報告されるようになった。ニッケル、カドミウム、水銀、パラフィル、鉛、錫などの、ヒ素についても同様の報告がある。しかしその影響は一様ではない。例えば、ニッケルはX線や紫外線で起こるDNA損傷の修復は、水銀は紫外線誘発のDNA損傷の修復は抑制しない。DNA損傷の修復は不完全な場合、細胞は細胞死し、突然変異などを引き起こすと考えられる。DNA損傷にはいくつかのタイプがあるが、特にDNA再修復（double strand break, dsb）が重要と考えられるが、DNA-dsbの修復に対する金属の効果についてはほとんど知られていない。そこで本研究ではX線に誘発されたDNA-dsbの修復に対するヒ素(As)とニッケル(Ni)の効果について検討した。一方、先端科学技術の急速な発展に伴い希土類元素の中には新たな利用目的が見出され産業界で大量に使用されているにも関わらず人に及ぼす影響が十分に検討されていない元素が幾つかある。ランタノイドの1元素であるガドリニウム(Gd)は磁性体として近年多種の工業製品に使用されており、また原子力の分野では中性子吸収体として、医学分野ではMRIの強力な造影剤として利用されるなど広範た多様な用途を有さず、体内に吸収した場合の毒性としては粒子状物質を吸引する能力(貯食能)を持っている細胞、特にマクロファージに特異的に取り込まれ、その細胞機能を防害することが分かっている程度であった。そこで環境毒性の観点からこのGdに着目し、その毒性をAMを使用した細胞毒性試験法で解明した。また、Gdは使用済み燃料の再処理工場で使用されているため、この工場での事故発生時にはブロットウム(Pu)とGdを同時に体内へ摂取する可能性が高く、Gdが生体に種々の影響を与えることはすでに報告されており、
Gd の共存により Pu の体内挙動が影響を受けることは十分に考えられる。そこで、ラットに Pu と Gd の共沈水酸化コロイドを気管内挿管法により投与し、その後の Pu の動きをみた。

実験方法
DNA-dsb の修復阻止

CHO-K1 細胞 1.5x10^3 を 35mm の培養皿ディッシュに植え、[14C] チミシンを添加して 24 時間培養し、DNA を標識した。種々の濃度のヒ素 (As)、ニッケル (Ni) を負荷して 2 時間培養後、15X Gy 線 40Gy を照射して DNA-dsb を誘発させた。更に培養を続けて DNA-dsb を修復させた後、トリプシン処理により細胞を回収して 0.5% アガロースゲルに懸濁し、氷冷下ホモゲナル注入してゲルを固め plug とした。plug はプロテナーゼ K で氷冷下、1 時間、更に30℃で一続処理して蛋白を分離し、逆洗操作を行った後リボソームクレアーゼで処理した。plug は TBE バッファーで多パルスフィールド電気泳動を 24 時間行った。ゲルを切り出し、plug と lane それぞれに含まれる 14C の放射活性を液体シンチレーションカウンターで測定し、次式により DNA-dsb の割合を FAR として算出した。

FAR (fraction of activity released) = lane 中の放射活性 / plug 中の放射活性 × lane 中の放射活性

カドミウムを種々の濃度で添加した。48 時間後、トリプシンブルー染色溶出試験により肺マクロファージの生存率を調査した。肺マクロファージの形態学的変化、DNA の断片化等は常法により検討した。食餌水は細胞にラテックス粒子を添加して一定時間培養し、固定染色後に顕微鏡下で細胞あたりの粒子数を計測することにより判定した。また、細胞内に取り込まれたガドリニウムの量は ICP-MS で測定した。

プルトニウムの体内挙動に対するガドリニウムの影響

2 種類の濃度 (Low(L), High(H)) の Gd を含む共沈コロイド (Pu-Gd-L 群、Pu-Gd-H 群) および Gd を含まないコロイド (Pu 群) をそれぞれ SD 系ラットに投与した。4 過間後に肺、肝、腎、脾、肺及大腸を摘出し、尿を試料とした。各器官および尿液中に含まれる Pu の放射活性を計測し、初期比率を用いた後肺に存在する Pu 量に対する肺、肝、腎及び尿液中の Pu 量の割合を算出した。一方、Gd の肺への影響は、重量の変化、肺洗浄細胞のタイプ数の変化に着目して検討した。

結果
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As(III) 800μM では一つの生存率が 90% 以上で毒性は見られないかった。Ni 20mM 以下では毒性はなかったが、200mM では生存率が 50% まで低下した（Fig. 1）。コロニー形成実験で見出された細胞の増殖能は Pu, Ni とともに濃度増加に伴って低下し、平均致死濃度は As が 0.12mM, Ni が 1.3mM であった（Fig. 2）。As を負荷した結果、0.12mM までは照射直後の FAR 値 (修復なし) は対照群と同じレベルであったが、それを以降濃度増加に伴って FAR 値は高くなった（Fig. 3）。30 分間の修復後は As 0.08μM 以上で有意に高い FAR 値を示し、DNA-dsb の修復が抑制された。

ガドリニウムの細胞毒性

肺洗浄によって採取したマウス及びラットの肺マクロファージを常法に従って培養し、ガドリニウムと、対照として重金属のカドミウムを種々の濃度で添加した。48 時間後、トリプシンブルー染色溶出試験により肺マクロファージの生存率を調査した。肺マクロファージの形態学的変化、DNA の断片化等は常法により検討した。食餌水は細胞にラテックス粒子を添加して一定時間培養し、固定染色後に顕微鏡下で細胞あたりの粒子数を計測することにより判定した。また、細胞内に取り込まれたガドリニウムの量は ICP-MS で測定した。

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Fig. 2. Cell survival as determined by the colony-forming assay after 2.5 h exposure to As or Ni. Vertical bars indicate the SD for 6 to 9 cultures obtained from at least three independent experiments.

Fig. 3. FAR values assayed immediately after irradiation (●) or 30 min after the repair incubation with As or Ni(II) (○). FAR values for the nonirradiated cells are also shown (△). Vertical bars indicate the SD of the 6 to 12 cultures from at least three independent cells.
Fig. 4. The viability of mouse and rat alveolar macrophages after 48 hr of exposure to Gd and Cd. The viability was expressed as the percentage of the viable cell number to that in control culture. Each data point is a mean of three separate experiments. Asterisks indicate the statistically significant difference from C3H and B6 mice at the corresponding concentration of Gd or Cd.

Fig. 5. Effects of filtration of Gd and Cd preparations on cell viability determined at 48 hr of culture in B6 mouse (upper) and SD rat (lower). Each data point is a mean of 3 separate experiments. Asterisks indicate the statistically significant difference from those exposed to filtered Gd.
Table 1. The uptake of Gd by B6 mouse and SD rat AM after 0, 4 and 10 hrs.

<table>
<thead>
<tr>
<th>Incubation (Time hr)</th>
<th>Incubation (Temperature °C)</th>
<th>AM</th>
<th>% Gd*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-</td>
<td>B6</td>
<td>0.06</td>
</tr>
<tr>
<td>0</td>
<td>-</td>
<td>SD</td>
<td>n.d.</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>B6</td>
<td>0.28</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>SD</td>
<td>0.22</td>
</tr>
<tr>
<td>4</td>
<td>37</td>
<td>B6</td>
<td>1.12</td>
</tr>
<tr>
<td>4</td>
<td>37</td>
<td>SD</td>
<td>0.58</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>B6</td>
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</tr>
<tr>
<td>10</td>
<td>4</td>
<td>SD</td>
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</tr>
<tr>
<td>10</td>
<td>37</td>
<td>B6</td>
<td>1.40</td>
</tr>
<tr>
<td>10</td>
<td>37</td>
<td>SD</td>
<td>0.78</td>
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*The amount of Gd per 10⁶ AM, expressed as a percentage of total amount of Gd added, i.e. 2ml of 300μM Gd

Fig. 6. Lung retention and cumulative excretion of 239Pu in feces and urine 4 weeks after intratracheal instillation of 239Pu-hydroxide, Pu-Gd-L, and Pu-Gd-H. Vertical bar shows SD of three rats.

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Fig. 7. Daily and cumulative excretion of $^{239}$Pu in feces (A) and urine (B) following intratracheal instillation of $^{239}$Pu-hydroxide (Pu), Pu-Gd-L and Pu-Gd-H. Vertical bar shows SD of 3 rats. *p<0.05, †p<0.02

或いはイオン化ガドリニウムの細胞内構成成分との反応における凝集によるものと考えられた。本研究では先端技術を駆使し急速な発展に伴い多量に広範な用途に使用されるようになった物質の一例としてガドリニウムを取り上げ、人びとを対象とした影響や細胞毒性を指標として検討したのであるが、その細胞毒性に実験動物間の顕著な差異を示すという予想外の成果を得た。このことから環境污染物質の影響を総合的な観点で評価するためには、多くの毒性試験それぞれの長短を正しく認識して可能な限り多くの毒性評価を行いその情報を得ることで、毒性発現機序の解明までを行うことが重要であると認識された。

Gdを肺に単独投与したラットの肺洗浄細胞中に炎症性細胞である好中球の存在が確認されたことから、PuとGdの共存により酸化コロイドを投与したラットでも炎症が引き起こされていることが推察された。一般に、炎症が起きていて肺では沈着粒子の肺からのクリアランスが遅くなることが知られおり、共存酸化コロイドを投与して4週間後のPuの残存量が高くなった。Puにより肺への炎症が起こったものと考えられる。Puの酸化化作用はlysosomeでの可溶化を経て細胞外へ放出されることやlysosomeが炎症に重要な役割を果たしていることを考慮すると、GdがlysosomeでのPuの可溶化に抑制的に作用している可能性があるが、現時点ではその経路は明らかではない。

結論
γ線により誘発されたDNA-dsbの修復がAsで阻害されたこと
は、Asと放射線の複合影響が環境中で起こることを考慮する
と非常に重要である。また、Niは本研究での実験条件では毒性を
示す高い濃度でのみDNA-dsbの修復を阻害したが、他のタイプの
DNA損傷の修復は低濃度で阻害されることからDNA-dsbの修復
に対する影響をより明らかにするためにさらに検討が必要であ
る。GdのAMに対する毒性はGdの細胞内への取り込み量と取り
込まれたGdの細胞内での溶存性に依存したが、マウスとラットの
AMに対するGdの放射線の大きさ依存性や表面作用に対する観察
法と関係がなく、取り込まれたGdの細胞内での溶存過程はにくいに依存することが示唆された。すなわち、ラットのAM

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Multitracer Studies on the Accumulation of Radionuclides in Mushrooms

TADAAKI BAN-NAI¹, YASUYUKI MURAMATSU¹, SATOSHI YOSHIDA¹, SHIGEO UCHIDA¹, SADAO SHIBATA², SHIZUKO AMBE³, FUMITOSHI AMBE³ and AKIRA SUZUKI⁴

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Mushroom/Multitracer/Accumulation/Cultivation experiment/Radionuclide

We used the multitracer technique to study the transfer of several radionuclides to two mushroom species. Radionuclides accumulated in the fruiting bodies of the mushrooms in the order of $^{87}$Rb > $^{60}$Zn > $^{40}$K > $^{22}$Na > $^{75}$Se and $^{85}$Sr > $^{60}$Co > $^{88}$Y, $^{103}$Rh, $^{135}$Cs, $^{144}$Pm, $^{146,152}$Gd and $^{159}$La > $^{157}$Hf. The concentration ratio values for $^{60}$Rb, $^{60}$Zn and $^{40}$Mn in the fruiting bodies were more than 10, whereas those for $^{87}$Rb, $^{88}$Y, $^{103}$Rh, $^{135}$Cs, $^{157}$Te, $^{157}$Hf and the rare earth elements were less than 1. There were major differences in the accumulations of the alkali elements.

INTRODUCTION

Mushrooms accumulate Cs, including radiocesium$^{1,2}$. After the Chernobyl reactor accident, high concentrations of radiocesium were found in European mushrooms$^{3-6}$. Cesium-137 concentrations in mushrooms are markedly higher than those in autotrophic plants$^{7-9}$. In a previous study$^{10}$, we calculated the contribution of mushrooms collected in Japan to the total Japanese intake of $^{137}$Cs (on average) as 32%, whereas the intake for $^{40}$K was only 1.7%. Mushrooms therefore are considered important in estimating the internal radiation dose of $^{137}$Cs obtained from foods.

In terms of the forest ecosystem, mushroom species have important functions in the decomposition of plant materials and the absorption of elements, such as K and P, from soil$^{11}$. In a previous study$^{12}$, we reported results of laboratory experiments on the accumulation of the radiotracers $^{137}$Cs, $^{85}$Sr, $^{40}$K, $^{40}$Co, $^{54}$Mn and $^{60}$Zn in three mushroom species and one higher plant (Medicago sativa). Mushrooms tended to accumulate

*Corresponding author
large amounts of Cs, but there were marked differences among the mushroom species. Accumulations were low for Sr and Co. There still is little data available on the transfer of various nuclides to mushrooms, in comparison to the known data on the transfer of other biological materials. Reliable data on the accumulation of other nuclides in mushrooms are needed for doing radioecological and environmental studies.

A multitracer, produced by irradiating targets with high-energy heavy-ions, can be used to study the behaviors of various elements in the environment. Ambe et al. made a multitracer study of the absorption of radionuclides through the roots of rice and soybean plants cultivated in nutrient solutions or soil and obtained the distributions of the radionuclides of 24 elements (Be, Na, Sc, Mn, Co, Zn, Se, Rb, Sr, Y, Zr, Nb, Ru, Rh, Ag, Te, Ce, Pm, Eu, Gd, Yb, Lu, Hf and Ir) in the grain, leaves, stems and roots.

We used the multitracer technique to study the transfer of several radionuclides (elements) from a medium (mixture of yeast extract and malt extract) to two mushroom species. The multitracers contained 17 nuclides, including ones related to fission products (Sr, Ce, Te), activation products (Mn, Co, Zn) and natural radionuclides (Rb).

MATERIALS AND METHOD

Multitracer

The radioactive tracers were produced by irradiating thin gold foil with a \(^{14}\text{N}\) beam accelerated to 135 MeV/nucleon in the RIKEN Ring Cyclotron. The irradiated foil was dissolved in \textit{aqua regia}, and the solution obtained evaporated to dryness. The residue was dissolved in 3 M HCl. Gold was extracted from the solution with ethyl acetate. The aqueous solution with the multitracer was adjusted to 0.05 M HCl. Details of the separation procedure are described in Ambe et al. In our study, the irradiated gold foil was cooled for more than 4 months before the separation. The radionuclides used were \(^{22}\text{Na}\), \(^{54}\text{Mn}\), \(^{60}\text{Co}\), \(^{65}\text{Zn}\), \(^{75}\text{Se}\), \(^{85}\text{Rb}\), \(^{89}\text{Sr}\), \(^{90}\text{Y}\), \(^{124}\text{Te}\), \(^{139}\text{Ce}\), \(^{140}\text{Pm}\), \(^{146}\text{Gd}\), \(^{152}\text{Gd}\), \(^{173}\text{Lu}\) and \(^{179}\text{Hf}\). Chloride was the chemical form of these nuclides in the multitracer.

Mushrooms Cultivation

Two mushroom species, \textit{H. vinosophyllum} and \textit{C. phlyctidosporus}, were used in the culture experiment. The medium solution was comprised of yeast extract (2 g), malt extract (10 g) and agar (15 g) dissolved in distilled water (1000 ml), then 25 ml portions were poured into flasks (200 ml), and the multitracer was added. The pH of the solution was adjusted to 6.0 with 0.1N–NaOH, and the mixture autoclaved. Three flasks per mushroom species containing the medium solution and multitracer were prepared and incubated at 25°C. After about one month, the mushrooms produced fruiting bodies. The concentrations of radionuclides in the mushroom samples (whole fruiting bodies) were measured with a Ge-detector. Details of the counting procedures are described elsewhere. The mean values of the three flasks were calculated for each species. The concentration ratio, defined as the radionuclide activity in the fruiting bodies of the mushroom (Bq/g, wet wt.) divided by the radionuclide activity in the medium at the beginning of the experiment (Bq/g, wet wt.), was calculated.
RESULTS AND DISCUSSION

The accumulations of the multitracer in the fruiting bodies of the two mushroom species are shown as concentration ratios in Table 1.

Figure 1 shows radionuclides that had mean concentration ratio values (with standard deviation) higher than 1. The ratios were in the order of Rb > Zn > Mn > Na > Se and Sr. Markedly high values of more than 10 were found for Rb (27), Zn (21) and Mn (11). The elements Co, Ce, Y, Rh, Te, Pm, Gd, Lu and Hf had concentration ratios lower than 1 (Fig. 2). The concentration ratios of Mn, Zn and Sr found for the mushrooms in this study were almost the same as the values reported previously. Because the concentration ratios of radioactive Rb, Zn and Mn are markedly high, in order to protect against radiation attention must be paid to the levels of these nuclides in contaminated forest ecosystems.

In a previous study, we found that radionuclide accumulation for mushrooms was in the order of Zn > Mn > Sr > Co, whereas for the higher plant (Medicago sativa) the order was Sr > Zn and Mn > Co. Mascanzoni also reported a tendency for the low accumulation of Sr in mushrooms collected from Swedish forests. Ambe et al. found a decreasing tendency in the transfer factor for soybean stem of the order Sr > Rb > Zn > Mn > Se > Na. Yoshida and Muramatsu analyzed many major and trace elements in mushrooms, higher plants (tree leaf) and soil samples collected from a pine forest and found a high accumulation of Rb and Cs and a low accumulation of Mg, Ca, Sr and Ba in mushrooms as compared with the higher plants. There is a characteristic difference in the accumulation of Sr between mushrooms and higher plants.

Interestingly, there are specific differences in the accumulation of alkali elements. We found that Rb is highly accumulated in mushrooms, whereas the concentration ratio of Na is significantly lower than that of

<table>
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<th>C. phylactosporus</th>
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<tr>
<td>$^{22}$Na</td>
<td>2.3 ± 0.3$^a$</td>
<td>1.8 ± 0.5$^a$</td>
<td>2.1</td>
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<tr>
<td>$^{54}$Mn</td>
<td>12 ± 2</td>
<td>11 ± 2</td>
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<tr>
<td>$^{65}$Zn</td>
<td>24 ± 4</td>
<td>18 ± 2</td>
<td>21</td>
</tr>
<tr>
<td>$^{75}$Se</td>
<td>2.7 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>1.7</td>
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<tr>
<td>$^{81}$Rb</td>
<td>32 ± 5</td>
<td>22 ± 3</td>
<td>27</td>
</tr>
<tr>
<td>$^{85}$Sr</td>
<td>2.0 ± 1.1</td>
<td>0.7 ± 0.2</td>
<td>1.4</td>
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<tr>
<td>$^{60}$Co</td>
<td>0.67 ± 0.48</td>
<td>0.64 ± 0.13</td>
<td>0.66</td>
</tr>
<tr>
<td>$^{88}$Y</td>
<td>0.52 ± 0.46</td>
<td>0.26 ± 0.08</td>
<td>0.39</td>
</tr>
<tr>
<td>$^{103}$Rb</td>
<td>0.47 ± 0.19</td>
<td>0.20 ± 0.05</td>
<td>0.34</td>
</tr>
<tr>
<td>$^{121}$Te</td>
<td>0.63 ± 0.19</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>$^{139}$Ce</td>
<td>0.47 ± 0.40</td>
<td>0.43 ± 0.09</td>
<td>0.45</td>
</tr>
<tr>
<td>$^{143,144}$Pm</td>
<td>0.48 ± 0.37</td>
<td>0.32 ± 0.07</td>
<td>0.40</td>
</tr>
<tr>
<td>$^{146,153}$Gd</td>
<td>0.45 ± 0.37</td>
<td>0.21 ± 0.01</td>
<td>0.33</td>
</tr>
<tr>
<td>$^{173}$Lu</td>
<td>0.47 ± 0.39</td>
<td>0.16 ± 0.03</td>
<td>0.32</td>
</tr>
<tr>
<td>$^{175}$Hf</td>
<td>0.27 ± 0.15</td>
<td>0.07 ± 0.03</td>
<td>0.17</td>
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</table>

$^a$The mean values with standard deviation for three flasks
Fig. 1. Concentration ratios of the multitracer in fruiting bodies of mushrooms (I)
Note: The mean values are for three flasks. Solid lines show the range of standard deviation.

Fig. 2. Concentration ratios of the multitracer in fruiting bodies of mushrooms (II)
Note: The mean values are for three flasks. Solid lines show the range of standard deviation.

Rb. There were no Cs isotopes in the multitracer used in this study. In a previous study using $^{137}$Cs, however, we found that it tended to be accumulated in mushrooms, the concentration ratios being $21 \pm 1$, $14 \pm 3$ and
2.6 ± 0.3, respectively for *H. vinosophyllum*, *F. velutipes*, and *C. phylctidosporus*. The concentration ratio of Rb for *H. vinosophyllum* (32) was somewhat larger than that of Cs. Differences in the concentration ratios among the species for Rb seemed to be smaller than for Cs. The concentration ratio of Cs for *H. vinosophyllum* was about 8 times that for *C. phylctidosporus*12, whereas the ratio of Rb in *H. vinosophyllum* in the experiment reported here was only about 1.5 times that for *C. phylctidosporus*. Ingroa et al.13 found no marked correlation between the concentrations of Cs and Rb in mushrooms collected in the field (R = 0.5).

In the present experiment, a small amount of Se was detected in the Silicon plug used to cover the flasks and in the activated charcoal supplemented with tetramethylendiamine in a dish placed in the incubator. We assumed that Se, added as an inorganic form, was converted to a gaseous organic species by the biological activity of the fungi. Chau et al.20 reported that inorganic and organic selenium compounds are converted to volatile selenium compounds by microorganisms in lake sediments. The volatile selenium compound produced would be dimethyl selenide.

The chemical composition of the medium was determined by inductively coupled plasma mass spectrometry (Yokogawa, PMS-2000). The element concentrations (on a wet weight basis) obtained (data for Na, K, Ca, Mn, Zn and Sr were published in our previous study12) are 2400 ppm for Na; 1000 ppm for K; 77 ppm for Ca; 2.6 ppm for Zn; 1.9 ppm for Sr; 0.5 ppm for Rb; 0.3 ppm for Mn; 0.03 ppm for Co; < 0.01 ppm for Se, Ce and Hf; and < 0.003 ppm for Y, Te, Gd and Lu. Elsewhere12 we found that the concentration ratios of mushroom to medium for Cs, Sr and Co were not markedly affected by coexisting stable elements (up to 10 ppm in the medium), whereas the ratio of Mn decreased as the amount of stable Mn in the medium increased. Because the concentrations of most elements (except Na, Sr and Zn) found in this study are very low, the concentration ratio of the relevant nuclide may not be much affected by coexisting stable elements. Sodium is known to function in the control of osmotic pressure and ionic strength in the cell. A high Na concentration could be the cause of the low Na concentration ratio.

The concentration ratios of Co, Y, Rh, Te, Ce, Pm, Gd, Lu and Hf are very low (Fig. 2). This suggests that there would be no particular accumulation of these elements in mushrooms in the case of contamination. The concentration ratios of the rare earth elements in *C. phylctidosporus* tended to decrease with increasing atomic number, but the data are limited.

Uptake experiments using a multitricer provided information about the concentration ratios of several nuclides (elements) in mushrooms. Because there is little available information on the accumulation of elements in mushrooms, our findings can be used to understand the behavior of radionuclides in the environment and to estimate possible radiation doses due to the consumption of mushrooms.

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LIFETIME RISK OF LUNG CANCER DUE TO RADON EXPOSURE PROJECTED TO JAPANESE AND SWEDISH POPULATIONS

Masahiro Doi,* Yuji Nakamura,* Tetsuya Sakashita,* Nobuko Ogiu,* Frédéric Lagarde,1 and Rolf Falk4

Abstract—Lifetime risk projections depend greatly on both background lung cancer rates and the selection of the risk model. Since background lung cancer rates differ from subject populations and the time, etiological risk of lifetime lung cancer mortality per unit radon exposure in WLM should be estimated for each subject population and the time of interest. To answer quantitatively how much are the differences among the projected risks for different populations, the Swedish case-control-study-based risk projection model was applied to the Japanese and Swedish populations from 1962 to 1997 as subject populations because of their distinct trends of lung cancer rates. To compare the results with the reference population and authorized risk projection models, U.S. population 1997 and the two risk projection models in BEIR VI report were applied, respectively. Lifetime risk of lung cancer mortality projected for Japanese, Swedish, and U.S. populations in 1997 per radon progeny exposure were estimated to range from 1.50 (0.40–3.19) × 10^{-4} WLM^{-1} to 9.86 (2.62–20.9) × 10^{-4} WLM^{-1}, which could be compared to the detriment associated with a unit effective dose. Conclusive dose conversion coefficients in this study ranged from 2.05 (0.55–4.37) to 13.5 (3.59–28.6) mSv WLM^{-1}, and within this range the discrepancy between dosimetric and epidemiological approaches was included.

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Key words: radon; risk analysis; epidemiology; alpha particles

INTRODUCTION

The Committee on the Biological Effects of Ionizing Radiations (U.S.) reviewed the four epidemiological studies (Lundin et al. 1971; Muller et al. 1983; Radford and Renard 1984; Howe et al. 1986; Hornung and Meinhardt 1987) in the BEIR IV report (NRC 1988). As the substantial revision of BEIR IV report, the recent BEIR VI report (NRC 1999) reviewed 11 miner studies and reexamined all approaches to assess the lung cancer risk posed by residential radon exposure. The BEIR VI report discussed uncertainties related to the induction of lung cancer at low level radon exposure and the combined impact of tobacco smoking, exposure to the mine dust, arsenic, diesel exhaust, Silica and Mycotoxins, and concluded that uncertainties posed by these known risk factors were not significant. And two risk projection models based on the pooled analysis of epidemiological studies of miners (Lubin et al. 1995) were authorized to extrapolate to estimate the potentially important public lung cancer risk posed by indoor radon exposure. It also emphasized that miner-based risk projection is an indirect evidence of lung cancer risk of residential radon exposure and must be validated by direct evidence of lung cancer risk of residential radon proved in the reliable epidemiological study.

BEIR committee reviewed some case-control studies on residential radon and lung cancer and their meta-analysis (Lubin and Boice 1997) and regarded them as inconclusive because of some potential limiting factors and errors in estimation of cumulative radon progeny exposure (NRC 1999). BEIR committee listed several formidable limitations of these case-control studies on residential radon and lung cancer studies and expressed a rather negative view of whether these limitations could be addressed to achieve more definitive results. But if they were not definitive, they showed direct evidence of indoor radon risk.

It is trivial that risk projections with an excess relative model depend greatly on background lung cancer rate of the subject population and also the selection of the risk model. But it is not answered quantitatively how much are the differences in the projected risks for different subject populations.

Lung cancer is one of the leading causes of cancer death in most of the developed countries, and several environmental toxins have been suspected to raise the background lung cancer risk. Particularly, daily cigarette smoking showed up as the most common cause of lung
cancer, and year trends of cigarette consumption, sales, and smoking prevalence are different by the population and the times.

ICRP proposed the reference population, for which survival probability and the age-specific lung cancer mortality rate were derived by Land and Sinclair (1991) from the average values of the five countries (i.e., Japan, the United States, Puerto Rico, the United Kingdom and China).

A conclusive lifetime lung cancer mortality coefficient was projected for the reference population with miner-based relative risk projection model and was estimated to be $2.83 \times 10^{-4}$ WLM$^{-1}$ (Working Level Month)$^{\text{[4]}}$ (ICRP 1994a). This value was compared to the estimate of BEIR IV, $3.5 \times 10^{-4}$ WLM$^{-1}$ projected for U.S. population 1980–1984 with time since exposure model (NRC 1988).

ICRP (1994a) divided this etiological lifetime lung cancer mortality coefficient by the detriment coefficient for effective dose of $5.6 \times 10^{-3}$ mSv$^{-1}$ for adult workers and $7.3 \times 10^{-5}$ mSv$^{-1}$ for members of the public (ICRP 1991) and recommended effective dose conversion factors of $5.06$ mSv WLM$^{-1}$ (or $1.43$ mSv per mJ h$^{-1}$) for workers and $3.88$ mSv WLM$^{-1}$ (or $1.10$ mSv per mJ h$^{-1}$) for members of the public. These reference dose conversion factors are general estimates, but it must be emphasized that the confidence intervals are included in these estimates substantially.

The ICRP has endorsed a dosimetric human respiratory tract model in Publication 66 (ICRP 1994b), and the modal value of 15 mSv WLM$^{-1}$ was evaluated by the model (Birchall and James 1994), which is 3 to 4 times larger than the above conversion conventions. Sensitivity analysis of each parameter in the respiratory tract model, including quality factor of 20 for alpha-particle exposure, was performed substantially by Birchall and James (1994) but could not explain such a discrepancy consistently. The BEIR VI report also reviewed the discrepancy between epidemiological and dosimetric approaches and discussed the uncertainty in the quality factor of alpha-particle in the dosimetric model (NRC 1999).

It must be reconfirmed before discussing about the discrepancy that the projected lung cancer risk of radon exposure is proportional to the survival probability and the age-specific lung cancer mortality rate of the subject population.

The first objective of this study is to estimate the etiological risk of lifetime lung cancer mortality for applying the Japanese and Swedish populations to a Swedish case-control study-based relative risk projection model in contrast to the miner-based risk projection models in BEIR VI report (NRC 1999). The second objective is to apply these models to the Japanese, Swedish, and U.S. populations in 1997, and to compare the dose conversion factors with their confidence intervals.

MATERIAL AND METHODS

Japanese and Swedish populations from 1962 to 1997 were selected as subject populations because Japanese population is just the opposite of Swedish population in the indoor radon exposure as well as the smoking rate. U.S. population was selected as a reference population because it was thoroughly examined in BEIR IV and VI reports (NRC 1988, 1999).

JAPANESE POPULATION

Residential radon concentrations in Japan were relatively low in comparison with those in other countries (UNSCEAR 1993). A recent nationwide indoor radon survey was commenced for 940 houses (Sanada et al. 1999). Radon concentration in the Japanese houses showed approximately a log-normal distribution of which arithmetic and geometric means were 15.5 and 12.7 Bq m$^{-3}$, respectively. Only one house exceeded the action level of 150 Bq m$^{-3}$ settled by the U.S. EPA (1986).

Smoking rate of Japanese male has been high in comparison with other countries. In 1965, 82.3% of Japanese males were smoking, and although it has tended to go downward, still 55.2% of Japanese males were smoking in 1997.

Smoking rate of total female had been very low, and it tended to be reduced gradually; it reached down to 15% in 1997. But the smoking rate of Japanese women in the 20–29 y age group has been constantly increasing since 1965, as illustrated in Appendix A.

By the census population study (Hirayama 1979), daily cigarette smoking is showed as the largest cause of lung cancer, of which excess relative risk and attributable risk in males were 3.76 and 67.2%, respectively. As the reflection of these conditions, malignant neoplasm by site is changing its pattern drastically in these 40 y in the Japanese population. In 1960, stomach cancer for males and females was the most common, accounting for 52.3% of all deaths from cancer for males and 39.2% for females, while lung cancer for males and females was 7.2% and 3.6%, respectively (Ministry of Health and Welfare 1962). Death rates by stomach cancer for both sexes are decreasing, and, instead, lung cancer has been increasing consistently for both males and females. According to the statistics in 1997, lung cancer was the most common type of cancer for males, accounting for 21.4% of all cancer deaths (for females lung cancer accounted for 11.7% of all cancer deaths). Fig. 1 shows the age-specific lung cancer mortality rates by sex, r$_{(a)}$, for the Japanese population from 1962–1997, which were calculated by the age-specific lung cancer cases in Vital Statistics of Japan (Ministry of Health and Welfare 1999).
1962–1997). A constant increase of background lung cancer mortality for Japanese males and females, which might reflect the year trend of smoking rates, is illustrated in Appendix A. Fig. 2 shows life expectancy of Japanese males and females shown in Vital Statistics of Japan (Ministry of Health and Welfare 1962–1997).

**SWEDISH POPULATION**

Residential radon concentrations in Sweden were regarded as some of the highest in the world (UNSCEAR 1993). As a rough guide to represent nationwide indoor radon concentration, 306 houses including 191 apartments in multi-family houses were measured in 1982, and the radon concentration showed approximately a log-normal distribution, of which arithmetic and geometric means of detached houses were 122 and 69 Bq m$^{-3}$, respectively (Swedemark and Mjönes 1984). Average radon concentration in the houses built since 1981 was surveyed to be 50 Bq m$^{-3}$, which might be caused by some social countermeasures to indoor radon, i.e., alms- hale concrete was not used in any houses built after 1975, and the building construction to decrease the inflow of soil gas has been improved by the Swedish 1980 building code (Swedemark and Akerblom 1994).

The proportion of smokers is steadily decreasing in Sweden. The proportion of daily smokers for men 16–84 y has decreased from 36% in 1980 to 22% in 1995, while
the proportion of daily smokers among women decreased during the corresponding period from 29% to 23% (Swedish National Board of Health and Welfare 1998). The proportion of smokers among men has decreased significantly since the 1960's, but among women has changed its trend to decline since the latter 1970's.

The Swedish Cancer Registry was established in 1958, and has collected statistics of cancer incidence in Sweden. Regulations of the National Board of Health and Welfare require that physicians send clinical reports of all cancer cases and pathologists send reports for every cancer diagnosis. This means that most of the cancer cases are noted twice in separate reports by the Registry. The background lung cancer mortality by age, \( r_s(a) \) for Swedish males and females are evaluated from Swedish Cancer Registry data from 1962–1997 (Swedish National Board of Health and Welfare 1964–1999, Statistiska Centralbyran 1964–1999), of which results are shown in Fig. 1. Recent vital statistics of Sweden are distributed by the Swedish National Board of Health and Welfare (http://www.sos.se/sos/publ/skrift/skrifte.htm). Fig. 1 shows background lung cancer mortality of Swedish males had almost been saturated during the 1990's, while that of females still increased gradually. These figures might reflect partly the trends of age-specific smoking rates in the 1960's and 1970's for Swedish males and females. As illustrated in Appendix A, smoking rates of Swedish males began to decrease in 1970, while those of Swedish females increased constantly. Fig. 2 shows the life expectancy of Swedish males and females from 1961 to 1997 (Statistiska Centralbyran 1964–1999).

U.S. POPULATION

As the reference of the above subject populations, recent U.S. population figures were selected. The lung cancer mortality rates of the U.S. population for 5-y age groups and sex in 1997 were derived from the number of lung cancer deaths reported by the U.S. Department of Health & Human Services (http://www.cdc.gov/nchs/data/gmwi/mwi_97.pdf) and populations by U.S. Census Bureau (http://www.census.gov/population/www/estimates/uspop.html). Life expectancy for females was 79.4 y and for males 73.6 y in 1997 (National Center for Health Statistics 1999).

RISK PROJECTION MODEL

Etiological risk of lifetime lung cancer mortality due to radon progeny exposure is evaluated at a radon progeny exposure rate of 0.1 WLM y\(^{-1}\) by adopting the relative risk projection model to the background lung cancer mortality of the subject population. Assuming 0.8 as an occupation factor and 0.4 as an equilibrium factor, 0.1 WLM y\(^{-1}\) is given by the indoor radon gas concentration of 22.4 Bq m\(^{-3}\).

According to the definition of relative risk, expected risk is expressed in proportion to the control (or background) lung cancer mortality of the subject population. This is a first simple approximation, and some modifying factors must be taken into consideration for more precise projection as the risk models in BEIR VI report (NRC 1999).

Excess relative risk and covered exposure period could be derived from each case-control study on residential radon and lung cancer or their meta-analysis (Lubin and Boice 1997; NRC 1999). For this study, a Swedish nationwide case-control study (Pershagen et al. 1994) was selected because it was based on the well-authorized cancer registry and radon monitoring, and the result showed the clear pattern of increasing relative risk with time-weighted mean radon exposure (Pershagen et al. 1993, 1994).

Because a subsequent study accounting for random error in the exposure assessment confirmed that measurements of radon concentration are more valid when study subjects who slept near open windows were excluded (Lagarde et al. 1997), a relative risk coefficient obtained by conditional logistic regression of all study subjects except those who slept near an open window was adopted in this study. Confounding by known risk factors, i.e., smoking, occupation, and urban living, was adjusted in the multivariate analysis. The resulting excess relative risk of lung cancer (thereafter abbreviated as ERR) was 0.020 (95% CI 0.0055–0.0433) WLM\(^{-1}\).

Since the Swedish epidemiological study (Pershagen et al. 1993, 1994) covered an average exposure period of 32.5 y for each case and control subject, estimated excess relative risk corresponds only to 32.5 y of cumulative radon exposure and does not reflect the lifetime risk of lung cancer mortality. Hence, radon exposure incurred 32.5 y or more before age at risk may be weighted more or less in the risk projection analysis, depending on hypotheses about the etiological relevance of such exposure.

Taking this into account, the risk projection model based on the Swedish case-control study is mathematically expressed as follows:

\[
r(\alpha) = r_s(\alpha)[1 + \beta \times (W_1 + RF \times W_2)],
\]

where

\[
r(\alpha) = \text{lung-cancer mortality rate for given age and calendar period by age } \alpha;
\]

\[
r_s(\alpha) = \text{background of lung cancer mortality rate in the population by age } \alpha;
\]

\[
\beta = \text{excess relative risk per unit radon progeny exposure per WLM}, = 0.020 \text{ (95% CI: 0.0055–0.0433) WLM}^{-1}. \text{(Pershagen et al. 1993, 1994)};
\]

\[
W_1 = \text{cumulative exposure in WLM incurred between 3 y and 32.5 y before age } \alpha;
\]

\[
W_2 = \text{cumulative exposure in WLM incurred 32.5 y or more before age } \alpha; \text{ and}
\]

\[
RF = \text{reduction factor for cumulative exposure, } W_2.
\]
The 3-y lag period in the model is based on the assumption that radon progeny exposures have no substantial effect on the lung cancer mortality for at least 3 y (Pershagen et al. 1994). The reduction factor, RF, is introduced in this model to account for modification of the risk coefficient related to exposure outside the 32.5-y period. This ad-hoc procedure allows conjectural extensions of the model to a wider time-frame in conjunction with values for RF varying from unity to zero. The lifetime risk of lung cancer mortality per unit WLM is the integration of [(r(a)−r0(a))] over the expected years of life divided by the lifetime exposure.

As the reference of the above model, two miner-based risk projection models authorized in BEIR VI report (NRC 1999) were also adopted to the subject populations. The Exposure-Age-Duration (EAD) model and the Exposure-Age-Concentration (EAC) model are the extrapolations from miner studies to the residential radon exposure and its lung cancer risk. Mathematical notations of EAD and EAC models are summarized in Appendix B.

RESULTS AND DISCUSSION

Fig. 3 shows the trend of etiological risk of lung cancer mortality per lifetime radon progeny exposure, projected to Japanese and Swedish populations by sex since 1962–1997, by applying the Swedish case-control-study-based risk projection model in the case of RF = 0. Error bars reflected the 95% confidence intervals of excess relative risk of the Swedish case-control-study (Pershagen et al. 1994).

Lifetime lung cancer risk for Japanese males increased constantly, from 0.99 (0.26–2.11)–4.87 (1.69–10.3) × 10⁻⁴ WLM⁻¹ from 1962–1997. For Swedish males, the lifetime lung cancer risk was estimated to be 1.77 (0.47–3.77) × 10⁻⁴ WLM⁻¹ in 1962, which was larger than that for Japanese male. It increased to reach the maximum of 2.93 (0.78–6.23) × 10⁻⁴ WLM⁻¹ in 1982, and then gradually decreased to 2.70 (0.72–5.75) × 10⁻⁴ WLM⁻¹ in 1997. This estimate was around half of that for Japanese males.

Lifetime lung cancer risk for Japanese and Swedish females was similar to increase constantly. From 1962–1997, it increased from 0.41 (0.11–0.87)–1.79 (0.47–

Fig. 3. Etiological risk of lung cancer mortality projected to the Japanese and Swedish populations from 1962–1997 by the Swedish case-control-study-based risk projection model in the case of RF = 0 at an exposure rate of 0.1 WLM y⁻¹.
3.82) × 10^{-4} \text{ WLM}^{-1} \text{ for Japanese females, and from } 0.51 (0.13-1.08)-1.50 (0.40-3.19) \times 10^{-4} \text{ WLM}^{-1} \text{ for Swedish females.}

The lung cancer mortality for Japanese males might be on the rise, while the total smoking rate decreased constantly. It may partly be explained that smoking rates in young age groups of Japanese males were more than 80%, and they did not decrease significantly before 1980 as shown in Fig. A2. In addition, per capita cigarette consumption of Japanese population increased constantly until 1980 as shown in Fig. A3. These may suggest that total exposure to tobacco of Japanese male was not necessarily decreased. Fig. A2 also shows the constant increase of smoking rates in the 20–29 y age groups of Japanese females since 1962, which may cause the increase of lung cancer risk for Japanese females as suggested in Fig. 3.

Swedish lung cancer mortality and smoking habits are discussed in the Swedish public health report (Swedish National Board of Health and Welfare 1998). Indeed, there are several complex reasons that influence the lung cancer mortality of each subject population, and careful epidemiological and biological analyses are required to understand the true situation. In the Swedish case-control study, a weak interaction was found between tobacco smoking and radon exposure, which was more than additive and less than multiplicative (Pershagen et al. 1994). Detailed discussions on tobacco smoking and radon exposure are shown in Appendix C, BEIR VI report (NRC 1999).

When the relative risk projection model was applied, etiological lung cancer risk due to lifetime radon exposure reflected the trend of background lung cancer mortality of each subject population by sex and age. Fig. 4 shows the age-specific etiological risk of lung cancer mortality for Japanese, Swedish, and U.S. populations by sex in 1997 at an exposure rate of 0.1 WLM y^{-1} by applying the three risk projection models. Swedish-case-control-study-based model with RF = 0 was applied, and the results were plotted with those of the two risk projection models in BEIR VI report (NRC 1999). Fig. 5 shows total etiological risk of lung cancer mortality per lifetime radon progeny exposure at an exposure rate of 0.1 WLM y^{-1} for Japanese, Swedish, and U.S. populations in 1997 projected by applying the above three risk projection models. Error bars in Fig. 5 reflect the 95% confidence intervals of excess relative risk for each risk projection model.

**Fig. 4.** Etiological risk of lung cancer mortality by age projected to the Japanese, Swedish, and U.S. populations in 1997 by the Exposure-Age-Duration (EAD) model, Exposure-Age-Concentration (EAC) model (BEIR VI, National Research Council 1999), and the Swedish case-control-study-based risk projection model in the case of RF = 0 at an exposure rate of 0.1 WLM y^{-1}.
As shown in Figs. 4 and 5, the largest risk projections were given by the Exposure-Age-Concentration model (NRC 1999) for all subject populations. For Japanese and U.S. populations, the minimum radon risk estimates were given by the Exposure-Age-Duration model (NRC 1999), while for Swedish populations they were given by the Swedish case-control-study-based model.

Since the Swedish case-control study corresponded to 32.5 y of exposure at a constant radon concentration of each case-control study subject (Pershagen et al. 1994), the risk related to exposure outside the 32.5-y period must be included in the lifetime lung cancer risk estimate by weighting the reduction factor, \( RF \), from unity to zero. Table 1 shows the etiological lifetime lung cancer mortality per lifetime radon progeny exposure projected to Japanese, Swedish, and U.S. populations (1997) by applying the risk projection model in the case of \( RF = 0 \), 0.5, and 1.0, respectively. In comparison with miner-based EAD and EAC models in Fig. 5, \( RF \) might be fixed to zero for Japanese and Swedish populations and 0.5 for the U.S. population at most. Consequently, lifetime lung cancer risk coefficients projected for Japanese, Swedish, and U.S. populations (1997) were included in the range from 1.50 (0.40–3.19) \( \times 10^{-4} \) WLM\(^{-1} \) (Swedish female, \( RF = 0 \)) to 9.86 (2.62–20.9) \( \times 10^{-4} \) WLM\(^{-1} \) (U.S. male, \( RF = 0.5 \)) as listed in Table 1.

The ICRP has recommended the conversion from radon progeny exposure to effective dose by a direct comparison of the detriment associated with a unit effective dose and a unit radon progeny exposure (ICRP 1994a). According to this method, the lifetime risk of lung cancer mortality per lifetime radon progeny exposure was compared with nominal risk coefficients for stochastic effects on atomic bomb survivors per unit effective dose, namely 7.3 \( \times 10^{-5} \) mSv\(^{-1} \) for the whole population (ICRP 1991). By a comparison of the above range of lung cancer mortality coefficients and the above nominal detriment coefficients, the possible range of conversion factors was estimated from 2.05 (0.55–4.37) to 13.5 (3.59–28.6) mSv WLM\(^{-1} \). ICRP proposed the nominal dose conversion factor of 3.88 mSv WLM\(^{-1} \) for the general public by an epidemiological approach (ICRP 1991), which was based on the lifetime lung cancer risk coefficient of 2.83 \( \times 10^{-4} \) WLM\(^{-1} \) for the reference.

<table>
<thead>
<tr>
<th>Population</th>
<th>Sex</th>
<th>Excess lifetime lung cancer mortality per 10,000 person WLM (95% CI)</th>
<th>Swedish-Epi-study-based risk projection model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( RF = 0 )</td>
<td>( RF = 0.5 )</td>
</tr>
<tr>
<td>Japan 1997</td>
<td>Male</td>
<td>4.87 (1.60–10.33)</td>
<td>8.40 (2.25–17.88)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>1.79 (0.47–3.82)</td>
<td>3.19 (0.85–6.79)</td>
</tr>
<tr>
<td>Sweden 1997</td>
<td>Male</td>
<td>2.70 (0.73–5.75)</td>
<td>4.40 (1.17–9.36)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>1.50 (0.30–3.19)</td>
<td>2.40 (0.64–5.11)</td>
</tr>
<tr>
<td>U.S. 1997</td>
<td>Male</td>
<td>5.94 (1.58–12.63)</td>
<td>9.86 (2.62–20.9)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>3.61 (0.96–7.69)</td>
<td>6.02 (1.60–12.8)</td>
</tr>
</tbody>
</table>
population (Land and Sinclair 1991) with the miner-based risk projection model.

On the other hand, the dosimetric approach by the computer-aided human respiratory tract model implies the arithmetic mean of effective dose per potential alpha energy exposure to be 17.2 mSv WLM$^{-1}$, with a modal value of approximately 15 mSv WLM$^{-1}$ (ICRP 1994b; Birchall and James 1994, 1999). The respiratory tract in vivo in vitro biodosimetry approach using rats by Brooks et al. (1999) found the absorbed dose per exposure for nasal epithelial cells and for deep lung epithelial cells to be 0.22–0.47 mGy WLM$^{-1}$ and 1.13–1.34 mGy WLM$^{-1}$, respectively. Taking a radiation-weighting factor for alpha particles of 10 to 20, these dosimetric approaches showed good agreement.

The estimated dose conversion coefficient in this study ranged from 2.05 (0.55–4.37) to 13.5 (3.59–28.6) mSv WLM$^{-1}$, and within this range the discrepancy between dosimetric and epidemiological approaches was included. It is important to assess the uncertainties within these alternative approaches and the selection of the models, and it is also important to account for a possible dependence on the background lung cancer mortality of subject populations and their time trends.

CONCLUSION

To answer quantitatively how much are the differences among the projected risks for specific populations with different risk projection models, the Swedish case-control study-based risk projection model was applied to the Japanese and Swedish populations 1962–1997 and U.S. population 1997.

When the relative risk projection model was applied, etiological lung cancer risk due to lifetime radon exposure reflected the trend of background lung cancer mortality of each subject population by sex and calendar year. For Japanese males, estimated lifetime lung cancer risk of radon exposure showed a significant increase from 0.99 (0.26–2.11) in 1962 to 4.87 (1.69–10.3) $\times 10^{-4}$ WLM$^{-1}$ in 1997.

For Swedish males it increased from 1.77 (0.47–3.77) $\times 10^{-4}$ WLM$^{-1}$ in 1962 to the maximum of 2.93 (0.78–6.23) $\times 10^{-4}$ WLM$^{-1}$ in 1982. For Japanese and Swedish females, it increased constantly from 0.41 (0.11–0.87)–1.79 (0.47–3.82) $\times 10^{-4}$ WLM$^{-1}$ for Japanese females, and from 0.51 (0.13–1.08)–1.50 (0.40–3.19) $\times 10^{-4}$ WLM$^{-1}$ for Swedish females. Significant increase of lifetime lung cancer risk for Japanese males during 1962–1997 reflected the increase of background lung cancer mortality rates, which may partly be caused by their trends of smoking habit from the 1930’s to the 1970’s.

Lifetime risk of radon exposure and lung cancer mortality projected for Japanese, Swedish, and U.S. populations in 1997 ranged from 1.50 (0.40–3.19) $\times 10^{-4}$ WLM$^{-1}$ to 9.86 (2.62–20.9) $\times 10^{-4}$ WLM$^{-1}$, which could be compared to the detriment associated with a unit effective dose, namely 7.3 $\times 10^{-5}$ mSv$^{-1}$ for the general population. Conclusive dose conversion coefficients in this study ranged from 2.05 (0.55–4.37) to 13.5 (3.59–28.6) mSv WLM$^{-1}$, which included the estimates of dosimetric and epidemiological approaches.

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The authors wish to express their deepest gratitude to Göran Pershagen, Institute of Environmental Medicine, Karolinska Institute, for his kind encouragement and invaluable academic direction throughout this work. The authors would also like to express their hearty appreciation to Anita Emf, Swedish Radiation Protection Institute, for her invaluable support to realize this collaborative work.

REFERENCES


APPENDIX A

Trends of smoking rate as a significant contributor to background lung cancer mortality of Japanese and Swedish populations from 1962 to 1998

As the health habit of the subject populations that may be tied-in with the background lung cancer mortality, the smoking rate of Japanese and Swedish populations by sex from 1962–1998 are illustrated in Fig. A1 (Japan Tobacco Inc. 1963, 1978, 1990; Swedish National Board of Health and Welfare 1998).

Trends of smoking rate of Japanese populations by 10-y age group from 1962 to 1998 was shown in Fig. A2 (Japan Tobacco Inc. 1963, 1978, 1990). Per capita cigarette consumption rate of Japanese population more

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**Fig. A1.** Smoking rate of Japanese and Swedish males and females from 1962–1998.
than 15 y old from 1920 to 1998 was shown in Fig. A3 (Japan Tobacco Inc. 1963, 1978, 1990). Detailed information on Japanese smoking rate and cigarette consumption rate are distributed by the Japanese Ministry of Health and Welfare (http://www.health-net.or.jp/kenkone/tobacco/front.html).

**APPENDIX B**

The miner-study based models in BEIR VI report, 1999

The BEIR committee developed the revised risk model of lung cancer risk associated with radon exposure, incorporating an updated assessment of dose-effect relationship from the latest review of historical miner cohort studies, and an associated extrapolations from miners to the general population.

The Exposure-Age-Duration (EAD) model is described as:

$$r(a) = r_s(a) \times [1 + \beta \times \phi(a) \times \gamma \times (W_1 + 0.72 \times W_2 + 0.44 \times W_3)],$$

where

- $\beta =$ excess relative risk, $0.553$ (95%CI $0.271-1.125) \times 10^{-2}$ per WLM;
- $r(a) =$ lung-cancer mortality rate for given age and calendar period; and
- $r_s(a) =$ background or baseline of lung cancer mortality rate in the population.

**Time-since-exposure Windows:**
- $W_1$: cumulative exposure in WLM incurred between 5 y and 14 y before age $a$;
- $W_2$: cumulative exposure in WLM incurred between 15 y and 24 y before age $a$; and
- $W_3$: cumulative exposure in WLM incurred 25 y or more before age $a$.

Attained age: $\phi(a)$: 1.0 when age $a$ is less than 55 y, 0.52 when $a$ is 55–64 y, 0.28 when $a$ is 65–74 y, and 0.13 when $a$ is 75 years or more.

Duration of exposure: $\gamma =$ 1.0 when duration is less than 5 y, 2.78 when duration is 5–14 y, 4.42 when duration is 15–24 y, 6.62 when duration is 25–34 y, and 10.20 when duration is 35 y or more.

The Exposure-Age-Concentration (EAC) model is described as

$$r(a) = r_s(a)\left[1 + \beta \times \phi(a) \times \gamma \times (W_1 + 0.78 \times W_2 + 0.51 \times W_3)\right],$$
where

\[ \beta = \text{Excess relative risk, } 7.681 \ (95\% \text{CI } 3.969 - 14.864) \times 10^{-2} \text{ per WLM;} \]

\[ r(a) = \text{lung-cancer mortality rate for given age and calendar period; and} \]

\[ r_\phi(a) = \text{background or baseline of lung cancer mortality rate in the population.} \]

Time-since-exposure windows:

- \( W_1 = \text{cumulative exposure in WLM incurred between 5 y and 14 y before age } a; \)
- \( W_2 = \text{cumulative exposure in WLM incurred between 15 y and 24 y before age } a; \) and

\[ W_3 = \text{cumulative exposure in WLM incurred 25 y or more before age } a. \]

Attained age: \( \phi(a) = 1.0 \text{ when age } a \text{ is less than 55 y; 0.57 when } a \text{ is 55–64 y; 0.29 when } a \text{ is 65–74 y; and 0.09 when } a \text{ is 75 y or more.} \]

Exposure rate (1 WL = 3,700 Bq m\(^{-3}\) EEC): \( \gamma = 1.0 \text{ when exposure rate is less than 0.5 WL; 0.49 when exposure rate is 0.5–1.0 WL; 0.37 when exposure rate is 1.0–3.0 WL; 0.32 when exposure rate is 3.0–5.0 WL; 0.17 when exposure rate is 5.0–15.0 WL; and 0.11 when exposure rate is 15 WL or more.} \]
Effects of \( \gamma \)-rays on the populations of the steady-state ecological microcosm

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Abstract. Purpose: To investigate the dose-response of an aquatic microcosm exposed to \( \gamma \)-rays and to test the suitability of micro-cosms for elucidation of the mechanisms that account for such ecological effects.

Materials and methods: The microcosm used in this study consisted of algae \textit{Euglena gracilis} Z as a producer, protozoa Tetrahymena thermophila B as a consumer and bacteria Escherichia coli DH5\(\alpha \) as a decomposer. After the steady-state microcosm was irradiated with \( \text{Co}^{60} \)-\( \gamma \)-rays at various dose levels, the population densities of each species were measured.

Results: Irradiation at up to 100 Gy did not affect the population of the microcosm except for a temporary decrease of \textit{E. coli} just after irradiation. At 500 or 1000 Gy, \textit{E. coli} died out just after irradiation. Only \textit{Eu. gracilis} and \textit{T. thermophila} could survive. Their populations, however, decreased compared with that of each control, except for a temporary increase of \textit{T. thermophila} after 1000 Gy irradiation. These population changes were attributable to the extinction of \textit{E. coli} in addition to the direct effects of radiation. Irradiation at 5000 Gy extinguished all species in the microcosm.

Conclusions: The response of the microcosm to radiation was dose-dependent over a range of high doses. The microcosm was also shown to be suitable for examining not only direct effects but also secondary effects.

1. Introduction

Ecosystems are exposed to various kinds of toxic agents derived from human activities. For conservation of ecosystems, the activities which are more harmful to ecosystems should be replaced with less harmful ones if possible. Therefore, the ecological effects of a toxic agent should be evaluated, as compared with other agents, in a proper way. For instance, the evaluation of the ecological effects of radiation compared with acidification will be a help to the decision-making of energy choice between nuclear power generation and thermal power generation.

The authors have started comparative studies with various toxic agents, in comparison with ionizing radiation. The reasons why radiation was chosen as a standard are: (1) radiation is one of the agents which potentially affects ecosystems, especially in the case of severe nuclear accidents such as Chernobyl and nuclear waste disposal to the deep sea or underground; and (2) dose estimation and the biological effects of radiation are investigated better than other toxic agents.

Radiation effects on wild organisms have been well investigated (IAEA 1976, 1988, 1992, NCRP 1991). However, the ecological effects of radiation have scarcely been investigated at the community level and there are few mechanistic studies of the ecological effects of radiation. Radiation may affect ecosystems, not only directly, but also secondarily because ecosystems consist of many species that have a wide range of radiosensitivities (Sparrow et al. 1967) and have complex interactions, for example predator-prey interactions. After one species has been damaged directly by radiation, another species which is more resistant to radiation may also be affected by the disappearance of interaction with that damaged species. Radiation effects on ecosystems cannot therefore be deduced from the effects on each organism isolated from the ecosystem, as shown in the case of ultraviolet radiation (Bothwell et al. 1994) or some chemicals (Mosser et al. 1972, Hutchinson and Czyska 1975, Taub 1976, Wilkes 1978, Crow and Taub 1979).

Radiation effects on ecosystems have been observed in high-background radiation areas (Léonard et al. 1981), nuclear test sites (Templeton et al. 1971) and natural fields irradiated experimentally (Woodwell 1967, Hinckley 1971) or contaminated by the accidents of nuclear facilities (Kozubov and Taskaev 1990, Krivolotsky and Pokarzhevsky 1990). These approaches, however, have not always led to correct evaluation of radiation effects and could not always contribute to the elucidation of the mechanisms which account for the ecological effects of radiation, because natural ecosystems are extremely complex and are affected by many variable factors.

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apart from radiation. An attempt has therefore been made to evaluate the radiation effects on ecosystems by using a microcosm.

Microcosms are experimental ecosystems constructed in the laboratory to have some of the physical, chemical and biological elements of natural ecosystems. Microcosms contain interactions among those elements as do natural ecosystems. Microcosms provide biotic or abiotic simplicity, controllability and replicability (Beyers and Odum 1993). It has been demonstrated that microcosms can act as fairly realistic models of natural ecosystems (Beyers 1963, Cooke 1967, Gordon et al. 1969). Microcosms have therefore been used for studies of basic ecology or the evaluation of ecological effects of toxic agents at the community level (Beyers and Odum 1993).

This paper investigated the effects of γ-rays on populations of a steady-state aquatic microcosm. The aim was: (1) to investigate the dose–response in an aquatic microcosm exposed to γ-rays; and (2) to test the suitability of microcosms for the elucidation of mechanisms that account for ecological effects.

2. Materials and methods

2.1. Microcosm

The microcosm used in this study was synthesized by Kawabata et al. (1995). It consisted of flagellate algae Euglena gracilis Z as a producer, ciliate protozoa Tetrahymena thermophila B as a consumer and bacteria Escherichia coli DH5α as a decomposer. The culture medium was a half-strength modified #36 Taub and Dollar’s salt solution (Taub and Dollar 1968) containing proteose peptone (Difco Laboratory, Detroit, MI, USA) of 500 mg/l. The microcosm was cultured in an incubator with fluorescent lamps under a 2500 lx and a 12 h light–dark cycle at 25°C.

In the microcosm, the population change of each organism reached a steady state 50 days after inoculation as a result of interactions among the species. All species can co-exist in the microcosm for as long as 1 year. When T. thermophila was cultured alone in the microcosm medium it died out without reaching a steady state and Eu. gracilis and E. coli when cultured alone in the microcosm medium could not exist so stably as they do in the microcosm.

In the microcosm, proteose peptone mainly contributes to the growth of Eu. gracilis and E. coli in the early stage of culture. After exhaustion of proteose peptone, the microcosm is maintained with energy which Eu. gracilis fixes by photosynthesis. Each species is supported with metabolites or the breakdown products of the other two species. T. thermophila cannot exist without E. coli because T. thermophila grazes E. coli as its staple food (Nakajima and Kawabata 1996).

2.2. Procedures

The microcosms were synthesized in 250 ml polypropylene bottles with screw caps (Nalgene, Rochester, NY, USA) containing 150 ml culture medium. They were irradiated with 60Co γ-rays (0, 50, 100, 500, 1000, 5000 Gy) at a dose rate of 50 Gy/min on the 56th day after the beginning of the culture. Four bottles of microcosm were irradiated with γ-rays at each dose and five bottles of microcosm were kept as unirradiated controls.

The population density of each organism was measured before irradiation and over 320 days after irradiation. The population of Eu. gracilis and T. thermophila was counted microscopically in a 1 mm deep chamber of 50 x 20 mm², having a 1 mm² grid. The number of viable E. coli was measured by counting colonies formed in a broth–agar medium (extract bonito: 3-0 g; polypeptone: 3-0 g; NaCl: 5-0 g; agar powder: 15-0 g; distilled water: 1 l; pH adjusted to 7-0) after incubation at 25°C for 5 days.

3. Results and discussion

3.1. Radiation effects on the cell populations of the microcosm

Figure 1 shows the changes in the population densities of the three species in the irradiated microcosm compared with non-irradiated controls. The patterns of changes observed can be separated into three groups as follows.

3.1.1. 50 or 100 Gy irradiation. At 50 or 100 Gy, the population of E. coli in the microcosm decreased just after irradiation compared with controls. Irradiation at 100 Gy decreased E. coli more significantly than at 50 Gy. However, in both cases E. coli soon recovered to control levels, and then its population density was maintained at control level. Irradiation at 50 or 100 Gy did not affect the populations of Eu. gracilis and T. thermophila. It is therefore concluded that the microcosm used in this study is resistant to up to 100 Gy γ-irradiation.

3.1.2. 500 or 1000 Gy irradiation. At 500 Gy, E. coli died out shortly after irradiation. After 500 Gy, the population density of Eu. gracilis in the microcosm was unchanged for a long time, and then became lower than that of the controls. This decrease was first observed on the 264th day after irradiation. This decrease is, therefore, thought to be a secondary effect rather than a direct effect of radiation. That is, it is
Figure 1. Population changes in the microcosm after irradiation with γ-rays. Solid lines represent results of irradiated microcosms. Broken lines represent results of controls. Error bars are standard deviations. Asterisks indicate statistically significant differences (p<0.05, Student's t-test).
thought that the extinction of *E. coli* in the irradiated microcosm decreased *Eu. gracilis* because metabolites of *E. coli* contribute the growth of *Eu. gracilis* in the microcosm (Nakajima and Kawabata 1996).

After 500 Gy irradiation, the population of *T. thermophila* in the microcosm decreased compared with that of controls. This decrease is not thought to be direct damage of radiation because lethal dose (LD₉₀) for pure-cultured *Tetrahymena pyriformis* is elucidated to be approximately 4000 Gy (Elliott 1959). It is thought that 500 Gy irradiation decreased *T. thermophila* by extinguishing *E. coli* in the microcosm; *T. thermophila* cannot exist without *E. coli*, which *T. thermophila* grazes as a staple food (Nakajima and Kawabata 1996).

At 1000 Gy, *E. coli* in the microcosm died out just after irradiation. After 1000 Gy irradiation, the population of *Eu. gracilis* in the microcosm decreased compared with controls. This decrease was more significant than the decrease after 500 Gy irradiation. Irradiation at 1000 Gy also began to decrease *Eu. gracilis* earlier than that after 500 Gy. These results suggest that the decrease of *Eu. gracilis* in the microcosm irradiated with 1000 Gy γ-rays was caused by direct radiation damage in addition to the secondary effects.

After 1000 Gy irradiation, the population of *T. thermophila* in the microcosm increased temporarily, and then decreased compared with controls. However, irradiation at 1000 Gy did not decrease *T. thermophila* as much as 500 Gy. This population change is thought to be a result of secondary effects rather than direct effects of radiation because *Tetrahymena* is radioreistant. The mechanism is thought to be that as a result of the direct effect of 1000 Gy irradiation, breakdown products of *E. coli* were left in the microcosm. These contributed to the growth of *T. thermophila*. Since dead *E. coli* also contribute to the growth of *Eu. gracilis*, the latter competes with *T. thermophila* (Nakajima and Kawabata 1996). The decrease of *Eu. gracilis* after 1000 Gy irradiation also provided *T. thermophila* with an advantage in the competition for breakdown products of *E. coli*. Therefore, *T. thermophila* temporarily increased in the microcosm after irradiation. When the consumption of breakdown products was largely complete, *T. thermophila* decreased. However, 1000 Gy irradiation did not decrease *T. thermophila* as much as 500 Gy irradiation because 1000 Gy irradiation decreased *Eu. gracilis*, whose breakdown products contribute to the growth of *T. thermophila* (Nakajima and Kawabata 1996).

Gamma irradiation at 500 or 1000 Gy extinguished *E. coli* in the microcosm. This resulted in the disappearance of interactions between *E. coli* and the other two species that are necessary to their survival. As a result of the disappearance of these interactions, in addition to the direct damage of radiation, the population of *Eu. gracilis* and *T. thermophila* changed compared with controls, and did not fully recover. Thus, 500 or 1000 Gy irradiation extinguished one species and changed the populations of the other two species in the microcosm.

3.1.3. 5000 Gy irradiation. At 5000 Gy, all species in the microcosm died out shortly after irradiation and the microcosm was completely destroyed.

The response of the microcosm was therefore dose-dependent within a high-dose range. A dose of 50 or 100 Gy was required for even temporary damage, 500 or 1000 Gy for severe damage, and 5000 Gy for complete destruction.

3.2. Significance and problems of microcosm tests for the evaluation of radiation effects on ecosystems

These results suggest that some species in ecosystems may respond to radiation differently from those in pure cultures. This phenomenon has been observed in natural ecosystems. For example, in the irradiated forest ecosystem the population and activity of micro-organisms decreased in spite of their radioreistance, a decrease that was thought to be the result of secondary effects of radiation, i.e. due to destruction of trees and shrubs by radiation (Poinset-Balagué et al. 1991). Populations of leaf mining insects and aphids increased in the irradiated forest ecosystem, though radiation is expected to damage those species directly. This increase was thought to be a result of radiation damage to trees (Woodwell and Brower 1967, Hinckley 1971).

Microcosm tests therefore may be useful to investigate the mechanisms of ecological effects of radiation, for instance, to determine whether the effects arise directly or secondarily.

In present study, some radiation effects on the microcosm were first observed long after irradiation. At 500 Gy, the decrease of *Eu. gracilis* was first observed on the 264th day after irradiation. At 1000 Gy, *T. thermophila* temporarily increased and then began to decrease compared with that of controls on the 130th day after irradiation. These results indicate that it takes considerable periods of time to express radiation effects at the ecosystem level, and long-term studies are needed for evaluation of radiation effects on entire ecosystems.

In general there is some conceptual danger in extrapolating directly from model ecosystem data to natural ecosystems. It is, however, reported that an aquatic microcosm responded to heavy metals in a
similar manner to natural ecosystems (Shen et al. 1986). It is also reported that an aquatic microcosm responded to anionic surfactant in a similar manner to a model ecosystem derived from lake water, which had a greater number of species and is thought to more closely resemble natural lake ecosystems than the microcosm (Takamatsu et al. 1997). Microcosm tests can be applied to the prediction of the ecological impact of radiation by using an adequate safety factor. In general, 1–1/3 is adopted as the safety factor for extrapolating from model ecosystem data to natural ecosystems, but 1/100 for extrapolating from single-species assessment to natural ecosystems (OECD 1981, CEC 1993, Nabholz et al. 1993). This means that microcosm tests are at least better for the evaluation of ecological impacts than single-species assessment.

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Different cytotoxic response to gadolinium between mouse and rat alveolar macrophages

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Abstract—The cytotoxicity of gadolinium (Gd) chloride was investigated in alveolar macrophages (AM) cultured in vitro. A marked difference in the cytotoxic response to Gd was found between mouse and rat AM. The viability of rat AM was decreased by exposure to Gd at doses more than 3 μM, while mouse AM appeared to be resistant even up to 1000 μM Gd exposure. The decrease in the viability of rat AM exposed to Gd at doses up to 1000 μM was mitigated by centrifugation and filtration of the culture medium containing Gd, or by the treatment of AM with lysosomotropic agents such as NH4Cl or chloroquine, suggesting that the cytotoxic response of rat AM to Gd at doses up to 1000 μM was dependent on the intracellular uptake and subsequent dissolution of Gd present in the culture medium in colloidal form. The phagocytic activity of mouse AM, evaluated by the uptake of latex particles, was higher than that of rat AM. Furthermore, quantitative analysis of Gd with inductively coupled plasma-mass spectrometry revealed that mouse AM took up a larger amount of Gd than rat AM. Therefore, the marked difference in the cytotoxic response to Gd between mouse and rat AM could not be attributed to the phagocytic activities for the colloidal form of Gd. The cytotoxic sensitivity of AM to Gd present in non-colloidal form was almost the same between mouse and rat AM. Therefore, it is suggested that the extent to which Gd-colloid phagocytosed is dissolved in the phagolysosome or the subsequent process to exhibit the cytotoxicity may be different between mouse and rat AM.

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Keywords: gadolinium; cytotoxic; macrophages; alveolar; colloidal; phagocytosis; cadmium; apoptosis.

Abbreviations: AM = alveolar macrophages; DMEM = Dulbecco's modified Eagle's medium; FCS = fetal calf serum; Gd = gadolinium; HBSS = Hanks' balanced salt solution; polyHEMA = poly 2-hydroxyethyl methacrylate.

INTRODUCTION

The use of gadolinium (Gd), a lanthanide element, has recently increased with the rapid development of advanced technologies (Hirano and Suzuki, 1996). Gd combined with chelating agents has been extensively used in the field of clinical medicine as a contrast agent for magnetic resonance imaging (Rocklage et al., 1991; Runge and Parker, 1997; Weimann et al., 1984). It has also been used medically and experimentally to block the reticuloendothelial system, since Lazar (1973) reported this action of rare earth metals in rats. Intravenous administration of Gd inhibited the phagocytic activity of Kupffer cells (liver macrophages) and so has been frequently used to study the role of Kupffer cells in physiological and pathological conditions. From these studies, it has been suggested that Gd affected not only the phagocytic activity of Kupffer cells (Suzuki et al., 1996; Ruttiger et al., 1996) but also their ability to produce interleukins (Callery et al., 1990; Rai et al., 1996), superoxide (Liu et al., 1995), NO (Roland et al., 1994, 1996) and chemoattractant (Hisama et al., 1996), as well as their viability (Kohno et al., 1997; O'Neil et al., 1994; Sarphie et al., 1996).

There have been a few studies on the effects of Gd on lung macrophages in vivo. The phagocytic activity of pulmonary intravascular macrophages was significantly depressed in sheep by the administration of Gd (Molina et al., 1995). It was shown that O2−-induced enhancement of O2− and NO production by alveolar macrophages (AM) was attenuated by Gd pretreatment of rats (Pendino et al., 1995), and that
lung injury after intratracheal instillation of lipopolysaccharide was alleviated by GdCl₃ in guinea pigs (Tasaka et al., 1996). It was obvious from these studies that Gd affected lung macrophages; however, the extent of toxicity of Gd for the macrophages remained obscure. Recently Mizgerd et al. (1996) demonstrated the lethal effects of Gd on rat AM cultured in vitro. We have also studied the cytotoxic effects of Gd on mouse AM, but did not find the same lethal effect. This prompted us to compare the cytotoxicity of Gd between mouse and rat AM, and we here report a marked difference in the cytotoxic response to Gd between macrophages from the two species.

MATERIALS AND METHODS

Materials

The following items were purchased: gadolinium chloride hexahydrate (GdCl₃·6H₂O) and cadmium chloride hemi pentahydrate (CdCl₂·2.5H₂O) from Wako Pure Chemical Industries, Ltd (Osaka, Japan); Dulbecco’s phosphate buffered saline (PBS), Dulbecco’s MEM (DMEM), Hanks’ balanced salt solution (HBSS), 100 bp DNA ladder and DNA MASS™ Ladder from Gibco BRL (Grand Island, NY, USA); poly 2-hydroxyethyl methacrylate (polyHEMA), NH₄Cl and chloroquine from Sigma Chemical Co. (St Louis, MO, USA); fetal calf serum (FCS) from C&C Lab (Tokyo, Japan); latex particles with 2 μm in diameter from Polyscience Inc. (Warrington, PA, USA); and ultrapure grade of HNO₃, HF and HClO₄ from Tama Chemicals Co., Ltd (Kawasaki, Japan). Complete culture medium consisted of DMEM supplemented with heat-inactivated FCS (10%) and 100 U penicillin/ml and 100 μg streptomycin/ml.

Alveolar macrophages

Two strains of male rats, Sprague–Dawley (SD) and Wistar, and two strains of male mice, C3H and C57Black/6 (B6) were purchased from the domestic breeder (SLC Co., Ltd, Shizuoka, Japan), and were housed in a controlled environment with access to food and water ad lib. All the animal experiments were carried out with permission and according to the regulations of the Institutional Committee for Animal Safety and Welfare of the National Institute of Radiological Sciences, and Regulations on Appropriate Animal Breeding and Treatment, Ministry Office of Japan. After the animals were anaesthetized by halothane and killed by exsanguination, resident AM were harvested by repeated post-mortem bronchoalveolar lavage with PBS according to the procedure described elsewhere (Kubota et al., 1994). 0.5–1.0x10⁶ cells per mouse and 0.5–1.0x10⁷ cells per rat were collected. The proportion of AM was greater than 95% of the lung lavage cells based on morphological criteria, the remainder being lymphocytes and neutrophils. More than 98% of AM were viable according to trypan blue exclusion. The cells were then washed and suspended in the complete culture medium.

Gd and Cd preparation

GdCl₃·6H₂O and CdCl₂·2.5H₂O were dissolved in sterile saline to be a concentration of 30 mm and then each preparation was diluted serially with complete culture medium. Where the effects of the particulate (colloidal) form of Gd and Cd preparations were studied, the preparations were centrifuged at 12,000 g for 30 min after diluting to the designated concentrations, and the supernatants were filtered with a 0.22 μm membrane filter (Millipore MILLEX, Millipore, Bedford, MA, USA) to remove the particulate form (the resultant solutions are referred in the following as filtered Gd and Cd preparations).

Cell culture

The collected AM were plated in 24-well microculture plates (Falcon 3046, B&D Lab, NJ, USA) precoated with polyHEMA to prevent AM from adhering, at a cell density of 5x10⁵ in 0.5 ml complete culture medium per well. 0.5 ml complete culture medium containing one or a combination of test agents (Gd, Cd, NH₄Cl, chloroquine) was then added to the well to reach the designated final concentrations and the incubation was started at 37°C in a humidified atmosphere of 5% CO in air (this time point was defined as 0 hr). In the experiment where filtered Gd and Cd preparations were used, 5x10⁵ cells pelleted in an Eppendorf tube were resuspended in 1 ml filtered Gd or Cd preparations and cultured in 24-well microculture plates.

Determination of cell viability

At the end of culture, cells and medium were transferred to an Eppendorf tube and centrifuged. Then 0.4% of trypan blue in saline was added to the cell pellets, and the number and viability of AM were determined microscopically. To compensate for variation in experimental conditions in each series of experiments, we calculated and used a standardized viability. Thus the term “viability” in the following denotes the percentage of the viable cell number in metal-loaded culture to that in control culture at the corresponding time point. Two wells were prepared for each data point. The mean and standard error of the cell viability were calculated from three separate experiments.

Morphological study of AM

The mode of cell death induced in AM by exposure to Gd was examined by light microscopy, as described previously (Kubota et al., 1994). After 48 hr of culture, the cells were collected by centrifugation, smeared on glass slides and dried in air. Then the cells were fixed with acetic acid–ethanol (1:3) for 10–20 min and stained with Giemsa. The percentage of cells showing pyknosis and/or nuclear fragmentation was calculated by observing at least 200 AM in each individual sample under the microscope.
DNA extraction and agarose gel electrophoresis

DNA was extracted by the method of Martin and Cotter (1991) with slight modification. After protease K and RNAse digestion of the cell lysate, the crude DNA was extracted twice using phenol–chloroform buffered with 0.1 M Tris–HCl (pH 7.8), then precipitated in the presence of 0.2 M NaCl and 70% ethanol at −20°C for 1 hr. Finally, the DNA was rinsed with 70% ethanol, and resuspended in 20–30 μl Tris-EDTA buffer. Electrophoresis of the DNA was carried out in a 1.2% agarose gel containing 0.5 μg of ethidium bromide/ml at 100 V for 1 hr in TAE buffer containing 0.5 μg of ethidium bromide/ml. As size markers, 100 bp DNA ladder and DNA MASS Ladder were used.

Phagocytic activity of AM

AM were prepared as described above. At the beginning of culture, latex particles 2 μm in diameter were loaded to reach a final concentration of 1.5×10⁷ particles/ml, that is the ratio of particles to AM 30:1. The cells were fixed and stained as described above 8, 24 and 48 hr after the beginning of culture. For each sample, the number of particles phagocytosed by each cell was counted under a microscope at least in 200 individual AM. Individual AM was classified according to the number of phagocytosed particles, namely 0, 1–5, 6–10, 11–20, or >20 particles. The number of classified AM was expressed as a percentage of the total AM counted. The mean and standard error were calculated from three separate experiments.

Quantitative analysis of Gd incorporated into AM

AM were exposed to 300 μM Gd in the same culture conditions as described above, except that the incubation time was 0, 4 and 10 hr, and that the incubation temperature was 4 or 37°C. At the end of culture, 10⁶ cells were recovered on the filter from two wells of the 24-well culture plates by the filtration through a 47-mm Nuclepore polycarbonate filter with 3 μm pore size (Corning Separations Division, Acton, MA, USA). In a preliminary experiment it was confirmed that Nuclepore filter with this pore size effectively trapped both mouse and rat AM by observing that no AM was found in the filtered solution. AM trapped on the filter were washed twice with 5 ml HBSS and lysed with 1 ml of 1% sodium dodecyl sulfate and 10 mM EDTA in PBS. The resultant cell lysate was digested with acids (HNO₃, HF and HClO₄) using a microwave digester (CEM, MDS2000). After digestion, the samples were heated on a hotplate, then redissolved in 1% HNO₃. The amount of Gd was measured by ICP-MS (Yokogawa PMS-2000, Yokogawa Analytical Systems, Tokyo, Japan) (Yoshida and Muramatsu 1997). Internal standards were used to compensate for the drift of analytical signals during the operation. A standard solution was prepared from SPEX Multi Element Plasma Standards (SPEX Industries Inc., XSTC-1,7,8 and 13) and used to make calibration curves. The amounts of Gd in serially diluted Gd preparations (Gd solution in complete culture medium) and the corresponding filtered Gd preparations were also determined by ICP-MS with the same protocol as described for the cell lysate, and the percentage of Gd existing in a non-colloidal form (amount of Gd in filtered Gd preparation) in total Gd (amount of Gd in Gd preparation) was calculated.

Statistical analysis

The mean and standard error were calculated from the data of independent samples; however, error bars were omitted in some figures because they overlapped on many data points. A two-sample Student’s t-test was used to evaluate the critical data. The difference between means was regarded as significant if \( P < 0.05 \)

RESULTS

Figure 1 shows the viability of mouse and rat AM after 48 hr of exposure to Gd or Cd. The viability of AM was decreased by increasing exposure to Cd, although the dose–response varied with species and strain. The cytotoxicity of Cd for Wistar rat AM appeared to be slightly higher than for AM from other animals examined. The viability of AM exposed to Gd was remarkably different between mice and rats. The viability of SD rat AM decreased linearly up to 10 μM Gd, and was less than 10% at 10 μM. In contrast, the viability of B6 mouse AM was not affected at doses up to 1000 μM. At 3000 μM, Gd killed both mouse and rat AM almost completely. As the cytotoxicity of Gd for AM was similar between B6 and C3H mice and between SD and Wistar rats, B6 mouse and SD rat AM were used in the following studies.

Plate 1 shows light microscopic photographs of AM exposed to Gd for 48 hr. SD rat AM exposed to 300 and 3000 μM Gd and B6 mouse AM exposed to 3000 μM Gd contained a number of cells with condensed nuclei and/or two to three fragmented nuclei within a cell, while B6 mouse AM exposed to 300 μM Gd appeared to be intact. To obtain quantitative data, the percentage of the apoptotic cells, that is the cells with condensed nuclei and nuclear fragmentation, was determined. As shown in Fig. 2, the percentage of the apoptotic cells was markedly higher in SD rat AM exposed to 300 and 3000 μM Gd and in B6 mouse AM exposed to 3000 μM Gd, compared with those in control AM and B6 mouse AM exposed to 300 μM Gd. Plate 2 shows the migration profile of DNA extracted from AM in agarose gel electrophoresis. DNA from B6 mouse AM exposed to 3000 μM Gd and from SD rat AM exposed to 300 and 3000 μM Gd revealed a distinct ladder migration pattern (lanes 3,5 and 6, respectively), indicating
Fig. 1. The viability of mouse and rat AM after 48 hr of exposure to Gd (left) and Cd (right). The viability was expressed as the percentage of the viable cell number to that in control culture. Each data point is a mean of three separate experiments. Error bars were omitted because they overlapped on many data points. Asterisks indicate the statistically significant difference from C3H and B6 mice at the corresponding concentration of Gd or Cd.

Fig. 2. The percentage of apoptotic cells showed pyknosis and/or nuclear fragmentation at 48 hr of culture. At least 200 AM were observed in each individual sample. The mean and standard error were calculated from three separate experiments. Asterisks indicate the statistically significant difference from the control (0 μM Gd) in B6 mice and SD rats, respectively.

Internucleosomal DNA fragmentation that is a representative feature of apoptosis. On the other hand, as expected from the data of Plate 1 and Fig. 2, the ladder pattern of DNA migration was not observed in the control AM (lanes 1 and 4) and B6 mouse AM exposed to 300 μM Gd (lane 2).

Observations of the culture under an inverted phase-contrast microscope revealed that Gd preparations (Gd in complete culture medium) contained particulate matter, the amount of which appeared to depend on Gd concentration, suggesting that Gd might exist as a colloidal form. Cd and Gd
Fig. 3. Effects of filtration of Gd (right) and Cd (left) preparations on cell viability determined at 48 hr of culture in B6 mouse (upper) and SD rat (lower) AM. Each data point is a mean of three separate experiments. Error bars were omitted because they overlapped on many data points. Asterisks indicate the statistically significant difference from those exposed to filtered Gd at the corresponding concentration of Gd. Note the scale of Gd concentration is different between upper and lower graphs.

Preparations were centrifuged and filtered to remove particulate matter, and then the cytotoxic effects of filtered and non-filtered preparations were compared (Fig. 3). Filtration did not alter the cytotoxicity of Cd preparations for both mouse and rat AM. In contrast, the filtered Gd preparations made by the filtration of originally 3–1000 μM Gd preparations exhibited a markedly reduced cytotoxicity for rat AM in comparison with non-filtered preparations. Filtration did not affect the cytotoxicity of
Fig. 4. The effect of NH₄Cl treatment on cytotoxicity of Gd for B6 mouse AM (center), Cd for B6 mouse (left) and SD rat (right) AM. Cell viability was determined at 48 hr of culture. Each data point is a mean of three separate experiments. Error bars were omitted because they overlapped on many data points. There was no statistically significant difference between NH₄Cl treated and non-treated samples. Note: the scale of horizontal axis (metal concentration) is different between the graphs.

Fig. 5. The effect of NH₄Cl (left) or chloroquine (right) treatment on the cytotoxicity of Gd for SD rat AM. Cell viability was determined at 48 hr of culture. Each data point is a mean of three separate experiments. Error bars were omitted because they overlapped on many data points. Asterisks indicate the statistically significant difference from non-treated AM at the corresponding concentration of Gd.

3000 μM Gd preparation for either mouse or rat AM.

Figures 4 and 5 show the effects of NH₄Cl or chloroquine treatment on the cytotoxicity of Cd and Gd for mouse and rat AM. These are well known lysosomotropic agents which elevate pH in lysosomes and consequently suppress the acidic lysosomal enzyme activities. The treatment of NH₄Cl had no effect on the cytotoxicity of Cd for either mouse or rat AM. On the other hand, the cytotoxicity of Gd at doses up to 1000 μM for SD rat AM was greatly reduced by treatment with NH₄Cl or chloroquine at appropriate concentrations. The cytotoxicity of Gd at a dose of 3000 μM was not altered by NH₄Cl or chloroquine treatment in either mouse or rat AM.

The phagocytic activity of mouse and rat AM was evaluated by adding latex particles to the culture of AM (Fig. 6). At all time points examined, both the percentage of AM phagocytosing more than one particle, and the percentage containing a relatively large number of particles, that is more than 11, were consistently higher in B6 mouse AM, indicating that the phagocytic activity of B6 mouse AM was higher than that of SD rat AM.
Different cytotoxic response to gadolinium between mouse and rat alveolar macrophages

Fig. 6. The percentage of AM classified according to the number of latex particles phagocytosed by individual AM. B6 mouse and SD rat AM were exposed to latex particles for 8, 24 and 48 hr. The mean and standard error were calculated from three separate experiments. Asterisks indicate the statistically significant difference from B6 mouse AM at the corresponding data points.

The amount of Gd incorporated into AM was shown in Table 1. The values at 0 hr of incubation, where $10^6$ AM were suspended in 2 ml of 300 $\mu$m Gd preparation and immediately separated from the Gd preparation through the filter, were approximately 0.1% of total Gd in the preparations, validating the experimental procedure with a very small amount of non-specific Gd contamination. The values at 4°C, which might be considered as non-specific adsorption of Gd to AM because phagocytosis is suppressed at 4°C, appeared to be similar between B6 mouse and SD rat AM. The uptake of Gd by B6 mouse AM incubated at 37°C was approximately twice that by SD rat AM at both 4- and 10-hr incubations.

Finally, the cytotoxicity of filtered Gd preparation made by the filtration of original 3000 $\mu$m Gd preparation was compared between mouse and rat AM at several time points. As shown in Fig. 7, SD rat and B6 mouse AM exhibited almost the same cytotoxicity to the particulate-free Gd preparation.

**DISCUSSION**

Gd has been utilized extensively as a functional blocking agent of the reticuloendothelial system. However, the extent and the mechanism of toxicity of Gd for macrophages has not been fully elucidated. Mizgerd et al. (1996) reported in SD rats that in vitro exposure of AM to Gd caused cell death in both dose- and time-dependent fashions, with a significant decrease in AM viability by the exposure to more than 27 $\mu$m Gd for 24 hr of culture. In the present
study, the viability of both SD rat and Wistar rat AM was diminished by 48 hr of exposure of 3–10 μM Gd (Fig. 1). Although direct comparison is difficult because of the different experimental protocols, the degree of Gd toxicity observed appeared to be similar to those observed in the present study.

Interphase cell death can be induced via either of two morphologically and biochemically distinct modes: necrosis or apoptosis (Kerr et al., 1972; Wyllie, 1980). Apoptosis has been defined on the basis of two hallmarks: characteristic cell morphology including nuclear condensation and fragmentation; and internucleosomal DNA fragmentation revealed as a ladder migration pattern in DNA electrophoresis. All observations presented in Plates 1 and 2 and Fig. 2 consistently indicated that the interphase cell death of AM induced by Gd exposure was apoptotic in both mice and rats. Mizgerd et al. (1996) also identified Gd-induced apoptosis in SD rat AM, and our findings were consistent with their findings.

Rare earth metals have been known to form colloidal macromolecules as carbonate, phosphate and hydroxide complex in a physiological condition.

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Table 1. Uptake of Gd by B6 mouse and SD rat AM after 6, 4 and 10 hr

<table>
<thead>
<tr>
<th>Incubation time (hr)</th>
<th>Incubation temperature (°C)</th>
<th>AM</th>
<th>% Gd(^a)</th>
<th>Exp. I</th>
<th>Exp. II</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>-</td>
<td>B6 mouse</td>
<td>0.06</td>
<td>n.d</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>-</td>
<td>SD rat</td>
<td>n.d</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>B6 mouse</td>
<td>0.28</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>SD rat</td>
<td>0.22</td>
<td>0.27</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>37</td>
<td>B6 mouse</td>
<td>1.12</td>
<td>0.96</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>37</td>
<td>SD rat</td>
<td>0.58</td>
<td>0.60</td>
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<tr>
<td>10</td>
<td>37</td>
<td>B6 mouse</td>
<td>0.27</td>
<td>0.40</td>
<td></td>
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<tr>
<td>10</td>
<td>37</td>
<td>SD rat</td>
<td>0.31</td>
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<tr>
<td>10</td>
<td>37</td>
<td>B6 mouse</td>
<td>1.40</td>
<td>2.00</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>37</td>
<td>SD rat</td>
<td>0.78</td>
<td>0.86</td>
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</tbody>
</table>

* Amount of Gd per 10⁶ AM, expressed as a percentage of total amount of Gd added, i.e. 2 ml 300 μM Gd.

Plate 2. The migration profile of DNA extracted from AM at 48 hr of culture. Lane M, a molecular size marker of 100 bp DNA; Lane 1, control B6 mouse AM; Lane 2, B6 mouse AM exposed to 300 μM Gd; Lane 3, B6 mouse AM exposed to 3000 μM Gd; Lane 4, control SD rat AM; Lane 5, SD rat AM exposed to 300 μM Gd; Lane 6, SD rat AM exposed to 3000 μM Gd; Lane m, a molecular size marker of DNA MASS™ Ladder.
(Hirano et al., 1996). We identified particulate matter on the bottom of culture plates with Gd preparations under an inverted phase-contrast microscope. A white cloudy substance was also observed immediately after adding Gd solution dissolved in sterile saline into the culture medium at high concentrations. From these observations, it is apparent that part of the Gd might exist as colloid. Gd was included in almost all experiments of the present study because it did not precipitate, and so to compare its cytotoxicity in this rather simpler case. The primary mechanism by which Gd has been considered as a benign phagocytic blocking agent (Lazar, 1973) without adverse effects on non-phagocytic cells, might be attributed to this chemical characteristic of Gd to form colloidal particles in vivo, which are exclusively taken up by phagocytic cells such as Kupffer cells. To determine whether the cytotoxicity was related to the phagocytosis of colloidal Gd in this study, Gd preparations were centrifuged and filtered to remove particulate matter. As shown in Fig. 3, this treatment greatly reduced the cytotoxicity for SD rat AM. This may be interpreted as indicating that the cytotoxicity was caused by the particulate form of Gd in the non-filtered medium, and that below an initial concentration of 1000 μM there was insufficient non-particulate Gd remaining after centrifugation and filtration to cause significant cytotoxicity. Furthermore, the cytotoxic effect of Gd was also diminished in a dose-dependent manner when rat AM were treated with NH₄Cl or chloroquine, well-known lysosomotropic agents (Brazil et al., 1997; Rao et al., 1995). NH₄Cl or chloroquine have a pharmacologic action to elevate pH in lysosomes and consequently to suppress the acidic lysosomal enzyme activities. On this basis, it may be concluded that the expression of Gd cytotoxicity in rat AM at doses up to 1000 μM largely depends on the phagocytosis of particulate forms of Gd and the subsequent dissolution of those in the phago-lysosomes. The cytotoxicity of Gd at 3000 μM was not significantly affected by the filtration of Gd preparation or the treatments of AM with NH₄Cl or chloroquine. Therefore, it is suggested that at a dose level as high as 3000 μM, significant amounts of Gd existed in a non-colloidal form and thus damaged the AM by the mechanism independent from the phagocytosis of particulate forms of Gd and subsequent dissolution of those in the phago-lysosomes. It could be supposed that the amounts of non-particulate Gd were so different between 1000 and 3000 μM Gd preparations that mouse AM were almost alive and dead, respectively. As shown in Table 2, the percentage of Gd existing as a non-colloidal form (the amount of Gd in the filtered Gd preparation) in total Gd (the total amount of Gd in Gd preparation) was not very different between 1000 and 3000 μM Gd, indicating that non-particulate Gd concentration in 3000 μM Gd preparation was roughly threefold that in 1000 μM Gd preparation, and that this threefold difference

<table>
<thead>
<tr>
<th>Table 2. Percentage of Gd existing as a non-colloidal form</th>
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<tr>
<td>Dose*</td>
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<tr>
<td>-------</td>
</tr>
<tr>
<td>100</td>
</tr>
<tr>
<td>300</td>
</tr>
<tr>
<td>1000</td>
</tr>
<tr>
<td>3000</td>
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</table>

*Concentration of total Gd (μM).

b Percentage of Gd existing as a non-colloidal form (the amount of Gd in the filtered Gd preparation) in total Gd (amount of Gd in original Gd preparation).

in non-particulate Gd concentration might be crucial for the viability loss in mouse AM. In the present study, it was not elucidated in what chemical forms the non-particulate Gd existed and which form of Gd was cytotoxic to AM. These are a matter for future research.

The present study demonstrates the marked difference in the cytotoxic effect of Gd on mouse and rat AM. Surprisingly, mouse AM were extremely resistant to Gd compared with rat AM. Gd did not affect the viability of mouse AM at doses up to 1000 μM, whereas the viability of rat AM was much reduced by exposure to Gd at doses more than 3 μM. Naito et al. (1996) reported that peritoneal macrophages from Balb/c mice were not killed for up to 48 hr by exposure to GdCl₃ at a dose of 5 mg/100 ml (135 μM). At this dose level, rat AM are remarkably damaged. The present study and that of Naito et al. (1996) suggest the consistent resistance of mouse macrophages to Gd toxicity. To explain the species difference, we hypothesized that there might be less phagocytosis of Gd-colloid by mouse AM compared with rat AM. To clarify this point, the phagocytic activity of AM was compared between mouse and rat AM. In the first experiment, the phagocytosis of latex particles was used as an indicator of phagocytic activity, since latex particles are non-toxic and often used for this purpose. As shown in Fig. 6, there was no indication that the phagocytic activity of B6 mouse AM was lower than that of SD rat AM. In the second experiment, the actual amount of Gd incorporated into AM was measured by ICP-MS. To avoid the influence of toxicity of Gd itself, the uptake was compared in relatively short incubation times of 4 and 10 hr. As shown in Table 1, the non-specific adsorption of Gd by AM, indicated by the uptake at 4°C, was similar for mice and rats. The amount of Gd taken up by AM at 37°C was larger in B6 mouse AM than SD rat AM. It thus appears that the amount of Gd incorporated into the cells by phagocytosis was not significantly less in mouse AM than in rat AM. Based on these two experiments, it could be concluded that the difference in phagocytic activity was not responsible for the species difference between mice and rats in the cytotoxicity of Gd.

Another possible explanation is that rat AM were inherently more vulnerable to Gd toxicity than
mouse AM. To examine this, the effect of the filtered Gd preparation (original Gd concentration of 3000 μM before the filtration) was compared between rat and mouse AM. As shown in Fig. 7, the viability of AM exposed to the filtered Gd preparation was almost same between mouse and rat AM at all the time points examined. Therefore, the cytotoxic sensitivity of AM to extracellular non-colloidal Gd appeared to be the same between mice and rats. Taking into account the above findings, the most plausible explanation for the species difference in Gd cytotoxicity, was that the dissolution rate or mode of Gd-colloid in the phago-lysosomes may differ between mouse and rat AM, and/or rat AM may be more easily impaired by Gd dissolved in the phagolysosomes of AM. There are a few reports that the intracellular particle dissolution rates in AM or intraphagolysosomal pH values differed between species (Heilmann et al., 1992; Kreyling et al., 1990, 1992).

In conclusion, a marked difference in the cytotoxic response to Gd at doses up to 1000 μM was found between mouse and rat AM. The cytotoxicity of Gd at the dose range was dependent on the intracellular uptake and subsequent dissolution of Gd-colloid formed in the culture medium. However, the marked difference in the cytotoxic response to Gd between mouse and rat AM could not be attributed to the phagocytic activities for colloidal form of Gd. Therefore, it is suggested that the extent to which Gd-colloid phagocytosed is dissolved in the phagolysosome or the subsequent process to exhibit the cytotoxicity may differ between mouse and rat AM. Rat AM may dissolve Gd-colloid more actively or may be more sensitive to Gd dissolved inside the phagolysosomes with respect to the cytotoxicity.


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Survival of Genetically Modified *Escherichia coli* Carrying Extraneous Antibiotic Resistance Gene through Microbial Interactions

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The advance of recombinant DNA techniques allows us to construct novel bacterial strains. The potential uses of such recombinant bacteria in the environment include biocatalysis of insects, environmental remediation and waste treatment (Alexander 1981). Intended release of such genetically engineered microorganisms (GEMs) into natural environments has, however, raised questions regarding their ecological impact and many studies are being conducted to evaluate their ecological effects (e.g. Goodnow et al. 1990; Min et al. 1998; Tiedje et al. 1989). The safety of releasing GEMs is still uncertain and, in Japan, the field application of GEMs is prohibited by law to prevent possible ecological impact. Recently, much more environmental concern has been paid to the unintended release of laboratory bacterial strain, which may be recombinant, for research purposes. (Av-Gay 1999). Although we can regulate the deliberate release of GEMs into environment, we are still exposed to unintended release of recombinant bacteria.

Bacteria such as *Escherichia coli* K-12 are intensively used as laboratory microorganisms in the area of molecular biology and their genetic characteristics are frequently modified for research purposes by introducing a foreign gene. Using *E. coli* K-12 as the parental strain for genetic manipulation has been deemed safe because not only have their modified characteristics led to loss of their ability to survive in natural environments but they are also burdened with the increased metabolic demands of the maintenance and expression of the introduced foreign genes. Several studies have been conducted to evaluate the effects of releasing genetically modified *E. coli* K-12 into the environment (e.g. Chao and Feng 1990; Helling et al. 1981) but the results were inconsistent. Some support the safety of releasing them and some cast doubt on their safety (Brill 1985; Sobeyck et al. 1992). To evaluate the impact of unintentionally releasing genetically modified *E. coli* K-12, it is necessary to focus on the factors, which are not site-specific, involved in its survival.

In this study, we focused on the effects of microbial interactions, which including the competition with the parental strain, and the existence of protozoa and algae, as factors which would be involved in the survival of genetically modified *E. coli* K-12. For this purpose, we used a species-defined microcosm, which consisted of the bacterium *E. coli*, a bacteria feeding protozoan *Tetrahymena thermophila* and a photosynthetic alga *Euglena gracilis* (Kawabata et al. 1995). In this system, *T.*
thermophila interact with E. coli directly through predation but E. gracilis interact with E. coli only through its metabolites (Matsui et al. in press). Therefore, we will be able to evaluate both direct and indirect effects of these processes on the survival of the genetically modified E. coli K-12 in this system. We also tried to evaluate the impact of the genetically modified E. coli K-12 for the population dynamics of the protozoan and the alga in this microcosm.

MATERIALS AND METHODS

E. coli K-12 DH5α (Sambrook et al. 1989), designated here as E. coli, is a laboratory strain commonly used for molecular biological studies and is used here as the parental strain of the genetically modified strain. Plasmid pBluescriptII SK(+) (Alting-Mees et al. 1989), encoding the antibiotics ampicillin resistance gene, is a plasmid vector widely used for DNA recombination. It was transformed into E. coli to construct the genetically recombinant strain, harboring the extraneous plasmid, designated here as E. coli (P). E. coli and E. coli (P) were maintained on Luria-Bertani (LB) agar medium (Sambrook et al. 1989) and LB with 50 μg/ml ampicillin agar medium respectively.

E. coli (P) was introduced into an E. coli single culture to test competition with the parental strain. Half strength #36 Taub's salt solution (Taub and Doller. 1968) without NaNO3, was supplemented with 500 mg/L of proteose peptone (Difco laboratory, USA) and used as a culture medium. The medium was decanted into a series of test tubes and autoclaved before inoculation with the organisms. To synchronize the condition of the cells, E. coli were precultured in LB medium until they reached the late-logarithmic growth phase, washed twice in half strength Taub's #36 salt solution, resuspended in the same solution and then inoculated into each test tube at 10^4 cells/mL, the initial population density. Each test tube was placed stationary, in an incubator under 2500 lx by fluorescent lamps with 12-12LD light regime at 25°C and were sacrificed for counting on each sampling day. E. coli(P) was introduced into E. coli cultures at the beginning and after 5-days of the cultivation period. The numbers of E. coli (P) were monitored by colony formation units (CFU) on broth-agar plate medium (extracted bonito 3.0 g, polypeptone 3.0 g, NaCl 5.0 g, and 15 g agar powder in 1 liter of distilled water; pH adjusted to 7.0) with 0.5mg/L of ampicillin. The total cell number was obtained by the CFU on nutrient agar without ampicillin. Each count was carried out for three petridishes and the mean values of three counts were used as CFU per mL.

E. coli (P) were also introduced into a simple aquatic microcosm, which was developed by Kawabata et al. (1995), to evaluate the effects of microbial interactions on the survival of the recombinant strain. The microcosm consisted of a photosynthetic alga Euglena gracilis (producer), a bacteria-feeding protozoan Tetrahymena thermophila (consumer) and bacterium E. coli (decomposer) and was thus, employed to represent a simplified aquatic microbial ecosystem (Kawabata et al. 1995). High reproducibility of this microcosm suggested its reliability as a model of a microbial community (Matsui et al. in press). The microcosm was constructed by inoculating the E. gracilis, T. thermophila and E. coli into culture medium by the same methods as used for E. coli single-culture except that the pre-culture of T. thermophila and E. gracilis was conducted in nutrient rich media (for T. thermophila; proteose peptone 2.5 g, yeast extract 2.5 g and dextrose 10 g in 1 liter of distilled water; pH adjusted to 7.0: for E. gracilis;
tryptone 10 g, yeast extract 1 g, dextrose 10 g and vitamin B12 100 μg in 1 liter of distilled water; pH adjusted to 3.0). *E. coli* (P) was inoculated into the microcosm at the beginning of cultivation (day-0) and after 5-days of cultivation. The methods used to obtain the numbers of *E. coli* (P) and the total cell numbers were as described above. The numbers of *T. thermophila* and *E. gracilis* cell were counted under a microscope according to Kawabata et al. (1995).

RESULTS AND DISCUSSION

Figure 1 shows the population changes of *E. coli* (P) in the *E. coli* single-culture system. No significant difference in growth was observed between *E. coli* and *E. coli* (P) when the latter was introduced to the *E. coli* culture at the beginning of cultivation (Fig. 1A). This indicates that *E. coli* (P) has the same growth potential as the parent *E. coli*. In contrast, *E. coli* (P) declined sharply when introduced into *E. coli* single-culture after 5-days of incubation (Fig. 1B). The disappearance of *E. coli* (P) from the culture may reflect an uncomfortable environment for its growth, such as a scarcity of nutrients because both strains have the same metabolite pathways and would compete for each others resources. Another possibility was accumulation of metabolites from *E. coli* which might negatively affect the growth for both *E. coli* and *E. coli* (P) (Matsui et al. in press). No significant difference was observed in the population density of the parental strain with the addition of *E. coli* (P). From these results in the *E. coli* single-culture system, it seems less possible that unintentionally released *E. coli* (P) survive at a release site, where its parental *E. coli* already existed, due to competition with the parental strain.

When *E. coli* (P) were introduced into the three species microcosm at the start of cultivation, they grew in the same manner as the parent *E. coli* (Fig. 2A). However, *E. coli* (P) were also able to maintain their population density over 20 days when introduced into a three species microcosm on day 5 of cultivation (Fig. 2B). The addition of *E. coli* (P) did not affect the population densities of the other species in the microcosm (Fig. 2).

It has been well documented that predation by protozoa is an important factor in reducing the population densities of bacteria in aquatic environments (Nakano et al. 1998) and this is supported by the decrease of the *E. coli* population densities from 10⁷ cells/mL (in single culture) to 10⁴ cells/mL (in three species microcosm). Here, we found additionally that the existence of algae and protozoa in the microcosm ameliorated the competition between *E. coli* and *E. coli* (P) and provided conditions which allowed the survival of *E. coli* (P) (Fig. 2B), which had disappeared from the five-day old *E. coli* single culture. The metabolites from algae and protozoa would ameliorate the self-toxicity of the *E. coli* metabolites and thus support the survival of *E. coli* (Matsui et al. in press). Although the exact mechanism by which *E. coli* (P) maintained its population in the three species microcosm is not clear. In a previous study, we found that, in addition to protozoan predation, a metabolite of the indigenous bacteria were factors affecting the decreases of both *E. coli* and *E. coli* (P) when introduced to a paddy field microcosm (Kawabata et al. 1998). The results in the present study suggest that a metabolite from the competitive *E. coli*, might be a stronger factor than protozoa in the decrease of the introduced genetically recombinant *E. coli* and that genetically recombined *E. coli* used in a laboratory have the possibility of surviving in aquatic environments through such microbial interaction.
Figure 1. Population changes of *E. coli* (P)(●) introducing into *E. coli* single culture at the same time as *E. coli* (A), and after 5 days cultivation of *E. coli* (B). Population changes of *E. coli* (P) + *E. coli* (○), are also represented and broken lines indicate results of control experiments.
Figure 2. Population changes of *E. coli* (P) (●) introduced into a three-species microcosms at the same time as the other three species (A) and after 5 days cultivation of the other three species (B). Population changes of *E. coli* (P) + *E. coli* (○), *T. thermophilia* (□), and *E. gracilis* (◇) are also represented. Broken lines represent results of control experiments.
The difference between intended and unintended release of GEMs are the genetic and physiological designs of the bacteria. With an intended environmental application the GEMs would often have as an objective their stable maintenance and function in a particular environmental niche. In contrast, the bacteria which were unintentionally released from a laboratory would originally be designed and recombined for a specific research purpose, such as cloning of a specific gene, and therefore, less attention would have been paid to its survival after experiments. In this study, we used the species defined microcosm and have demonstrated that the survival potential of a laboratory *E. coli* strain, through microbial interaction which may attenuate competition with the parental strain. Since there are several reports that *E. coli* is one component of the resident bacteria in aquatic environments (Brettar et al. 1992), our results indicate that the survival of laboratory used genetically recombined *E. coli* in aquatic environment is possible, where parental *E. coli* are resident.

**Acknowledgments.** This work was partly supported by a Japanese Ministry of Education, Science and Culture Grant-in-Aid for Scientific Research on Priority Areas (4319), Project "Symbiotic Biosphere: An ecological interaction network promoting the coexistence of many species" and also by a Research Fellowships of the Japan Society for the Promotion of Science for Young Scientists.

**REFERENCES**


15. Effect of Acidification on the Population of Growth Stage Aquatic Microcosm

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INTRODUCTION

Acidification phenomenon of natural ecosystem caused by human activity such as industrial energy production or motorization is recognized as one of the most serious ecological stress. It is necessary to establish a reasonable method to evaluate the ecological effect of acidification comparing with the other environmental toxicants, if human beings want to maintain a sustainable development. As the natural ecosystem consists of various biological species and includes the complex mutual interaction among them, it is not easy to assess any effects on ecosystem from accumulated knowledge of the effect on single species. Experimental studies on model ecosystem might have an ideal possibility, by which we can get valuable information that cannot be induced from either experiments for single species in a laboratory, or observation of complicated phenomena in natural ecosystem. In this study an aquatic microcosm system was adopted as a model ecosystem for ecological assessment of acidification effect. This system is a biological community, which has capability to demonstrate an indirect effect by any ecological stress on the species composing the ecosystem.

MATERIAL AND METHODS

The aquatic microcosm system consists of three species of microorganism in a small container like a test tube or a small plastic bottle. This system was composed by Kawabata et al.1, and the mutual interaction of materials among the three species incubated in a regular condition has been investigated. This microcosm consists of flagellate algae Euglena gracilis Z as a producer which has chloroplast to do photosynthesis, ciliated protozoa Tetrahymena thermophila B as a consumer which grazes bacteria, and bacteria Escherichia coli DH5α as a decomposer which decomposes metabolite and dead bodies of the other two species. They can survive by exchanging materials each other in a closed container with limited nutrients at the start of incubation, and their population densities are kept in a steady-state for a long time, usually for more than a year.

An experiment of acidification was carried out as follows: Each microorganism was preincubated following the method of Kawabata et al. Then three species of microorganisms were inoculated into a culture medium (0.05 % protease peptone in half strength of modified Taub and Dollar's solution) in test tubes. The culture medium was in advance acidified to pH 4.0 by adding the volume equivalently mixed solution of 1N nitric acid and 1N sulfuric acid, while the control medium was originally pH 7-8. Population densities of each organism were determined at various time intervals after starting incubation under a 2500 lx and 12-12 hrs. LD light regime at 25 °C. The population density of Eu. gracilis and T. thermophila were counted microscopically, and that of E. coli was measured by counting colonies formed in the broth-agar medium.

RESULTS AND DISCUSSION

Figs. 1-3 show the time dependent variations in the population densities of three
microorganisms after inoculation in the control microcosm and in the microcosm acidified to pH 4.0, which are compared with the changes in the population densities of each microorganism incubated in the single pure culture.

I. Acidification effect on each three microorganism in the single pure culture

*Eu. gracilis* (Fig. 1): *Eu. gracilis* in the pH 4.0 single pure culture increased more than that in the control. The population density of *Eu. gracilis* in the steady-state from the twentieth day after inoculated into the pH 4.0 medium reached to $10^4$ cells/ml order, while that in the control is $10^4$ cells/ml order. This is because *Eu. gracilis* is not stressed by acidification but promoted to increase by addition of nitric acid and sulfuric acid.

*T. thermophila* (Fig. 2): The population density of *T. thermophila* in the pH 4.0 single pure culture was not so much different from that in the control. Although *T. thermophila* is not stressed by acidification to pH 4.0, it was extinguished after thirty days passed from inoculation, in the same way as the control. Because it cannot survive alone with neither organic matters which were consumed during the thirty days, nor *E. coli* which plays a role of feed for *T. thermophila*.

*E. coli* (Fig. 3): The population density of *E. coli* in the pH 4.0 single pure culture kept the approximate same order of that at the time of inoculation, which means $10^{-3}$ times lower than the control in the steady-state. *E. coli* was not extinguished but could not increase in the stressed condition by acidification.

II: Acidification effect on each three microorganism in the microcosm

*Eu. gracilis* (Fig. 1): The population density of *Eu. gracilis* in the pH 4.0 microcosm is higher than that in the control microcosm. Consequently, the population density of *Eu. gracilis* in the steady-state was highest in both cases of single pure culture and microcosm system, when the medium was acidified to pH 4.0. It is concluded that *Eu. gracilis* is not stressed by acidification but promoted to increase. It is interesting that the control microcosm system is better condition for
*E. gracilis* to increase and to keep the high population density, compared with the control single pure culture.

*T. thermophila* (Fig. 2): It is clearly recognized that the population density of *T. thermophila* in the microcosm increased and kept a steady-state of $10^5$ cells/ml even after thirty days from the inoculation. This is because *T. thermophila* can survive without protease pepton by grazing *E. coli*. Although the population density of *T. thermophila* was not affected by the acidification to pH 4.0 in the case of single pure culture, the population density of *T. thermophila* in the pH 4.0 microcosm is one order lower than that in the control microcosm in the growth stage until twenty days passed after inoculation. It is considered that the population density of *E. coli*, which plays a role of feed for *T. thermophila* was not enough during this period, compared with the control, as is described later.

*E. coli* (Fig. 3): The population density of *E. coli* in the pH 4.0 microcosm decreased and did not increase until seven days passed after inoculation, while in the control microcosm it increased and reached a steady-state of $10^6$ cells/ml. Then the population density of *E. coli* in the pH 4.0 microcosm had increased till the tenth day and reached the same order as that of the control. As *E. coli* cannot increase in the pH 4.0 medium in the case of single pure culture, it is considered that the condition in the acidified microcosm changed to be suitable for increasing of *E. coli* until ten days passed, as is described later.

On the other hand, as mentioned above, the population density of *T. thermophila*, the predator for *E. coli*, in the pH 4.0 microcosm was lower than that in the control in the growth stage. As *E. coli*, the feed for *T. thermophila*, did not increase during this period, *T. thermophila* was inhibited to increase by the low population density of *E. coli*. This phenomenon is recognized to be an indirect effect resulted from interspecific interaction occurred in a model ecosystem, microcosm. *T. thermophila* was affected secondarily by low population density of *E. coli*, which was directly affected by acidification.

### III. The change of pH of microcosm

Fig. 4 shows the time dependent variation of pH of microcosm. The pH of the control microcosm increased from 7.8 to 8.2 until ten days passed after inoculation. The pH of the acidified microcosm also increased from 4.0 to 7.1 until ten days passed after inoculation. As the pH was changed to be suitable for increasing of *E. coli*, the population density of *E. coli* reached to the same $10^5$ cells/ml order as that of the control on the tenth day after inoculation in the pH 4.0 microcosm. What worked for elevating pH of the microcosm?

Fig. 5 shows the time dependent variation of pH of single pure culture of *E. gracilis*. It
was also elevated from 4.0 to 6.5 until ten days passed after inoculation. It is, therefore, concluded that in the pH 4.0 microcosm the pH was elevated by *Eu. gracilis*, and as a result, *E. coli* started increasing. This is thought to be one kind of demonstration of an interspecific interaction occurred in a model ecosystem, microcosm.

**Fig. 4**  *The change of the pH of microcosm*

**Fig. 5**  *The change of the pH of single pure culture of Eu. gracilis*

**CONCLUSION**

The present study showed that acidification did not affect on the microcosm, consequently. Although one of the species was directly affected, the other contributed to remediation of the condition of microcosm system. This microcosm system could be expected to be applied to examine other various ecological toxicants, by its advantage of demonstrating indirect effects originated from mutual interaction among the organisms.

**REFERENCE**

Determination of plutonium concentration and its isotopic ratio in environmental materials by ICP-MS after separation using ion-exchange and extraction chromatography

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An analysis method for $^{239}$Pu and $^{240}$Pu in environmental samples (including four certified reference materials) by ICP-MS was studied. Two types of chromatographic resin, Dowex 1X8 and TEVA, were examined for their applicability to the separation of Pu from the matrix elements. Sufficient decontamination factors ($10^5$--$10^6$) for many matrix elements including U, which interferes with the detection of mass 239, were obtained with both resins. The detection limit of Pu by ICP-MS was about 0.02 pg ml$^{-1}$ (0.05 mBq ml$^{-1}$ for $^{239}$Pu; 0.17 mBq ml$^{-1}$ for $^{240}$Pu) in the sample solution or 0.1 pg in the sample. Analytical results of $^{239+240}$Pu in certified reference materials (IAEA-135, -SOIL-6, -368 and -134) indicated that the accuracy of the method was satisfactory. Data on the $^{240}$Pu/$^{239}$Pu atom ratios in these reference materials, which are scarce in the literature, were also obtained, i.e., 0.211 for IAEA-135, 0.191 for IAEA-SOIL-6, 0.043 for IAEA-368 and 0.200 for IAEA-134. Compared with alpha-spectrometry, the ICP-MS method has significant advantages in terms of its simple analytical procedures, prompt measurement time and capability of determining the $^{240}$Pu/$^{239}$Pu ratio.

Introduction

Plutonium is one of the most important nuclides from the viewpoints of radioecology and toxicology. There are different anthropogenic sources of Pu in the environment. Atomic weapons testing introduced the major deposited fraction of Pu onto the Earth's surface. It was estimated that 10$^9$ TBq of $^{239+240}$Pu have been injected into the atmosphere through weapons testing.\(^1\) Plutonium has also been released into the environment due to accidents at nuclear facilities such as Sellafield (UK),\(^2\) Kyshtym (Russia)\(^3\) and Chernobyl (Ukraine).\(^4\)

The isotopes of Pu range from $^{233}$Pu to $^{248}$Pu, of which $^{238}$Pu (half-life: 87.7 years), $^{239}$Pu (half-life: 24110 years), $^{240}$Pu (half-life: 6584 years) and $^{242}$Pu (half-life: 14.35 years) are thought to be the most important in terms of environmental studies. Among them, $^{239}$Pu and $^{240}$Pu are the two most abundant Pu isotopes in the environment. The $^{240}$Pu/$^{239}$Pu atom ratio in global fallout reported by Krey et al.\(^5\) is, on average, 0.176 ± 0.014. It is known that the $^{240}$Pu/$^{239}$Pu ratio is a good indicator to identify the source of the contamination.\(^6\) However, data for the $^{240}$Pu/$^{239}$Pu ratio in environmental samples are still limited. There are no values for the $^{240}$Pu/$^{239}$Pu ratio in certified reference materials for environmental research, as far as we know, in a survey report prepared by the IAEA.\(^7\)

The analytical methods usually used for Pu determination are alpha-spectrometry,\(^8-12\) LX/ez ray measurement,\(^13\) fission track,\(^14,15\) TIMS (thermal ionization mass spectrometry)\(^16,17\) and ICP-MS (inductively coupled plasma mass spectrometry).\(^15,18-21\) The most widely used method is alpha-spectrometry. However, this method is very time consuming and it cannot resolve the $^{239}$Pu and $^{240}$Pu peaks because of their similar energies (5.15 and 5.16 MeV). Analytical results from alpha-spectrometry are therefore expressed as the sum of the $^{239}$Pu and $^{240}$Pu activity concentration (i.e., $^{239+240}$Pu).

TIMS provides very accurate and precise determinations of Pu isotopes at very low concentrations. However, the instrument is very expensive and its operation requires much experience. In this context, ICP-MS, which is increasingly being used in environmental analysis for the determination of several elements\(^22,23\) and isotopes,\(^24\) has advantages in terms of ease of operation and prompt analysis, although the precision is not as high as that in TIMS. Kim et al.\(^15\) applied ICP-MS to the determination of $^{239}$Pu and $^{240}$Pu in soil and silt samples after chemical separation of the nuclides by solvent extraction and ion-exchange. Crain et al.\(^16\) reported the determination of actinides (including Pu) in soil leachates by ICP-MS and alpha-spectrometry after separation by extraction chromatography using a resin which is known to absorb actinides.\(^25-27\)

Recently, Yamamoto et al.\(^16\) successfully used ICP-MS in the determination of Pu isotopes in samples collected from the former Semipalatinsk Nuclear Site. Chiappini et al.\(^20\) improved the detection limits for actinides through improvements in the interface pumping system in ICP-MS and by using a high-efficiency nebulizer system. However, there are still only a few papers dealing with the application of ICP-MS to the determination of Pu and its isotopes in environmental materials. No details of separation procedures suitable for ICP-MS or of the validation of the analytical qualities are given in these papers. Therefore, further investigations of the details of the analytical procedure are necessary in order to establish an appropriate method for ICP-MS. Because there is almost no information on the $^{240}$Pu/$^{239}$Pu ratio in international certified reference materials, it is also necessary to obtain these isotopic data.

In this paper, analytical procedures for Pu in environmental samples (including certified reference materials of soil, sediment and fish flesh) by ICP-MS were studied. Particular
attention was paid to the separation procedures. Two different techniques, viz., ion-exchange and extraction chromatography, were compared for their efficiency in the separation of Pu from the matrix. The following points were specifically studied: (1) chemical separation of Pu from the matrix; (2) determination of Pu by ICP-MS; and (3) determination of Pu and the $^{241}$Pu/$^{239}$Pu atom ratio in international certified reference materials. Several soil samples collected in Japan with background Pu levels were also analyzed using the developed method.

**Experimental**

**Standards, reagents and samples**

Plutonium-242 (CRM 130, Plutonium Spike Assay and Isotopic Standard, New Brunswick Laboratory, USA) was used to spike the samples. The standard solution of $^{242}$Pu was diluted to obtain stock solutions with a concentration of 0.2 ng ml$^{-1}$ (29 mBq ml$^{-1}$) in 2 M HNO$_3$ solution. To check the $^{242}$Pu/$^{239}$Pu ratio, a Pu isotope standard solution (NIST SRM 947) was used. Standard solutions of other elements (e.g., U, Th, Ce, Fe) were prepared from Multielment Plasma Standards (SPEx Industries). As an internal standard, Bi (20 ng ml$^{-1}$) was used to assay any changes in analytical signals during the measurement.

The ion-exchange resin used was Dowex 1X8 (anion-exchange resin, 100–200 mesh, Dow Chemical Co.). The resin was first conditioned in a large column (ca. 250 ml) by passing solutions in the following order: 1 M NaOH (ca. 5 l), H$_2$O (washing), 0.5 M (NH$_4$)$_2$SO$_4$ (ca. 0.5 l), H$_2$O (washing), 3 M HCl (ca. 2.5 l) and H$_2$O (washing). After this procedure had been repeated, the conditioned resin was placed in a plastic bottle and kept in a cool, dark place. For the analysis, 2 ml of the resin were placed in a mini-column (diameter: 8 mm; length: 80 mm). The extraction chromatography resin used was TEVA-Spec (Eichrom Industries). This resin consisted of methylbenzimidazolium ion sorbed on an inert polymeric support (Amberlite XAD-7). A ready-to-use mini-column (diameter: 7 mm; length: 80 mm) packed with 2 ml of TEVA resin was applied for the analysis. Both resins, Dowex 1X8 and TEVA, retain only the tetravalent form of Pu quantitatively.

All reagents used were of analytical-reagent grade. Nitric acid used in the final solution for ICP-MS was of super-pure grade (AA-100, Tama Chemicals). The certified reference materials analyzed were IAEA-135 (sediment from the Irish Sea), IAEA-SOIL-6 (soil from Austria), IAEA-368 (ocean sediment from Mururoa Atoll) and IAEA-134 (fish flesh from the Irish Sea). Two soil samples were collected from a forest in Ohmagari (Akita Prefecture, Japan) and from a rice paddy field in Hitachinaka (Ibaraki Prefecture, Japan). They were air-dried and sieved through a 1 mm sieve.

**Sample pre-treatment**

A known amount of $^{242}$Pu (0.1–0.5 ml of a 0.2 ng ml$^{-1}$ stock solution) was added to samples (2–50 g, depending on the concentration level) as a spike. The sample was ashed at 500°C for 4–6 h in a muffle furnace to decompose organic matter. (If the sample amount is low, less than 5 g, the ashing procedure can be omitted.) The ashed sample was placed in a Pyrex beaker and 8 M HNO$_3$ (more than five times the sample weight) was added. The mixture was boiled on a hot-plate (180–200°C) for at least 3 h. During heating, the beaker was covered with a watch-glass to prevent significant evaporation, and the sample was stirred occasionally with a glass rod. The warm supernatant (leachate) was filtered through a glass-fibre filter (Whatman GF/F). The residue in the beaker was boiled again with 8 M HNO$_3$ (about twice the sample volume) for about 20 min, then filtered. This leaching procedure was usually repeated twice. Samples with a large volume had to be centrifuged prior to filtration so that the procedure could be completed rapidly. The residue, as obtained after centrifugation, was again boiled with 8 M HNO$_3$ (about twice the sample volume), then filtered. This procedure was repeated twice.

All the filtrates were collected in a beaker, and heated on a hot-plate until a thick wet paste was obtained. (If the paste was completely dried, it could not easily be dissolved in HNO$_3$ in the following processes.) The wet paste was dissolved by adding HNO$_3$ while warming on the hot-plate. The sample solution was diluted with de-ionized water to adjust the acidity of the solution to 8 M HNO$_3$ (for Dowex 1X8) or 2 M HNO$_3$ (for TEVA). The solution volume should be maintained at 40–200 ml; the actual volume depended on the amount of sample. Any residue appearing was removed by filtration. In order to select an appropriate reagent for converting Pu to Pu(iv), which is the only retainable form in the chromatography columns (both for Dowex 1X8 and TEVA), NaNO$_2$ was added to the filtered leachate. (For comparison tests, reagents such as NH$_4$OH·HCl and H$_2$O$_2$ were also used instead of NaNO$_2$; see under Results and discussion.)

**Separation by resins**

Anion-exchange resin (Dowex 1X8). The sample solution (8 M HNO$_3$) was loaded onto a column containing 2 ml of Dowex 1X8 at a rate of <2 ml min$^{-1}$. The resin was washed with 8 M HNO$_3$ (40 ml), then with 10 M HCl (40 ml). Tetravalent Pu should be retained on the resin and most of the U and matrix elements should pass through the column. Finally, NH$_4$Cl (5%–10 M HCl solution, 40 ml, <2 ml min$^{-1}$) was added to reduce Pu(iv) to Pu(III). Since Pu(III) is not retained on the resin, Pu was eluted from the column. HNO$_3$ (4 ml) and HClO$_3$ (0.2 ml) were added to the eluate which was heated on a hot-plate to decompose any organic material (e.g., fine resin particles) and to expel the excess of iodine. In order to remove iodine completely from the beaker, H$_2$O$_2$ (1 ml) was also added to convert iodide to I$_2$ and volatilize it. After the solution had been taken to dryness, the residue was dissolved in HNO$_3$.

**Extraction chromatography (TEVA)**

The sample solution (2 M HNO$_3$) was loaded onto a column containing 2 ml of TEVA resin at a rate of <2 ml min$^{-1}$. The resin was washed with 2 M HNO$_3$ (40 ml), then with 9 M HCl (40 ml). Tetravalent Pu should be retained on the resin and most of the U and matrix elements should pass through the column. Finally, hydroquinone (0.1 M)–9 M HCl solution (40 ml, <2 ml min$^{-1}$) was added to reduce Pu(iv) to Pu(III) and to elute Pu from the column. The separation chemistry using TEVA was based on the procedure recommended in the information sheet provided by Eichrom Industries for this resin. To the eluate, HNO$_3$ (4 ml) and HClO$_3$ (0.2 ml) were added and the solution was heated on a hot-plate to decompose any hydroquinone that remained. After the solution had been taken to dryness, the residue was dissolved in HNO$_3$.

**Decontamination factor.** The decontamination factor, defined as the number of atoms in the initial solution divided by the number of atoms in the final solution, was determined for selected elements (U, Th, Ce, Zn, Fe, Mn, Ca, Al, Mg and Na) in solutions which were passed through the two different resins.

Determinations by ICP-MS

The sample solution separated by the above-mentioned procedure was adjusted to an acid concentration of 4% m/m HNO₃. The total volume of the final solution was usually 5 mL. As an internal standard, Bi was added (20 ng mL⁻¹) to the sample and standard solutions. (The concentration of Bi can be reduced, if necessary.) Concentrations of Pu and other elements were determined by ICP-MS using a quadrupole-type mass spectrometer (Yokogawa PMS-2000) with a conventional liquid nebulizer (Meinhard type). Measurement conditions are shown in Table 1. The total counting time was selected after due consideration of the isotopic concentrations as follows: 50-100 s for ²³⁹Pu, 100-200 s for ²⁴⁰Pu, 20-40 s for ²⁴²Pu, 1-5 s for other elements. From the results of isotopic ratios to the spike (²⁴⁰Pu), concentrations of ²³⁹Pu and ²⁴⁰Pu were calculated (isotope dilution method). A Pu isotopic standard solution (NIST SRM 947) with a known ²⁴⁰Pu/²³⁹Pu ratio was used to check the accuracy. The abundance sensitivity based on ²³⁸U measurement (masses 237 and 238) was about 1×10⁶. Mass bias per unit mass, as determined for a U standard (natural) and a Pu isotopic standard solution (NIST SRM 947), was usually <0.5%.

Results and discussion

Separation procedures

Chemical yields in the chromatographic separation. The major chemical forms of Pu extracted from the samples by HNO₃ on heating are expected to be the oxidized forms, Pu(vi) and/or Pu(v). Since both resins, Dowex 1X8 and TEVA, retain only tetravalent Pu quantitatively, the chemical species of Pu should be converted to Pu(v) by adding appropriate reagents. Three reagents, NaNO₃, NH₄OH-HCl and H₂O₂, were examined. The results obtained for the chemical yields in the chromatographic separation by using these reagents are shown in Table 2. The highest chemical yields in the separation procedures were found by using NaNO₃, followed by NH₄OH-HCl. Use of H₂O₂ led to a poor chemical yield, although this reagent is recommended for use in some literature. From these results it was decided to use NaNO₃ (0.2 M) to convert Pu to Pu(iv) in the sample solution prior to loading it onto the columns.

Decontamination factors using two different resins. Total cation concentrations in the final solution for ICP-MS should be less than 1000 ppm (µg mL⁻¹) to avoid matrix suppression and/or interface cloaking. Therefore, it is necessary to reduce the element concentrations in the sample solution without losing the Pu content. In addition to the separation of major matrix elements, the element U should also be eliminated to avoid peak overlap on ²³⁹Pu due to polyatomic ion production (uranium hydride ions; ²³⁵UH⁺) and/or to the effect of up-mass tailing during the ICP-MS measurement. The results for the decontamination factors for ion-exchange chromatography (Dowex 1X8) and extraction chromatography (TEVA) obtained from soil samples [Akita-soil (Japan) and IAEA-SOIL-6 (Austria)] are shown in Fig. 1. Elements showing high concentrations in the initial solutions (soil leachates) were usually Fe (10⁻⁴-10⁻³ µg mL⁻¹) and Al, Mg, Na and Ca (10⁻⁴-10⁻² µg mL⁻¹). In the final solution (after the separation procedures), the element showing the highest concentration was usually Na (up to 5 µg mL⁻¹) and the total concentrations of the measured cations were significantly less than 20 µg mL⁻¹ with both resins. The decontamination factors for many elements (including U) were of the order of 10⁻⁴-10⁻⁶. Concentrations of U in the final solution were, in many cases, 0.04-0.2 ppb (0.4-2×10⁻⁶ µg mL⁻¹). These results indicated that the matrix elements and also U were successfully separated by both resins. Comparison of the two resins showed that the decontamination factor for U was better with Dowex 1X8 than with TEVA for the samples studied here.

The influence of U concentrations on ²³⁹Pu determination by ICP-MS was assessed. A solution containing U (100 ng mL⁻¹) was used and the increase in the 239 mass peak was measured. During normal operation, the influence of U on mass 239 was about 3×10⁻⁵. If it is assumed that the maximum U concentration in the final solution is 1 ppb

![Decontamination factor comparison](image)

**Fig. 1** Decontamination factors of selected elements with Dowex 1X8 and TEVA for (a) Akita-soil and (b) IAEA-SOIL-6. (Error bars: standard deviations of 2–5 separate runs.)

(ng ml⁻¹), the expected influence is calculated to be only about 0.03 pg ml⁻¹, which is almost the same as the detection limit. Because the U concentration in the final solution was usually much less than 1 ng ml⁻¹, there would be almost no effect due to U.

Using a sample (50 g) collected from a deeper soil layer, in which the Pu level should be very low, no Pu peaks (²³⁹Pu, ²⁴⁰Pu, ²⁴ⁱPu) were found after the separation procedure, indicating that there were no influences due to blanks from the reagents used and from soil substances.

These results suggested that our separation procedure was appropriate and applicable to the determination of environmental levels of Pu.

Measurements by ICP-MS

The sample solution separated by the above-mentioned procedure was subjected to ICP-MS measurements of Pu. The measurement time for one sample was 10–20 min. For alpha-spectrometry, the counting time is normally 1–5 d. ICP-MS has a significant advantage in terms of analytical time. The sensitivity, in counts per second per ng ml⁻¹ of ²⁴²Pu, was about 3 × 10⁴ cps under normal operating conditions. Background counts on the mass were 2–4 cps. The detection limit, defined as three times the standard deviation of the blank solution (0.07 µg HNO₃), was about 0.02 pg ml⁻¹ (ppt) Pu or (²³⁹Pu: 0.05 µg HNO₃ ml⁻¹, ²⁴⁰Pu: 0.17 µg HNO₃ ml⁻¹) for a counting time of 100 s. Since the volume of the final solution was 5 ml, the absolute detection limit (in pg) was calculated to be about 0.1 pg. Using a 50 g sample, the concentration factor of Pu in the final solution (usually 5 ml) would be 10, which would result in detection limits of about 0.005 Bq kg⁻¹ for ²³⁹Pu and 0.017 Bq kg⁻¹ for ²⁴⁰Pu. In this study, Pu was measured by ICP-MS with a normal nebulizer. However, if other types of nebulizer such as ultrasonic or micro-concentric nebulizers or electrothermal vaporization were used, much better sensitivities might be obtained. Chiappini et al. detected down to 35 fg of Pu in environmental samples by using a high-efficiency nebulizer system (Mistral) together with an improved interface pumping system.

The relative standard deviation (RSD) for three analyses of the same sample solution was usually 1–5% for a Pu concentration of > 2 pg ml⁻¹. The RSD obtained from the measurements of the isotopic ratio to the spike (i.e., ²³⁹Pu/²⁴⁰Pu and ²³⁹Pu/²⁴⁲Pu) was better than the above-mentioned value, i.e., usually 0.5–2%. The ratios to ²³⁵Pu were used and the concentrations of ²³⁹Pu and ²⁴⁰Pu in the samples were calculated on the basis of the isotope dilution method. These data indicated that the precision of the ICP-MS measurements was reasonable in the determination of Pu in environmental samples.

Analysis of certified reference materials

In order to validate the analytical method and to obtain data on the Pu isotopic composition, four international certified reference materials (2 sediments, 1 soil and 1 fish flesh) having recommended values for ²³⁹+²⁴⁰Pu (but no data on ²⁴²Pu/²⁴⁰Pu ratios) were analyzed. The analytical method finally used is summarized in Fig. 2. Results obtained for ²³⁹Pu and ²⁴⁰Pu in certified reference materials are shown in Tables 3–6, together with information on the resin types, chemical yields and sample weights. The ²⁴⁰Pu/²³⁹Pu atom ratios were also calculated and are also given in Tables 3–6.

Table 3 shows the results obtained for IAEA-135 (Irish Sea sediment). Only about 2 g of the sample were used because of its high Pu concentration due to contamination from the Sellafield nuclear facility. Six analyses were performed on this material: three analyses were by Dowex 1X8 and three were by TEVA. There were no significant differences between the analytical data obtained by the two resins. The sum of ²³⁹Pu and ²⁴⁰Pu concentrations (²³⁹+²⁴⁰Pu) was calculated for comparison with the value recommended by the IAEA. Our analytical results for ²³⁹+²⁴⁰Pu (mean value: 210 ± 12 Bq kg⁻¹) agreed well with the recommended value (213 Bq kg⁻¹).²⁸ The ²⁴⁰Pu/²³⁹Pu atom ratio was calculated as 0.211 ± 0.004. Since there is no IAEA information on the ²⁴⁰Pu/²³⁹Pu ratio for the reference materials, it was not possible to make a comparison. Our value was comparable to the ratio (0.20) reported by Yamamoto et al.²⁹ in a sediment sample originating from the Sellafield area.

Table 4 shows analytical results for IAEA-SOIL-6 (surface soil collected from Austria). The value obtained for the ²³⁹+²⁴⁰Pu concentration (1.00 ± 0.04 Bq kg⁻¹) agreed well with the recommended value (1.04 Bq kg⁻¹) given by the IAEA.²⁹ A comparison of the results obtained with Dowex 1X8 and with TEVA showed that there were no marked differences between the analytical results obtained. The mean ²⁴⁰Pu/²³⁹Pu atom ratio obtained for IAEA-SOIL-6 was 0.191 ± 0.003, which was comparable to the global fallout value of 0.176 ± 0.014 reported by Krey et al.²⁹

Table 5 shows the results for IAEA-368 (ocean sediment from Mururoa Atoll). The mean ²³⁹+²⁴⁰Pu value of the eight analyses, 29.7 ± 3.0 Bq kg⁻¹, agreed with the recommended value (31 Bq kg⁻¹).³⁰ There were also no significant differences between the mean values obtained with Dowex 1X8 and TEVA. The standard deviation for the eight analyses was larger than that of the previous two samples (IAEA-135 and SOIL-6). This sample had a high calcium carbonate content and was derived from ocean sediments (coral rich) near a nuclear weapons test site. The homogeneity of the sample might not be as good as that of the other two samples. It was noted that the ²⁴⁰Pu/²³⁹Pu atom ratio obtained for IAEA-368 was very low, i.e., 0.043 ± 0.008. This suggests that the Pu was derived from the testing of a Pu-bomb in which ²³⁹Pu was enriched.

Table 6 shows the results for IAEA-134 (fish flesh from the Irish Sea). The mean value for the ²³⁹+²⁴⁰Pu concentration for the four analyses (15.4 ± 0.5 Bq kg⁻¹) agreed well with the IAEA recommended value (15 Bq kg⁻¹).³¹ This indicated that the present method is also applicable to biological materials, which are ashed prior to analysis. The ²⁴⁰Pu/²³⁹Pu atom ratio
Table 3 Analytical results for $^{239}$Pu, $^{240}$Pu and their atom ratios in IAEA-135 (Irish Sea sediment)*

<table>
<thead>
<tr>
<th>Resin</th>
<th>Sample weight/g</th>
<th>Yield (%)</th>
<th>$^{239}$Pu/ $^{240}$Pu (Bq kg$^{-1}$)</th>
<th>$^{240}$Pu/ $^{239}$Pu (Bq kg$^{-1}$)</th>
<th>$^{239}$+ $^{240}$Pu/ $^{239}$Pu (atom ratio)</th>
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<tr>
<td>Dowex 1X8</td>
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<td>97</td>
<td>124 ± 5</td>
<td>99 ± 2</td>
<td>224 ± 5</td>
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<tr>
<td>Dowex 1X8</td>
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<td>82</td>
<td>114 ± 6</td>
<td>88 ± 6</td>
<td>201 ± 8</td>
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<tr>
<td>TEVA</td>
<td>1.7</td>
<td>53</td>
<td>120 ± 3</td>
<td>91 ± 2</td>
<td>210 ± 4</td>
</tr>
<tr>
<td>TEVA</td>
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<td>72</td>
<td>114 ± 5</td>
<td>87 ± 5</td>
<td>201 ± 7</td>
</tr>
<tr>
<td>TEVA</td>
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<td>80</td>
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<td>98 ± 4</td>
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</tr>
<tr>
<td>Mean (Dowex)</td>
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<td>116 ± 8</td>
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<td>208 ± 13</td>
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<tr>
<td>Mean (TEVA)</td>
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<td>120 ± 6</td>
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<td>213</td>
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</table>

*±: standard deviation.

Table 4 Analytical results for $^{239}$Pu, $^{240}$Pu and their atom ratios in IAEA-SOIL-6 (Austrian soil)*

<table>
<thead>
<tr>
<th>Resin</th>
<th>Sample weight/g</th>
<th>Yield (%)</th>
<th>$^{239}$Pu/ $^{240}$Pu (Bq kg$^{-1}$)</th>
<th>$^{240}$Pu/ $^{239}$Pu (Bq kg$^{-1}$)</th>
<th>$^{239}$+ $^{240}$Pu/ $^{239}$Pu (atom ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dowex 1X8</td>
<td>25</td>
<td>90</td>
<td>0.58 ± 0.01</td>
<td>0.41 ± 0.02</td>
<td>0.99 ± 0.02</td>
</tr>
<tr>
<td>Dowex 1X8</td>
<td>25</td>
<td>89</td>
<td>0.56 ± 0.02</td>
<td>0.40 ± 0.01</td>
<td>0.96 ± 0.02</td>
</tr>
<tr>
<td>Dowex 1X8</td>
<td>50</td>
<td>87</td>
<td>0.58 ± 0.03</td>
<td>0.40 ± 0.02</td>
<td>0.98 ± 0.03</td>
</tr>
<tr>
<td>Dowex 1X8</td>
<td>50</td>
<td>92</td>
<td>0.59 ± 0.02</td>
<td>0.41 ± 0.01</td>
<td>1.00 ± 0.02</td>
</tr>
<tr>
<td>TEVA</td>
<td>25</td>
<td>69</td>
<td>0.62 ± 0.02</td>
<td>0.46 ± 0.03</td>
<td>1.08 ± 0.03</td>
</tr>
<tr>
<td>TEVA</td>
<td>25</td>
<td>69</td>
<td>0.58 ± 0.02</td>
<td>0.39 ± 0.01</td>
<td>0.97 ± 0.02</td>
</tr>
<tr>
<td>Mean (Dowex)</td>
<td>23</td>
<td>89</td>
<td>0.58 ± 0.01</td>
<td>0.41 ± 0.01</td>
<td>0.98 ± 0.02</td>
</tr>
<tr>
<td>Mean (TEVA)</td>
<td>69</td>
<td>69</td>
<td>0.60 ± 0.03</td>
<td>0.43 ± 0.05</td>
<td>1.03 ± 0.06</td>
</tr>
<tr>
<td>Mean (total)</td>
<td>83</td>
<td>83</td>
<td>0.59 ± 0.02</td>
<td>0.41 ± 0.03</td>
<td>1.00 ± 0.04</td>
</tr>
<tr>
<td>IAEA recommended</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.04</td>
</tr>
</tbody>
</table>

*±: standard deviation. *The IAEA recommended value was originally described as 28 pCi (or 1.04 Bq kg$^{-1}$) for $^{239}$Pu on the information sheet and related publication (IAEA 1984). However, we confirmed with IAEA that the value should be for $^{239}$+ $^{240}$Pu.

Table 5 Analytical results for $^{239}$Pu, $^{240}$Pu and their atom ratios in IAEA-368 (Ocean sediment, Mururoa Atoll)*

<table>
<thead>
<tr>
<th>Resin</th>
<th>Sample weight/g</th>
<th>Yield (%)</th>
<th>$^{239}$Pu/ $^{240}$Pu (Bq kg$^{-1}$)</th>
<th>$^{240}$Pu/ $^{239}$Pu (Bq kg$^{-1}$)</th>
<th>$^{239}$+ $^{240}$Pu/ $^{239}$Pu (atom ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dowex 1X8</td>
<td>5.4</td>
<td>86</td>
<td>27.3 ± 0.9</td>
<td>3.7 ± 0.1</td>
<td>31.0 ± 0.9</td>
</tr>
<tr>
<td>Dowex 1X8</td>
<td>5.4</td>
<td>54</td>
<td>28.3 ± 1.1</td>
<td>5.5 ± 0.2</td>
<td>33.8 ± 1.1</td>
</tr>
<tr>
<td>Dowex 1X8</td>
<td>5.0</td>
<td>92</td>
<td>23.9 ± 0.6</td>
<td>3.4 ± 0.1</td>
<td>27.3 ± 0.6</td>
</tr>
<tr>
<td>Dowex 1X8</td>
<td>5.0</td>
<td>88</td>
<td>21.8 ± 0.6</td>
<td>3.3 ± 0.1</td>
<td>25.1 ± 0.6</td>
</tr>
<tr>
<td>Dowex 1X8</td>
<td>5.0</td>
<td>93</td>
<td>27.6 ± 0.8</td>
<td>3.9 ± 0.1</td>
<td>31.5 ± 0.9</td>
</tr>
<tr>
<td>Dowex 1X8</td>
<td>5.0</td>
<td>86</td>
<td>28.4 ± 0.9</td>
<td>3.9 ± 0.1</td>
<td>32.3 ± 0.9</td>
</tr>
<tr>
<td>TEVA</td>
<td>5.4</td>
<td>46</td>
<td>26.4 ± 0.7</td>
<td>3.4 ± 0.2</td>
<td>29.7 ± 0.7</td>
</tr>
<tr>
<td>TEVA</td>
<td>5.4</td>
<td>47</td>
<td>22.1 ± 0.6</td>
<td>4.5 ± 0.2</td>
<td>26.6 ± 0.6</td>
</tr>
<tr>
<td>Mean (Dowex)</td>
<td>83</td>
<td>83</td>
<td>26.2 ± 2.7</td>
<td>4.0 ± 0.8</td>
<td>30.2 ± 3.3</td>
</tr>
<tr>
<td>Mean (TEVA)</td>
<td>47</td>
<td>47</td>
<td>24.3 ± 3.1</td>
<td>4.0 ± 0.8</td>
<td>28.2 ± 2.2</td>
</tr>
<tr>
<td>Mean (Total)</td>
<td>74</td>
<td>74</td>
<td>25.7 ± 2.7</td>
<td>4.0 ± 0.7</td>
<td>29.7 ± 3.0</td>
</tr>
<tr>
<td>IAEA recommended</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>31</td>
</tr>
</tbody>
</table>

*±: standard deviation.

Table 6 Analytical results for $^{239}$Pu, $^{240}$Pu and their atom ratios in IAEA-134 (fish flesh, Irish Sea)*

<table>
<thead>
<tr>
<th>Resin</th>
<th>Sample weight/g</th>
<th>Yield (%)</th>
<th>$^{239}$Pu/ $^{240}$Pu (Bq kg$^{-1}$)</th>
<th>$^{240}$Pu/ $^{239}$Pu (Bq kg$^{-1}$)</th>
<th>$^{239}$+ $^{240}$Pu/ $^{239}$Pu (atom ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dowex 1X8</td>
<td>5</td>
<td>69</td>
<td>9.6 ± 0.3</td>
<td>6.5 ± 0.1</td>
<td>16.1 ± 0.3</td>
</tr>
<tr>
<td>Dowex 1X8</td>
<td>5</td>
<td>96</td>
<td>8.6 ± 0.2</td>
<td>6.4 ± 0.2</td>
<td>15.0 ± 0.3</td>
</tr>
<tr>
<td>Dowex 1X8</td>
<td>10</td>
<td>87</td>
<td>8.6 ± 0.1</td>
<td>6.5 ± 0.2</td>
<td>15.1 ± 0.2</td>
</tr>
<tr>
<td>Dowex 1X8</td>
<td>10</td>
<td>89</td>
<td>8.7 ± 0.2</td>
<td>6.6 ± 0.2</td>
<td>15.3 ± 0.3</td>
</tr>
<tr>
<td>Mean (Dowex)</td>
<td>85</td>
<td>85</td>
<td>8.9 ± 0.5</td>
<td>6.5 ± 0.1</td>
<td>15.4 ± 0.5</td>
</tr>
<tr>
<td>IAEA recommended</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>15</td>
</tr>
</tbody>
</table>

*±: standard deviation.

for this material was 0.200 ± 0.010. This value was almost the same as the ratio obtained for the Irish Sea sediment sample (IAEA-135), suggesting that the origin of the contamination was the same.

As mentioned above, good agreements were found between the present results for $^{239}$+ $^{240}$Pu concentrations in the certified reference materials and the recommended values. This indicated that the accuracy of the method was reasonable and also suggested that Pu in the sample was almost completely extracted from the matrix by boiling the sample with HNO$_3$ for at least 3 h. However, further tests on the extraction procedure for other matrices might be necessary to confirm

---

the applicability of the method to a variety of samples and Pu forms. A comparison of the two resins showed that the chemical yields using Dowex 1X8 (24 analyses) were 54–99% (mean: 89%) and using TEVA (eight analyses) 46–80% (mean: 64%). The decontamination factor for U with Dowex 1X8 was also better than that with TEVA. The concentrations of HNO₃ used in the column separation were 2 M for TEVA and 8 M for Dowex 1X8; hence, if it is desirable to reduce acid consumption, the method using TEVA is more appropriate. However, the solubility of Pu should be better in concentrated acid (i.e., 8 M). Samples with certain matrices (e.g., high Fe, Mn, Ce) might be effective for TEVA.

Application of the method to the analysis of common soil

The method was applied to the determination of Pu in soil samples in ordinary natural environments. Samples were collected from a forest in Akita Prefecture and from a rice paddy field in Ibaraki Prefecture. The results are shown in Table 7. For the Akita-soil, six analyses were performed. The mean value of the ²³⁹⁺²⁴⁰Pu concentration for this sample was 1.37 ± 0.04 Bq kg⁻¹. The RSD (i.e., precision) of the six determinations was about 3%. The concentration of ²³⁹⁺²⁴⁰Pu in the rice field soil from Ibaraki was 0.39 ± 0.01 Bq kg⁻¹, which was markedly lower than that in the forest soil from Akita. The ²⁴⁰Pu/²³⁹Pu ratios in the Akita-soil (0.168 ± 0.06) and Ibaraki-soil (0.171 ± 0.03) were almost identical and were comparable to the global fallout value² mentioned above.

Comparison with other methods

Compared with alpha-spectrometry, the present method using ICP-MS has significant advantages in the determination of the Pu isotopic composition (i.e., ²⁴⁰Pu/²³⁹Pu ratio). The separation procedure for ICP-MS is fairly simple and the analytical time is considerably shorter than for the method using the alpha-spectrometer. For example, the presence of iron and lanthanides resulted in thick deposits which gave poor resolution of alpha spectra.¹ Incomplete chemical separation of a nuclide having a similar alpha energy (e.g., U, Th) interferes with the measurements. More time is also necessary for the electrodoposition procedure and measurement with an alpha-counter. A typical detection limit by alpha-spectrometry with a 4000 min counting time is of the order of ¹₀⁻⁶ Bq L⁻¹ which corresponds to about 0.05 pg of ²³⁹Pu. This value is similar to the detection limit by ICP-MS with a conventional nebulizer obtained in this study. If another type of nebulizer were used, as mentioned above, the detection limit should be improved. If high resolution ICP-MS (HR-ICP-MS), which is known to have a higher mass resolution and better detection limits for many elements,² was used, a better sensitivity for Pu would also be expected. For the TIMS method, the separation procedure is also critical to achieve a precise analysis, i.e., the separation procedure by column chromatography should be repeated 2 or 3 times to separate Pu as cleanly as possible. For ICP-MS, the chemical separation required is not so severe in comparison with the procedures for alpha-spectrometry and TIMS. This method has the advantages of simple separation chemistry, shorter analytical time, lower costs and easier operation. However, the detection limit and precision of the isotopic measurement are better in the TIMS method.

Conclusion

From the above-mentioned results for the certified reference materials and also field samples, it was found that the use of ICP-MS after a chemical separation with Dowex 1X8 or TEVA is applicable to the determination of both ²³⁹Pu and ²⁴⁰Pu in environmental samples. Compared with alpha-spectrometry, the ICP-MS method has significant advantages in terms of its simple analytical procedures, prompt measurement time and capability of determining the ²⁴⁰Pu/²³⁹Pu ratio. Because there are almost no data on the ²⁴⁰Pu/²³⁹Pu ratios in the certified reference materials, the analytical results obtained for IAEA-135 (0.211), IAEA-SOIL-6 (0.191), IAEA-368 (0.043) and IAEA-134 (0.200) may contribute to the acquisition of information on the isotopic compositions of Pu in these materials. The determination of the ²⁴⁰Pu/²³⁹Pu ratio is important in understanding the source of the contamination.

Acknowledgements

We thank Professor M. Yamamoto (Kanazawa University) for his valuable comments on the separation chemistry and Dr. Y. Igarashi (Meteorological Research Institute) for his advice on the use of ICP-MS in the determination of long-lived nuclides. We also thank Dr. K. Gunji (JAIERI) and Dr. M. Yamada (NIRS) for their useful comments on Pu standards, Mr. Y. Ito and Mr. H. Sato (NIRS) for their help in

Table 7 Analytical results for ²³⁹Pu, ²⁴⁰Pu and their atom ratios in soil samples collected in Japan*

(A) Forest soil from Ohmagari/Akita (0-41)—

<table>
<thead>
<tr>
<th>Resin</th>
<th>Sample weight/g</th>
<th>Yield (%)</th>
<th>²³⁹Pu/²⁴⁰Pu Bq kg⁻¹</th>
<th>²⁴⁰Pu Bq kg⁻¹</th>
<th>²³⁹ + ²⁴⁰Pu Bq kg⁻¹</th>
<th>²⁴⁰Pu/²³⁹Pu (atom ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dowex 1X8</td>
<td>24</td>
<td>97</td>
<td>0.83 ± 0.06</td>
<td>0.51 ± 0.04</td>
<td>1.33 ± 0.07</td>
<td>0.170 ± 0.009</td>
</tr>
<tr>
<td>Dowex 1X8</td>
<td>24</td>
<td>96</td>
<td>0.83 ± 0.04</td>
<td>0.53 ± 0.03</td>
<td>1.36 ± 0.05</td>
<td>0.174 ± 0.002</td>
</tr>
<tr>
<td>Dowex 1X8</td>
<td>24</td>
<td>92</td>
<td>0.83 ± 0.05</td>
<td>0.52 ± 0.02</td>
<td>1.34 ± 0.05</td>
<td>0.170 ± 0.003</td>
</tr>
<tr>
<td>Dowex 1X8</td>
<td>24</td>
<td>96</td>
<td>0.83 ± 0.04</td>
<td>0.52 ± 0.05</td>
<td>1.36 ± 0.06</td>
<td>0.172 ± 0.005</td>
</tr>
<tr>
<td>Dowex 1X8</td>
<td>50</td>
<td>99</td>
<td>0.86 ± 0.08</td>
<td>0.50 ± 0.02</td>
<td>1.36 ± 0.08</td>
<td>0.157 ± 0.008</td>
</tr>
<tr>
<td>TEVA</td>
<td>24</td>
<td>75</td>
<td>0.89 ± 0.02</td>
<td>0.54 ± 0.02</td>
<td>1.44 ± 0.03</td>
<td>0.166 ± 0.005</td>
</tr>
<tr>
<td>Mean (Dowex)</td>
<td>96</td>
<td></td>
<td>0.83 ± 0.02</td>
<td>0.52 ± 0.01</td>
<td>1.35 ± 0.01</td>
<td>0.169 ± 0.007</td>
</tr>
<tr>
<td>Mean (total)</td>
<td>93</td>
<td></td>
<td>0.84 ± 0.03</td>
<td>0.52 ± 0.01</td>
<td>1.37 ± 0.04</td>
<td>0.168 ± 0.006</td>
</tr>
</tbody>
</table>

(B) Paddy soil from Hitachinaka/Ibaraki (P-53)—

<table>
<thead>
<tr>
<th>Resin</th>
<th>Sample weight/g</th>
<th>Yield (%)</th>
<th>²³⁹Pu/²⁴⁰Pu Bq kg⁻¹</th>
<th>²⁴⁰Pu Bq kg⁻¹</th>
<th>²³⁹ + ²⁴⁰Pu Bq kg⁻¹</th>
<th>²⁴⁰Pu/²³⁹Pu (atom ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dowex 1X8</td>
<td>50</td>
<td>95</td>
<td>0.24 ± 0.01</td>
<td>0.16 ± 0.01</td>
<td>0.40 ± 0.01</td>
<td>0.173 ± 0.003</td>
</tr>
<tr>
<td>Dowex 1X8</td>
<td>50</td>
<td>93</td>
<td>0.23 ± 0.00</td>
<td>0.15 ± 0.01</td>
<td>0.38 ± 0.01</td>
<td>0.169 ± 0.005</td>
</tr>
<tr>
<td>Mean (total)</td>
<td>50</td>
<td>94</td>
<td>0.24 ± 0.01</td>
<td>0.16 ± 0.01</td>
<td>0.39 ± 0.01</td>
<td>0.171 ± 0.003</td>
</tr>
</tbody>
</table>

* ±: standard deviation.
obtaining authorization for the use of Pu isotopes for the analysis, and Mr. A. Tanaka (Kaken Co.) and Ms. K. Ebine for their technical assistance.

References


Paper 9/00071B
人類が受ける放射線の主たるものは、宇宙線、自然放射性核種など地球環境自体に由来する。しかし、原子力利用、医療用放射線利用など、人間活動がもたらす新たな放射線源も増加している。

1. はじめに

地球の放射線環境を作り出している放射線はそのsourceから見ると、
a. 宇宙線（太陽からの紫外線も含む）
b. 自然・人工放射性核種からの放射線
c. 人为的に作り出される放射線
に大別される。一方、人の放射線被曝の場という側面からは、
1) 人間の活動に依存しない放射線場　
   a. 宇宙線場（主に陽子、中性子）
   b. 宇宙線誘導放射性核種による線源場　
      内部被ばく・外部被ばく
   c. 自然放射性核種による線源場　
      内部被ばく・外部被ばく
2）人間活動に由来する放射線場　
   人为的に作り出される放射線場　
   一 医療放射線　
   一 核技術による放射線　
   一 原子力利用に伴う放射性核種　
      内部被ばく・外部被ばく
   b. 人为的に高められた自然放射線場
に分類される。

これらの放射線場、放射線環境が現在どのような大きさであるか、また人間活動に伴いどのように変化してきたか、また今後どのような変化が予想されるか概観してみる。

2. 現在の放射線環境

2-1. 自然放射線の線量

自然放射線による成人一人当たりの実効線量は、世界平均で年間約2.4mSvと推定される（表1）。このうち約1.1mSvは、宇宙線、地殻γ線、人体内の放射性核種（40K等）に由来するものである。また約1.3mSvはラドン及びその崩壊生成核種による寄与である。
表1 自然放射線による成人の年間実効線量

<table>
<thead>
<tr>
<th>線源</th>
<th>年間実効線量 (mSv)</th>
<th>高い値</th>
</tr>
</thead>
<tbody>
<tr>
<td>宇宙線</td>
<td>0.39</td>
<td>2.0</td>
</tr>
<tr>
<td>地殻起源のγ線</td>
<td>0.46</td>
<td>4.3</td>
</tr>
<tr>
<td>体内放射性核種</td>
<td>0.22</td>
<td>0.6</td>
</tr>
<tr>
<td>ラドン及び崩壊生成物</td>
<td>1.3</td>
<td>10</td>
</tr>
</tbody>
</table>

(UNSCEAR1993年報告から①)

宇宙線による寄与は、人が居住する緯度・高度により変動し、また地殻γ線寄与は地質による自然放射性核種の量によって変動する。また、自然放射性核種の体内量は食習慣に依存し、ラドンによる寄与は居住地域の地質とともに、家屋の材質・構造に強く依存している。このため自然放射線による線量は、局所的に変動することになる。

2-2. 人工放射線の線量

1）医療被ばく

医療に伴う放射線被ばくは、診断・治療を受けた患者と非患者、国ごとの医療レベルによって大きく異なっている。放射線治療による被ばく線量は、患者一人当たりの診断に係わる放射線被ばく線量に比べて遜色に大きいが、患者数が少ないため平均的被ばく線量としての寄与は小さい。表2に、先進工業国（レベルI）における医療放射線（診断）被ばく線量の推定値を示す。

（診断）被ばく線量の推定値を示す。

放射線被ばくは、人工放射線源からの被ばくのうち最も大きな線量寄与を与えるものである。

2）原子力利用に伴う放射線被ばく

原子力利用に伴う放射線被ばくの主たるものは、i）大気圏内核実験と核兵器製造及び、ii）原

図1 平均年実効線量

放射性降下物
0.02

原子力発電
0.00015

医療放射線
(先進工業国)
1.0

自然放射線
2.4

単位：mSv/年
（一般人：世界平均）

1988年国連科学委員会報告より

表2 医療放射線被ばく線量の年平均推定値（診断）

<table>
<thead>
<tr>
<th>線源</th>
<th>平均値</th>
<th>レベルI 平均値</th>
<th>レベルII, III 平均値</th>
<th>世界平均値</th>
</tr>
</thead>
<tbody>
<tr>
<td>X線診断</td>
<td>0.3〜2.2</td>
<td>1.0</td>
<td>0.02〜0.2</td>
<td>0.3</td>
</tr>
<tr>
<td>放射性薬剤</td>
<td>0.09</td>
<td></td>
<td></td>
<td>0.03</td>
</tr>
<tr>
<td>全放射線診断</td>
<td>1.1</td>
<td></td>
<td></td>
<td>0.3</td>
</tr>
</tbody>
</table>

(UNSCEAR1993年報告から①)
子力発電からの被ばくである。
現在では大気圏内核実験は行われておらず、フォールアウトの量も過去に比べ低下している。しかし実験開始以来、2200年までに世界全人口に与えられる平均線量は、約1.4mSvと見積もられている。この積算線量（預託）は、年平均線量とすると約0.02mSvに対応する。
一方、原子力発電に伴う放射線被ばくは、採鉱・精錬・燃料製造・原子炉運転・廃棄物処理等様々なプロセスで発生する。原子力利用に伴う放射線被ばくは、事故等の突発的事象の危険性のため、広く懸念されることが多い。しかし、このリスクは、潜在的リスクであり、その線量寄与そのものは極めて小さく、世界平均で年間約0.00015mSv程度と見積もられている。表3に、原子力発電に伴う被ばく線量寄与を示す。
図1に、自然放射線・人工放射線源からの年実効線量寄与を示す。

図2は、海洋堆積物中のBe-10濃度分布を示す。

3. 放射線環境の変遷

前節のように、人間を取り巻く放射線環境は、当然の事ながら自然放射線の寄与が最も大きい。人間の活動が与える影響は、極近年になってからである。

3-1. 放射線技術利用以前

人類が放射線利用をする以前においては、放射線源を含む野生動物、宇宙線と自然放射性核種による放射線場のみである。これらの放射線源のうち、地殻に含まれる放射性核種の量は人類の歴史的な時間では、大きな変動はない。宇宙線強度の時間変化に関しても、大きな宇宙線強度の変化は認められていないが、地球磁場の変動による宇宙線強度の変動が指摘されている。宇宙線強度の変動の可能性を示す1, 2の例を示す。

図2は、レースバックらによる海洋堆積物中のBe-10のプロファイルである。Be-10は、高エネルギー宇宙線による大気中の酸素及び窒素の核破砕反応で生成される。通常、大気中の放射能モニタリング等で検出されるBe-7と似た生成過程より、定常に生成されている。この核種は、半減期が長く通常の放射能測定では検出が困難であるが、加速器質量分析法などによって測定されている。この実験では、ほぼ1500万年にわたり比較的一定のBe-10降下量が得られている。即ち、過去1500万年にわたっては、それほど大きな宇宙線強度変動は認められない。しかし、著者らは、1500万年以前には降下量即ち生成量が大きかった可能性があると推論している。最も、1500万年前後では、人類発生以前ということになる。

一方、より最新にBe-10降下量の異常が認められた例もある。図3は、氷中のBe-10濃度を示す。上方試料中のBe-10濃度、下部は酸素同位体比であり、相互に良好同期していることがわかる。酸素同位体比の変動は、平均気温（水温）の変化による同位体効果を示す。つまり、この同位体比は、氷の堆積速度（平均気温）の違いに基づき氷期と間氷期を示している。ところが、36,000年ほど前に、Be-10濃度異常が認められる。この原因については、一時的な堆積（氷積）速度の違いという解釈もなされているが、Be-10の生成速度が上昇した可能性も指摘される。この原因の一つに「地
球近傍でのSuper-Novaの可能性が論じられている。もしそうであるなら、地上での宇宙線度が2〜3倍に上昇した可能性を示すものといえよう。

Enhanced radiation（高められた自然放射線場）の1例が、「鉱山労働者の肺損傷問題」である。これは、鉱山労働という閉鎖された場で、空気中のラドン濃度が高められる事により、高濃度ラドンの吸入による発癌の発生の例である。

しかしながら、おしなべて見ると核技術時代以前には、非常に高密度に住む集団や特殊な職種に伴う集団という制限された集団以外、地上の放射線環境は当然の事ながら、かなり定常的であったと考えられる。

3-2. 核技術時代の放射線環境

1. 医療被曝

X線の発見以来、人間は放射線を利用することを始め、放射線の医学利用は急速に普及しそのとともに、放射線障害も発生している（表4）。現在では、これらの障害発生の経験から放射線発生装置の改良が進め、被ばく線量は以前に比べ低下している。

前節で述べたように、一人当たりの平均的な医療被ばく線量は、年平均1mSvと評価されています。これは、自然放射線被曝の1/2程度であり、

<table>
<thead>
<tr>
<th>年代</th>
<th>人物</th>
<th>事項</th>
</tr>
</thead>
<tbody>
<tr>
<td>1895年</td>
<td>Roentgen</td>
<td>X線の発見</td>
</tr>
<tr>
<td>1896年</td>
<td>Grubbe</td>
<td>X線管の製作とX線の実験</td>
</tr>
<tr>
<td></td>
<td>Edison</td>
<td>X線透過実験：眼障</td>
</tr>
<tr>
<td></td>
<td>Daniel</td>
<td>X線による頭髪の脱落</td>
</tr>
<tr>
<td>1902年</td>
<td></td>
<td>X線による発癌報告（慢性腫瘍）</td>
</tr>
<tr>
<td></td>
<td></td>
<td>その他。放射線の影響への影響 (Albers-Schonberg)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>小児の甲状腺障害（放射線による甲状腺障害）</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ショウジョウバエを用いた放射線による突然変異発生の確認</td>
</tr>
</tbody>
</table>

他の人工放射線源（原子炉事故等の特別な被ばくを除いて）よりも大きい。医療被ばくは、直接的なbenefitを伴っており、また診断・治療を受ける特定個人の被ばくという側面を持っているため、他の放射線源と同様には論じられないが、前述したレベルIの国について放射線場としての一応の評価をしてみよう。

X線検査においては、上部消化管（平均実効線量：7.2mSv）、血管造影（同：6.8mSv）、CT撮影（4.3mSv）、下部消化管（4.1mSv）等が高く、これに対してより一般的に行われる胸部直接撮影（0.25mSv）や、同間接撮影（0.52mSv）などは従来に比べ、かなり低減されている。

2. 人工的に高められた自然放射線被ばく
放射線、核エネルギーの利用は、人をとりまく放射線環境を大きく変化させた。これらの計画活動はいずれも、本来の地球環境には存在していない新たな放射線環境を作りだす。従って、特異な集団における放射線環境の変化にとどまらず、人類全体の放射線のがく線量の増大、あるいは増大の可能性（潜在的のぼく）をもたらしている。また、活動域の拡大（高層・極地の居住、大気中に飛行、地下深部利用など）は、自然放射線環境を高めている。

人間活動による放射線環境で、医療放射線とともに重要な要素は原子力利用による放射線場である。このような放射線場は、前述したように線量寄与として極めて大きいが、原子力施設における事故等の見られるように、潜在的かつ極めて局所的に高い放射線環境を作り出す可能性をもっている。また、鉱工業における放射線利用、核技術利用による放射線環境等もあるが、極めて限られた空間・対象（人）に対する放射線場であり、一般公衆に対する放射線場としての寄与は小さい。

このような放射線環境に伴って、人為的に高められた自然放射線環境がある。

エネルギー生産における石炭・石油・天然ガス・地熱等の利用に伴う放射線環境は、核放射線環境のそれぞれ10, 0.25, 0.015, 1%程度と評価されている。

また、航空機の利用がより一般的になるともに、一般公衆の宇宙線放射線の増加も起こっている。大気圏に伴う宇宙線放射線は、飛行高度・コース・時間により様々である（表5）。

3-3. 将来の地球放射線環境

地球規模での放射線環境は、本質的には自然放射線環境である。この自然放射線環境自体は今後相当長期間にわたって常態的であろうと予想される。これにより、人間活動が与える放射線環境は、極めて局所的であろう。原子力利用は、局所的に人間生活に連帯的な打撃をもたらすのが放射線環境を作り出す可能性が潜んでおり、十分な予防措置を講じる必要があることは当然である。しかしながら平常的には、地球規模で見ると必ずしも大き
話 題
先端技術に由来する有害物質の比較影響評価に関するワークショップ

中村 裕 二

1. はじめに
近年の科学技術の進展とともに、従来自然環境中にはない、あるいは極めてまれにしか存在しない様々の物質が自然環境中に負荷される可能性が増大している。エネルギー産業から放出される各種の放射性物質による人・生態系への影響に関する研究は、これまで多くの場合を上げてきた。放射線による影響では、その負荷量（dose）概念が比較的明瞭であり、因果関係解析が有効に機能してきた。しかしながら人・生態系への影響を及ぼす環境負荷因子は、放射性物質だけではない。むしろ、人の健康や自然環境の保全という観点からは、これまで自然界には存在しなかった化学物質や、自然界に存在するものの局在化により極度に濃縮された重金属等、様々な環境負荷因子の影響を適切に評価する必要がある。

放射線医学総合研究所は、日本保健物理学会、日本放射線影響学会の共催の下、科学技術庁及び科学技術国際交流センターの支援を受け、平成10年1月28日から30日までの3日間で行われるプラセントラル・プラセンホールにおいて、「先端技術に由来する有害物質の比較影響評価に関する国際ワークショップ」を開催した。このワークショップは、エネルギー産業及び先端科学技術の発展に伴って排出される様々な有害因子（放射性物質、重金属、レアメタル等）に関して、環境中での分布や挙動（環境研究）、人及び生物への影響（毒性学的研究）、生態系への影響（生態学研究）において先導的な研究を行っている研究者を一堂に会し、境界領域を含めた総合研究への発展、将来展望について議論することを目的としたものである。

2. 国際ワークショップ
ワークショップでは、佐々木優人放射線医学総合研究所所長による開会挨拶に引き続き、国際から参加した7名及び国内から参加した14名、計21名の専門家による講演が行われた。ワークショップのプログラムを第1表に示す。

（基調講演及び特別講演）
ワークショップ初日には、ウクライナから招聘したG.G. POLIKARPOV教授及び大阪大学医学部の森本兼義教授による基調講演が行われた。

G.G. POLIKARPOV教授（Academy of Science, Ukraine）は、「放射生態学研究の生物の側面：現状と将来展望」と題して、放射生態学研究の枠組み及び放射性有害物質の影響評価への適用について、広範な視点を行った。放射生態学研究は、子孫利用が進んでいる現代において基礎研究・応用研究の両側面においてより一層重要性が増していること、生物影響研究から得られてきた電離放射線の定量的「線量・効果」関係を基本とする影響評価の方法論は、非放射性有害物質の影響評価のモデルとなり得ること、また、生態系影響評価においても有用な手段となり得ることが述べられた。

森本兼義教授（大阪大学医学部）は、「ライフスタイルと健康：健康指標としての染色体変化と免疫学的変化について」と題して、「不健康な」ライフスタイルが及ぼす影響について、種々の疾患に対する分子生物学的、細胞学的、免疫学的研究の実例を示し、影響評価における重要性を強調した。喫煙の有無、過度の睡眠、定期的な運動、睡眠時間、食事の栄養バランス、過度の労働時間、飲酒、ストレスの有無の8項目を、「健康」「非健康」なるライフスタイルの指標として、染色体異常、免疫能など様々な指標との相関が示された。

同日午後には、R. Setz博士（IAEA）による「放射性廃棄物及び化学毒性物質からの健康・環境影響アセスメントについて」と題する特別講演が行われた。この中で、原子力発電やその他の発電システムに伴う放射性及び非放射性廃棄物の環境・健康影響の比較評価を行うための戦略・方法論について紹介された。この講演では、放射性物質（線）と化学毒性物質の人の健康及び環境への影響を評価するための共通の尺度（基準）を確立すること、廃棄物の量的評価、毒性評価、物理的・化学的特性、再利用の可能性、廃棄物処分の方式等についての評価が重要であることが指摘された。

3. 一般講演
特別講演に引き続き、3日間内にあたり一般講演が行われた。


放射線医学総合研究所第4研究グループ：千葉市稲毛区穴川4-9-1（〒263-8555）
Department of Environmental & Toxicological Sciences, National Institute of Radiological Sciences; 4-9-1, Anagawa, Inage-ku, Chiba 263-8555, Japan.
International Workshop on Comparative Evaluation of Health Effects of Environmental Toxicants Derived from Advanced Technologies

Wednesday, January 28, 1998

I. Opening Session
   1. Opening remarks
      Yasuto Sasaki (or Jiro Inaba), Director General, NIRS, Japan

II. Key-note Address
    Chairpersons: A. Hsie (USA), Y. Ohmomo (IES)
    1. G. Polikarpov (Ukraine)
       Biological Aspect of Radioecology: Objective and Perspective
    2. K. Morimoto (Osaka Univ.)
       Life Style and Health: Chromosome Alterations and Immunological Potentials as Health Indices of Overall Lifestyles

III. Special Lecture
    Chairpersons: G. Polikarpov (Ukraine), S. Takahashi (NIRS), R. Seitz (IAEA)
    Assessment of Health and Environmental Effects from Radioactive and Chemically Toxic Waste

IV. Session A: Environmental Behavior of Toxicants
    Chairpersons: W.R. Schell (USA), Y. Muramatsu (NIRS)
    1. S. Yoshida and Y. Muramatsu (NIRS)
       Behavior of Trace Elements and Radionuclides in Soil-Plant Systems
    2. W.R. Schell (USA)
       Behavior and Modeling of Radionuclide and Non-Radionuclide Toxicants in the Environment
    3. S. Hisamatsu (IES)
       Behavior of Tritium in the Environment—Tritium in the Food and Human Body—
    4. A. Kudo (Kyoto Univ.)
       Fate of Nagasaki $^{239,240}$Pu and Interaction of $^{239}$Pu and $^{237}$Np with Bentonite and Sulfate Reducing Anaerobic Bacteria
    5. Y. Shibata (NIES)
       Chemodynamics of Arsenic in Marine Environment

Thursday, January 29, 1998

V. Session B: Effects on Eco-system
   Chairpersons: H. Jones (UK), K. Komura (Kanazawa Univ.)
   1. Z. Kawabata (Ehime Univ.)
      Evaluation of the Effects of Biological Perturbations on an Ecosystem Using Aquatic Microcosms
   2. H. Jones (UK)
      The Ecotron Controlled Environment Facility: Microcosm Studies on the Effects of Elevated CO$_2$ on Terrestrial Communities
   3. I. Aoyama (Okayama Univ.)
      Ecotoxicity Assessment and Bioassay of Chemicals
   4. S. Fuma (NIRS)
      Ecological Effects of Radiation and Other Environmental Stress on Aquatic Microcosm

VI. Session C: Modeling and Methodology in Environmental Risk Studies
    Chairpersons: H. Maubert (IPSN), Y. Nakamura (NIRS)
7. 地球規模のフォールアウトに由来する$^{137}$Cs摂取量に基づく線量評価と日本人大感染におけるリスク評価（京都大学地球環境工学：鳥田洋次他）
8. 食品及び食事栄養の多元素分析に基づく一般公共者の健康影響比較評価（放射線医学総合研究所：白石久二他）
9. 3種の土壤型におけるキュウリによる$^{137}$Cs及び$^{90}$Srの吸収（理化学研究所：S. GOUTI 他）
10. 必須元素及び微量元素の土壤から農作物への移行（環境科学技術研究所：塚田祥文）
11. 放射性核種の土壤から野菜への移行に関する研究（放射線医学総合研究所：坂内光明他）
12. テクネチウムの水田土壌から米粒への移行（放射線医学総合研究所：柳澤啓也）
13. 水田土壌における$^{99m}$Tcの挙動に関する考察（放射線医学総合研究所：田上敏子他）
14. 水性マイクロロジズムを用いたγ線及び紫外線の生態学的影響に関する比較評価（放射線医学総合研究所：武田洋他）
15. 酸性化による成長期の水性マイクロロジズムの集団密度に対する影響（放射線医学総合研究所：宮本薰子他）
16. マイクロロジズム系を用いた水生生態系に対する表面活性剤の毒性評価（筑波大学：高松良江他）
17. 複雑系の自己保存システムとしてのマイクロロジズムに関する計算機シミュレーション（放射線医学総合研究所：土居雅広他）
18. ラドン誘発肺ガンの実在するか？（放射線医学総合研究所－スウェーデン国立放射線防護研究所：Anita Enflo）
19. 肺マクロファージにおけるガドリニウムの細胞毒性に関する研究（放射線医学総合研究所：久保田善久他）
20. Rhodes ocellatus ocellatusの胎細胞を用いた突然変異性試験（宇都宮大学：松浦浩高他）
21. MSTO-211H セルラインにおけるアスベストとγ線の細胞毒性の比較（東京理科大学：沖津希世他）
22. ヒトの皮膚線維芽細胞のHPRT遺伝子座における放射線誘発突然変異の分子生物学的解析（放射線医学総合研究所：山本和他）
23. 多環芳香族炭化水素の光化学反応毒性（食品薬品安全センター：中川ゆずき他）
24. ウミを用いた水質汚染の発生毒性モニタリングシステム（横浜市立大学：早乙女京子他）

3.5 セッションD（環境毒性学研究）
1月30日には、午前中から午後までにわたり生物影響、環境毒性研究に関するセッションが開催された。

古谷圭一教授（東京理科大学理学部）は「粒子状物質の化学特性とその細胞毒性」と題し、酸化ニッケルの塩類溶液やラジウムの培養細胞マクロファージ、C3H マウスの呼吸系などにおける解離性、石炭ファイアッシュの物理化学的特性とラットの肺マクロファージに対する細胞毒性などについて詳しい報告を行った。

A.W. Hsieh 教授（The University of Texas Medical Branch, USA）による「哺乳動物細胞における電離放射線及び化学物質によって誘発される遺伝子突然変異の分子マーカー」と題する講演では、突然変異を誘発する反応性酸素分子（ROS）による HPRT 遺伝子座の突然変異スペクトルが詳細に報告された。放射線による ROS がもたらす突然変異誘発の機構は、ブレオマイシン、アドリアマイシンなどの化学物質による変異発現機構と同一と考えられ、比較的毒性の方法論を確立する上でも重要で情報である。

高橋千太郎博士（放射線医学総合研究所）による「放射線と環境汚染物質の毒性影響の比較：トランスジェニックマウス、培養肺マクロファージ、DNA 二重鎖切断解析」と題する講演では、in vivo における突然変異検出のためのトランスジェニックマウスモデルの有効性、培養肺マクロファージを用いた細胞毒性試験法、マウス肺マクロファージや MSTO 細胞における DNA 二重鎖切断の誘発など、新しい毒性評価法・試験法について、極めて興味深い報告がなされた。

G. Patrick 教授（MRC Toxicology Unit, UK）による「肺マクロファージ初期培養を用いた吸入物質の毒性影響の予防法」と題する講演では、吸入物質の毒性影響試験法について、広範なレビューが行われた。さらに、粒子状テルビウムやコバルトの肺マクロファージによる解離性と毒性、アスペスト曝露による呼吸器細胞の免疫学的反応、呼吸器障害の誘発メカニズムとの関連等について詳しい報告がなされた。

平野清司郎博士（国立環境研究所）による「肺に及ぼす塩化イットリウムの影響」と題する講演では、先端科学技術産業に伴う環境有害物質として希土類元素、特にイットリウムの毒性について報告された。$^{40}$Y は β線放出核種であり臨床応用されているが、イットリウム自体の体内代謝、毒性についての情報は少ない。講演では、塩化イットリウムの呼吸器毒性について、SPF ウィスタンラットを用いた豊富な実験データが紹介された。

荻生俊昭博士（放射線医学総合研究所）による「実験
動物における化学物質及び放射線による発癌」と題する講演では、ラットを用いた化学物質及び放射線（X線）の発ガン性の比較が紹介された。N-ニトロソ化合物は、強い発ガン性を持ち、かつその前駆物質は自然環境中に存在しているため、環境安全評価上重要である。講演では、MNU、ENU、PNU、BNUなどのN-ニトロソ化合物による発癌性、発癌部位、放射線による発癌との対比が詳しく述べられた。

4. 総合討論
1月30日午後からは、「比較影響評価研究の今後の展開」に関する総合討論が行われた。国立病院総合研究所センター長隆渉行雄博士から、水俣病からチェルノブイル事故にまでわたる広範な環境毒性物質に関する講演が行われ、引き続いてW.A. Schell教授、G.G. Polikarpov教授、A.W. Hse教授、R. Sett博士その他のワークショップ参加者から、今後の研究動向に関する提言、コメントなどが提出された。これらの提言に関しての熱心な討論が行われた。

最後に、本ワークショップ企画運営委員長である福寺次郎放射線医学総合研究所研究総務官より、閉会の辞が述べられ会社のうちに閉幕した。

5. おわりに
このワークショップでは、放射線・環境放射線のみならず、種々の環境有害物質に関して、環境研究から生物毒性研究にわたる様々な分野の研究者による講演、議論が行われた。講演内容のプロシーディングは、近々刊行される予定である。

共催していただいた日本保健物理学会及び日本放射線影響学会、また後援していただいた科学技術庁、科学技術国際交流センターの諸機関に感謝を表す。

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Effects of Atmospheric Transport on Temporal Variations of $^{222}$Rn and Its Progeny Concentration in the Atmosphere

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The purpose of this study was to estimate the effects of atmospheric transport on temporal variations in the concentration of $^{222}$Rn and its progeny in the air by using a three dimensional atmospheric dispersion model. The objective region for this study was the Chubu district in Japan and the objective period was from November 1 to 20, 1991. It is well known that the diurnal variation of concentration is due to the diurnal cycle of the growth and decay of the mixed layer. In addition, this study led to the following conclusions:

1. Comparing the observed results with one- and three-dimensional models showed that the atmospheric transport of $^{222}$Rn and its progeny affects the temporal variation of concentrations for limited area sources like the islands of Japan.

2. Time series analysis of observed and calculated results revealed that a periodicity of several days was included in the variation of the concentration of $^{222}$Rn from a remote source. Moreover, it suggested that it is dependant on the long-range transport of $^{222}$Rn by the air-flow caused by the changing high and low pressure systems.

KEYWORDS: radon 222, progeny, atmospheric transport, advection, time series analysis, seasonal adjustment model, remote source, exhalation rate, equilibrium factor, atmospheric diffusion

I. INTRODUCTION

In the UNSCEAR 1988 Report[13], it was estimated that the inhalation of $^{222}$Rn progeny accounts for about one half of the effective dose equivalent from all natural sources of radiation. Therefore, to estimate the radiation dose to the public, it is important to study $^{222}$Rn progeny's behavior in the atmosphere.

For several decades, many field studies have been carried out, in which the variations of the outdoor atmospheric concentration of $^{222}$Rn and its progeny have been cleared with local time, season, altitude and location, quantitatively. Moreover, numerical analysis were performed by several investigators to reveal diurnal variations and vertical diffusion theoretically[13]-[14].

However, their studies using one dimensional model have concentrated on the variation caused by vertical diffusion, although atmospheric transport could also greatly contribute to the variation of the $^{222}$Ra and its progeny concentration at sites adjacent to large bodies of water, like Japan. This study therefore aims at estimating the effects of atmospheric transport on the temporal variation of $^{222}$Rn and its progeny concentration by using a three-dimensional numerical atmospheric dispersion model. In particular, the effects of advection in local and synoptic areas were investigated.

II. MODEL

In general, Atmospheric dispersion models are divided into two types; the Eulerian model which solves in advection and diffusion equations, and the Lagrangian model in which the transportation of materials is expressed by the movement of a large number of marker particles. One of the advantages of the Lagrangian model is that it is free from the numerical diffusion which occurs in the Eulerian model[13]. However, if the Lagrangian model is applied to an area source like $^{222}$Rn, a very large number of maker particles would be needed to keep the statistical error small. Therefore, an Eulerian model that included a highly accurate finite differencing scheme for numerical diffusion was used in this study.

1. Coordinates

Rectangular coordinates were used for the horizontal plane, and a uniformly spaced computational grid was employed. For the vertical direction, we used a terrain-
following-coordinate \((z^\ast)-\text{coordinate}\) in order to take the effect of terrain in the calculational area into consideration. The \(z^\ast)-\text{coordinate}\) is represented by

\[ z^\ast = \frac{H(z - z_p)}{(H - z_p)}, \quad (h = H - z_p), \quad (1) \]

where \(z_p(x, y)\) is the terrain height and \(H\) the reference altitude for the upper boundary. The reference altitude was set at 3 km. In the vertical direction, a variable spacing grid was employed.

2. Wind Field Model

We used a mass-consistent wind field model (WSYNOP) that was developed by Ishikawa\(^{(6)}\). Firstly, the model wind components for each section of grid are calculated by the simple interpolation of the surface and aerological data. The wind field used in the transport calculation needs to satisfy the equation of continuity,

\[
\frac{\partial (hu)}{\partial x} + \frac{\partial (hv)}{\partial y} + \frac{\partial (hw^\ast)}{\partial z^\ast} = 0, \quad (2a)\]

where \(u, v\) and \(w^\ast\);

\[
w^\ast = \frac{1}{(H - z_p)} \left\{ (z^\ast - H) \left( u \frac{\partial x}{\partial x} + v \frac{\partial x}{\partial y} \right) + Hw \right\} \quad (2b)\]

are components of wind velocity along the \(x, y\), and \(z^\ast\) axes. The interpolated wind field is adjusted by the variational method so that it satisfies Eq. (2a). (See detail in Ishikawa\(^{(6)}\))

3. Atmospheric Transport Model

We solved advection and diffusion equations which included horizontal and vertical advection, vertical diffusion, sink and source. Equations for the transport-model for \(^{222}\text{Rn}, ^{218}\text{Po}, ^{214}\text{Pb}\) and \(^{214}\text{Bi}\) are

\[
\frac{\partial h C_i}{\partial t} + \frac{\partial (hu C_i)}{\partial x} + \frac{\partial (hv C_i)}{\partial y} + \frac{\partial (hw^\ast C_i)}{\partial z^\ast} = \frac{\partial}{\partial z^\ast} \left[ \frac{H^2}{(H - z_p)^2} \frac{\partial h C_i}{\partial z^\ast} \right] - h \lambda_i C_i + \lambda_{i-1} C_{i-1}, \quad (i = 1, \ldots, 4), \quad (3)\]

where \(C_i\) is the concentration of the \(i\)-th member of the decay chain (\(i=1\) for \(^{222}\text{Rn}, i=2\) for \(^{218}\text{Po}, i=3\) for \(^{214}\text{Pb}\), and \(i=4\) for \(^{214}\text{Bi}\)) and \(\lambda_i\) the corresponding radioactive decay constant. The second term, \(-h \lambda_i C_i\), is the sink to radioactive decay. The third term, \(\lambda_{i-1} C_{i-1}\), is the generation d由于 the decay of the \((i-1)\)th nuclide in the decay chain. For \(^{222}\text{Rn}\) when \(i=1\), instead of this term, we used the following boundary conditions to consider the exhalation of \(^{222}\text{Rn}\) from the ground;

\[
-\frac{H^2}{(H - z_p)^2} K \frac{\partial h C_1}{\partial z^\ast} = h E \quad \text{at} \quad z = z_g, \quad (4)\]

where \(E\) corresponds to the \(^{222}\text{Rn}\) exhalation rate and \(K\) the vertical diffusion coefficient. As for deposition, we assumed that the \(^{218}\text{Po}, ^{214}\text{Pb}\) and \(^{214}\text{Bi}\), which reached the ground surface by molecular diffusion would never be resuspended. This assumption gives the following boundary conditions:

\[ C_i = 0 \quad \text{at} \quad z = 0 \quad i > 1. \quad (5)\]

In this model, advection and diffusion equations for \(^{222}\text{Rn}\) progeny were transformed to similar form to \(^{222}\text{Rn}\) by using the numerical technique introduced by Beck so that computations can be easily performed (See detail in Ref. (3)).

The advection term was solved using the NIC scheme\(^{(7)}\). In this method, the flux at the grid-interval boundary \(x_i\) was calculated assuming the local density in the whole integration-domain had a continuous-first-derivative. The local density can be represented by a parabolic curve within a mesh width, \(\Delta x\). Integral variables, when normalized to a unit grid interval, are

\[ \phi_i = \frac{1}{\Delta x} \int_{x_{i-1/2}}^{x_i} \phi(x, t) dx = \frac{1}{\Delta x} [\psi(z_i) - \psi(z_{i-1})], \quad (6)\]

where \(\phi\) is the local density, \(\psi\) the primitive function of \(\phi\) and the subscript \(i\) the mesh number. Cubic spline interpolation is applied to \(\psi\) to obtain \(\phi\). As a result, the flux at the grid-interval boundary is

\[ F_i = a \frac{\Delta x}{\Delta t} \left[ \phi c + (3\phi_{i-1/2} - 2\phi_i - \phi_{i-1})c^2\right] + (\phi_i + \phi_{i-1} - 2\phi_{i-1/2})c^3], \quad (7)\]

where \(a = sgn(u_i)\) governs the direction of the fluxes, and \(c\) the Courant number. The average \(\phi\) within the integral grid interval for the next time step is calculated using the following equation,

\[ \phi_{i+1/2, i+1} = \phi_{i-1/2, i+1} - \frac{\Delta t}{\Delta x} (F_i - F_{i-1}), \quad (8)\]

where \(\Delta t\) is the time step. The time step used in the transport model was set to 60 s. The wind field was calculated every 3 h and linearly interpolated to the time step of the transport model.

The diffusion term was solved using the Crank Nicolson method\(^{(10)}\). Diffusion coefficients were calculated with the turbulence closure model (Level 2) of Mellor and Yamada\(^{(9)}\). The Level 2 model reduces the entire turbulence model to the algebraic relation of turbulent quantities and can be cast in a traditional eddy coefficient format modified by Richardson-number-dependent functions. The diffusion coefficient on the surface was set to the molecular diffusion constant of 5.4\(\times10^{-6}\text{m}^2/\text{s}\). An upper limit (70 m/s) for the diffusion coefficient was imposed to avoid unlikely strong vertical diffusion in unstable conditions. A lower limit was also introduced as seen in Fig. 1.
4. Computational Area

Figure 2 shows the computational area. This area is centered at Nagoya city, and covers the Chubu-district. The grid size for the simulation was 51×51×21 with a horizontal interval of 4 km. In the vertical, the variable mesh width was used, in which the depth of the lowest layer was 2 m.

5. Meteorological Data and Calculational Period

To estimate the diffusion coefficients, we used wind and air temperature data from Chukyo-TV monitoring station of the Aichi Prefectural Office (15, 17, 135 and 137 m), the electric power plant at Hamaoka (20 and 100 m) and Environmental Science Research Center of Fukui prefecture (7.5 and 185 m).

Aerological wind and air temperature data from Hamamatsu, Wasima and Shionomisaki stations of the Japan Meteorological Agency were used to estimate the upper wind-field above about 1,500 m (850 hPa). Furthermore, wind data from the Japan Meteorological Agency's Automated Meteorological Data Acquisition System (AMeDAS) were used to calculate horizontal wind field near the surface.

The calculations were done for the period from November 1 to 20, 1991.

6. Boundary Conditions

The inflow of $^{222}$Rn and its progeny at the lateral boundary was assumed to be zero in this simulation. However, the measured concentrations of $^{222}$Rn are the summation of $^{222}$Rn emanating in the computational area and $^{222}$Rn flowing into the computational area from the outside of the area. Therefore, the value of 2.3 Bq/m$^3$ was added to calculated concentrations to account for $^{222}$Rn flowing into the area. As for the radon progeny, the concentration balancing to 2.3 Bq/m$^3$ of $^{222}$Rn was added to the computed value to account for the contribution from remote sources.

III. RESULTS

1. Analysis of Observations

Outdoor $^{222}$Rn concentration, which was averaged for 1 h, was measured with an electrostatic radon monitor (ERM)$^{11}$ at a height of 1 m above the ground every hour. Polonium-218, $^{214}$Pb and $^{214}$Bi concentrations, which were averaged for 7 min after evaluation time, were measured using a filter method monitor (MGR)$^{12}$ at the same height every 3 h.

Calculated results for all the objective nuclides were averaged for 1 h.

Since there was little rain over the calculational period in Nagoya, it was supposed that the contribution of the precipitation to the observed concentration was minor.

In order to eliminate short term fluctuations from observed $^{222}$Rn and its progeny concentrations, and to estimate the distribution of observations, a trend model was used.

The trend model$^{13}$ assumes that time series of observation $Y_n$, which is the $^{222}$Rn concentration measured in outdoors, consists of the trend-component-model in Eq.(9) and the observational-model in Eq.(10). The trend model is written as

$$\Delta^k t_n = v_n, \quad \text{Eq.(9)}$$
$$Y_n = t_n + w_n, \quad \text{Eq.(10)}$$

where $t_n$ is the trend at the $n$-th time step, $\Delta^k$ the $k$-th order of the finite difference, and $v_n$ the white noise whose standard deviation is $\tau$ (system noise) and the average is zero. $w_n$ is the white noise of which the standard deviation is $\sigma$ (observational noise) and its average is zero. This represents the observational error. For $k$, we used a value of 2. When $k=2$, Eq.(9) is $t_n = 2t_{n-1} - t_{n-2} + v_n$. This allows the trend component to vary linearly in a local aspect. The trend was calculated using Kalman filtering and smoothing. $\sigma$ was calculated using the max-
imum likelihood method. Figure 3 shows the results applied trend model analysis to $^{222}$Rn concentration. Most of the observed points are within ±3σ of the trend component.

2. Comparison of Three Simulations with Observed Data

The trend of the observed data were compared with those of the following three simulations: (1) one-dimensional $^{222}$Rn simulation (1D); (2) three-dimensional $^{222}$Rn simulation assuming a constant-exhalation-rate (3D(CE)); (3) three-dimensional $^{222}$Rn simulation taking the exhalation-rate map into consideration (3D(ME)).

To clarify the effects of advection, 1D was compared to 3D(CE). While the one-dimensional model assumes essentially an infinite plane source, the three-dimensional model must consider the source distribution on land and sea. Thus, in 1D and the land areas of 3D(CE), the $^{222}$Rn exhalation rate was set to 0.48 atom/cm$^2$·s$^{13}$. Since the oceanic emission was 100-1,000 times smaller$^{13}$ than the emission from the land areas, the flux of $^{222}$Rn from the ocean was assumed to be zero in 3D(CE). Furthermore, a constant value of 2.3 Bq/m$^3$ was added to the calculated value of the 3D(CE) to sum up the inflow from the outside of the area. As shown in Fig. 4(a) and (b), it is clear that the variations in 3D(CE) are closer to the trend of observed than those of 1D. The trend of the observed results was not shown from 0:30 13th November to 9:30 14th November, because of the lack of observed data.

The radon exhalation rate varies widely according to the soil, atmospheric conditions, and so on$^{13}$. Although, in 3D(CE), the radon exhalation rate was assumed to be constant at 0.48 atom/cm$^2$·s, the geographical distribution of the source was also considered in 3D(ME). The source was estimated by the following equation introduced by Ikebe et al.$^{13}$:

$$Q = 0.48E + 2.3,$$

where $Q$ is the annual average of $^{222}$Rn concentration (Bq/m$^3$) and $E$ the exhalation rate (Bq/m$^2$·s) depending on the geographical location. From observed data at some sites in the objective region$^{13}$, $Q$ was estimated at each grid point by simple interpolation with the weight of the reverse square of the distance between the site and grid. The second term on the right hand of the equation, 2.3 Bq/m$^3$, corresponds to the remote source component. Figure 5 is a map of $^{222}$Rn exhalation rate.

The result of the three-dimensional simulation that takes the exhalation rate map into consideration (3D(ME)) is shown in Fig. 4. In Fig. 4, it can be seen that 3D(ME) showed better agreement with the trend of observed than 3D(CE).

3. Comparison of Observed and Simulated Concentrations for $^{222}$Rn, $^{218}$Po, $^{214}$Pb and $^{214}$Bi

Comparisons of observed concentrations of $^{222}$Rn,
of "Trend +3σ" and "Trend −3σ" was presented because fluctuations in the observed results were very large. The observed and calculated concentration averages over the calculational period (1991.11.1-11.20) are shown in Table 1. As can be seen in Table 1, the average calculated concentration for each nuclide is approximately the same as what was observed. This means that our calculational model worked well.

Table 2 shows the observed and calculated equilibrium factors. The calculated equilibrium factor was very close to the observed one. Both are also close to the value

<table>
<thead>
<tr>
<th>Term</th>
<th>Observed</th>
<th>Calculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>222Rn</td>
<td>6.93</td>
<td>5.06</td>
</tr>
<tr>
<td>214Pb</td>
<td>5.25</td>
<td>7.90</td>
</tr>
<tr>
<td>214Bi</td>
<td>6.39</td>
<td>7.20</td>
</tr>
</tbody>
</table>

Table 2 Equilibrium factor

<table>
<thead>
<tr>
<th>Term</th>
<th>Observed</th>
<th>Calculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1991.11.1-11.20</td>
<td>0.87</td>
<td>0.89</td>
</tr>
</tbody>
</table>

Fig. 5 Radon-222 exhalation rate distribution map (×10^{-1} atom/cm^{2}·s=2.1 mBq/m^{3}·s)

218Po, 214Pb and 214Bi with calculated ones in 3D(ME) are shown in Figs. 6-9, respectively. In each figure, calculated results, observed results, Trend +3σ and Trend −3σ are presented. However, in Fig. 7, "Trend" in place

Fig. 6 Comparison of observed and calculated 222Rn concentration on (a) November 1-10, 1991 and (b) November 11-20, 1991

Fig. 7 The same as Fig. 7, except for 214Po
reported in UNSCEAR 1988(1) Report.

Although the calculated concentrations agreed well with the measurements on average, there are still some discrepancies between the calculated concentrations and the observed range from Trend +3σ to Trend -3σ in Figs. 6-9. In the following section, the discrepancy between the observed and calculated $^{222}\text{Rn}$ concentrations will be investigated with time series analyses of the observed and calculated results, because the variation of $^{222}\text{Rn}$ progeny are just like that of $^{222}\text{Rn}$.

**IV. DISCUSSION**

In this chapter, the causes of the discrepancies of $^{222}\text{Rn}$ concentrations found in Figs. 6 is investigated by using the following time series analysis: (1) Autocorrelation function and power spectrum of observed and calculated $^{222}\text{Rn}$ concentration; (2) Application of seasonal adjustment model(20) to observed and three calculated concentrations; (3) Correlation between observed $^{222}\text{Rn}$ concentration and atmospheric pressure; (4) Scale analysis of a variation of $^{222}\text{Rn}$ concentration.

1. **Autocorrelation Function and Power Spectra**

As the first step to clarify the reasons of the discrepancies, the autocorrelation function between the observed and the calculated concentrations were evaluated for $^{222}\text{Rn}$. We used the data with the interval of 3 h by the following reasons: (1) The data with the interval of 3 h had almost the same correlation and the power spectrum as those of 1 h data; (2) The variation with in longer cycles than 3 h was discussed, because the input interval of meteorological data was 3 h in the calculation.

Figures 10(a) and (b) shows the autocorrelation functions of the observed and calculated $^{222}\text{Rn}$ concentrations. Time-lag (in days) was represented by the abscissa. As shown in Figs. 10(a) and (b), both have the strong correlation with the time lag of 1 day. In addition, the correlation with the time lag of about 5 days, shown by the dotted line in Fig. 10(b), overlap daily correlation in the calculated result, whereas observed result did not show the correlation with such a time-lag. To inspect this difference in more detail, power spectra of the variations of the observed and calculated concentrations was evaluated.

The power spectra of the observed and calculated $^{222}\text{Rn}$ concentrations, which represent the magnitude of various periodical components included in the variations, are shown in Fig. 11. The shapes of both spectra were basically similar. In the spectrum of the observed result,
Effects of Atmospheric Transport on $^{222}$Rn and Its Progeny Concentration

Fig. 10 Autocorrelation function of the observed and calculated $^{222}$Rn concentration

Fig. 11 Observed and calculated power spectra

there was the distinguished peak of 22.9 (h). The rest of peaks were also higher harmonics of 22.9 (h). In contrast, many peaks at 120, 48 and 24 (h) were appeared in the calculated spectrum. The first two spectral peaks were not appeared in the observed spectrum. From these results, it is concluded that the variation of the calculated concentration had conspicuous long-term cycles, including 5-day appeared in Fig. 10(b), whereas the effect of such a cycle was not shown in that of the observed. The reason of this discrepancy was studied by using the seasonal adjustment model which could decompose a variation into some components with the characteristic cycle.

2. Application of the Seasonal Adjustment Model

To estimate components of a variation and investigate the effects of diffusion, advection and the geographical distribution of the source on the components, we applied the seasonal adjustment model to observed and three calculated variations, 1D, 3D(CE) and 3D(ME) (See detail in Sec.III-2). This model is almost similar to the trend model. Observations $Y_n$ are written as

$$Y_n = t_n + d_n + p_n + w_n$$

$$t_n = t_{n-1} + \sigma_t \quad (n = 1, \ldots, m),$$

$$d_n = d_{n-1} + \sigma_d$$

$$p_n = \sum_{k=1}^{2} a_{n-k} C_{n-k} + \sigma_p,$$

where $t_n$, $d_n$, $p_n$ and $w_n$ are a trend component (TC), a daily component (DC), a stationary autoregressive component (AR) and an observational noise component at a time step $n$, respectively. $\sigma_t$, $\sigma_d$ and $\sigma_p$ are white noises. $m$ is the total number of the time steps. A daily component represents a daily variation. A stationary AR component represents a variation dominantly affected by concentrations within 6 h before an evaluating time. In addition, it represents a variation with a short-term cycle. A trend component is different from that of the trend model mentioned in the Sec.III-1. It is a variation except daily and AR components from a variation, and represents a variation with a long-term cycle.

The power spectra of components, DC, AR and TC and variations of them were investigated.

(1) Daily Component

Figure 12(a) shows power spectra of daily components of observed, 1D, 3D(CE) and 3D(ME). All the spectral shapes were almost the same. However, by looking into the daily variations of the observed and three calculated concentrations, the discrepancies between them became apparent as shown in Fig. 12(b). The variation of 3D(ME) was the closest one to that of observed, and the concentrations of 3D(ME) are almost the same value as observed ones. This result shows that 3D(ME) can simulate the daily variation of observed concentrations and it is important to consider the effects of advection and source's distribution.

(2) Autoregression Component

Figure 13(a) shows the power spectra of autoregression components. Power spectra of calculated results,
1D, 3D(CE) and 3D(ME) had almost the same shape. In the region of the long-term cycles, the considerable discrepancies between observed and calculated results were appeared. This discrepancy is more apparent in the variation of autoregression components shown in Fig. 13(b). As shown Fig. 13(b), all calculated results had basically similar patterns in the variation. In addition, every several days, the overestimations of the calculated concentrations happened to all calculated results.

This means that the calculated variations have the several-days cycle. Thus, the several-days cycle of calculated results shown in Fig. 13(a) was caused by this overestimations. Moreover, it seemed that the discrepancies on several-days cycle in Fig. 11 (See detail in Sec.IV-1) was caused by the same reason. The root of the problem that the calculated variations had the several-days cycle was the insufficient modeling. However, as shown by the arrows, the discrepancies became small by the improvement of the model. Introducing a more realistic model is proper. Nevertheless, a little discrepancies were left in these term. In addition, there was no improvement in the term from 2nd to 4th November. As for these discrepancies, although Level 2.0 would be probably one of causes on these problems, they need to be investigated in detail.

(3) Trend Component

Figure 14(a) shows power spectra of trend compo-
nents of observed, 1D, 3D(CE) and 3D(ME). The spectrum of observed results had the distinguished peak at several-days cycle. The spectrum of 1D's results was also shown.

The variations of the trend components are shown in Fig. 14(b). The trend component of 1D's result gradually increased. It was caused by a transition to a steady state, because of the initial condition that $^{222}\text{Rn}$ concentrations were equal to zero in the whole domain. The trend component of observed results varied with a several-days cycle. However, the trend components of 3D(CE) and 3D(ME) were constant. Thus, the trend components seems important to explain the discrepancies between the observed and calculated variations. Two explanations are possible. One reason of the discrepancy is the changes in meteorological conditions in the local area, affecting the exhalation rate of $^{222}\text{Rn}$. It will be discussed in the next section. Second is a contribution of $^{222}\text{Rn}$ (remote source component) migrating from the outside of the calculational area, which is not considered in the calculation.

3. Correlation between Observed $^{222}\text{Rn}$ and Atmospheric Pressure

For changes of atmospheric pressure, it is possible that $^{222}\text{Rn}$ exhalation changes over a few days. To investigate the effect, the cross-correlation function between observed $^{222}\text{Rn}$ and the atmospheric pressure were evaluated. Figure 15 shows the result of it.

In the previous section, it was pointed out that the trend component of $^{222}\text{Rn}$ concentration varied with several days cycle. In relation to this, it should be noted that the $^{222}\text{Rn}$ exhalation rate increases with the low atmospheric pressure. However, since the cross-correlation between the local atmospheric pressure and observed $^{222}\text{Rn}$ concentration is positive, as seen in Fig. 15, there is no evidence of such influence. Therefore, the time variation of observed $^{222}\text{Rn}$ concentration, like the trend component in Fig. 14(b), must be due to variations on the remote source component.

4. Scale Analysis

A simple analysis was performed in order to relate the time scale of the periodical variation, i.e. $^{222}\text{Rn}$ concentration, to the geographical scale.

If the effect of eddy diffusion on long-range transport is smaller than that of advection, the transport equation can be approximated as

$$\frac{\partial C}{\partial t} = -u \frac{\partial C}{\partial x},$$

where $C$ is the concentration and $u$ the wind velocity along the $x$-axis. One of the solutions of Eq.(13) is

$$C = A \cos \left( \frac{2\pi}{\lambda} (x - ut) \right),$$

where $A$ is a constant and $\lambda$ the wave length. The dispersion relation is

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\[ \lambda = uT, \]  
(15)

where \( T \) is the periodicity. This relation is shown in Fig. 16. This means that a cycle of several days corresponds to a wave length of several hundred to several thousand km. This scale coincides with the scale of an ordinary high or low pressure system. It suggests that the time variation of the remote-source component of \( ^{222}\text{Rn} \) concentration depends on the change in air-flow due to high or low pressure systems and long-range transport that accompanies the change.

V. CONCLUSION

The effects of atmospheric transport on temporal variations of \( ^{222}\text{Rn} \) and its progeny concentration in the atmosphere were estimated using a numerical model. The major findings are as follows:

1. Advection and geographical variations in \( ^{222}\text{Rn} \) exhalation rate affect a variation of \( ^{222}\text{Rn} \) concentration.

2. The daily component of the observed variation were simulated well by our model.

3. The long-term cycle of the observed variation could not be simulated by a local calculational model. The calculation for a nuclide with a half-life time like \( ^{222}\text{Rn} \) needs the regional calculational area.

Furthermore, time series analyses suggested that the time variation of the remote-source component of \( ^{222}\text{Rn} \) concentration depends on the change of air-flow due to high or low pressure systems and the long-range transport accompanied with these changes.

Decomposing an original variation into some components with the characteristic cycle, the discrepancies between observed and calculated variations became more apparent. On each components, if the causes of the discrepancies would be investigated in more detail, simulating the observed variation by the computational model will be possible.

In addition, the eddy diffusion coefficient should be improved as the limitations of the level 2 model under unstable and extremely stable conditions were pointed out by Takagi et al. (1989). The influences of meteorological factors must be also studied in detail.

Numerical simulations of this study provide detailed information on the source, diffusion, deposition and equilibrium factors, which are useful for clarifying the behavior of \( ^{222}\text{Rn} \) and its progeny in the environment.

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![Fig. 16 Dispersion relation](image-url)
EFFECTS OF GADOLINIUM ON THE RETENTION AND TRANSLOCATION OF $^{239}$Pu-HYDROXIDE

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Abstract—The effect of gadolinium on the lung retention, excretion, and translocation of plutonium was studied in rats instilled intratracheally with plutonium hydroxide with and without gadolinium. Three types of plutonium hydroxide were prepared: pure $^{239}$Pu-hydroxide colloid, that containing a high concentration of gadolinium, and that containing a low concentration of gadolinium. The lung retention of $^{239}$Pu was higher and the fecal excretion was lower in the rats administered $^{239}$Pu-hydroxide containing a high concentration of gadolinium than those administered pure $^{239}$Pu-hydroxide colloid. The translocation of $^{239}$Pu from lung to other organs including the liver, spleen, femur, and kidney was not affected by gadolinium. The cytological examination of bronchoalveolar lavage cells showed that the administration of $^{239}$Pu-hydroxide containing a high concentration of gadolinium induced the inflammatory reactions in the lung. The delayed alveolar clearance of plutonium in the rats administered $^{239}$Pu-hydroxide colloid containing a high concentration of gadolinium may be attributable to the change in physicochemical characteristics of colloid and the inflammation induced in the lung by gadolinium.

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Key words: plutonium; rat; lungs; rodent; biokinetics

INTRODUCTION

GADOLINIUM (Gd), one of the rare earth elements, is used as a material in semiconductor and magnetic substances in industry, and as a contrast agent for magnetic resonance imaging in clinical medicine (Hirano and Suzuki 1996; Weinmann et al. 1984; Runge and Parker 1997). Gadolinium is also used as a neutron absorber in the nuclear industry. In a newly developed facility for reprocessing of nuclear fuel in Japan, gadolinium nitrate is added to the nitric acid solution as a neutron absorber during the fuel-dissolution process. There is concern, therefore, that workers could be simultaneously exposed to gadolinium and radioactive nuclides in an accident.


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Gadolinium is a toxic element, especially for reticuloendothelial cells such as alveolar macrophages (O’Neill et al. 1994; Yoneda et al. 1995; Suzuki et al. 1996; Ruttigser et al. 1996), and thus may affect the metabolism of radionuclides in the human body. However, little is known about the effects of gadolinium on the retention and metabolism of radionuclides in the human body.

Inhalation may be a possible route for the accidental contamination by gadolinium and radionuclides. In such a case, plutonium would be one of the important radionuclides. In the present study, therefore, an animal experiment was carried out to elucidate whether or not the co-existence of gadolinium may affect the retention and translocation of plutonium in the lung. Plutonium hydroxide with and without gadolinium was instilled into the rat lung, and the lung retention and translocation of plutonium were compared.

MATERIALS AND METHODS

$^{239}$PuO$_2$ was obtained from the Japan Atomic Energy Research Institute and converted to $^{239}$Pu nitrate by a procedure described elsewhere (Black and Drummond 1965). Then gadolinium nitrate, Gd(NO$_3$)$_3$, was added to the plutonium nitrate solution, and the pH of the solution was adjusted to 7.2 with 0.1 M sodium hydroxide under vigorous agitation. By this neutralization, a colloidal form of plutonium hydroxide containing gadolinium was formed. The resultant hydroxide colloids were stabilized with 0.2% gelatin and made isotonic with 0.25 M glucose. Two types of $^{239}$Pu-hydroxide colloid containing gadolinium were prepared. One was $^{239}$Pu-hydroxide containing a high level of gadolinium (0.9 mg Gd mL$^{-1}$ in the final solution, referred to as Pu-Gd-H in the following), and another containing a low level of gadolinium (0.22 mg Gd mL$^{-1}$, referred to as Pu-Gd-L). $^{239}$Pu-hydroxide colloid was also prepared by the same procedures described above without addition of gadolinium. The radioactive concentration of $^{239}$Pu in the final colloidal solutions was 15 kBq mL$^{-1}$ in all three solutions. The reagents used for these procedures were analytical grade (Wako Pure Chemical Industries$^5$).

$^5$ Wako Pure Chemical Industries, Ltd., 5-13, Honcho 4-chome, Nihonbashi, Chuo-ku, Tokyo 103-0023, Japan.
Nine-week-old male Sprague-Dawley rats, weighing about 300 g, were obtained from a domestic breeder. All animals were kept in an air-conditioned room (22±2°C), and given a commercial rat chow and drinking water ad libitum. The animals were divided into three groups consisting of fourteen rats each, and the colloidal solution of Pu and Gd was administered into lung with intratracheal instillation. The detail of the procedure has been described elsewhere (Sato et al. 1994). In brief, the animals were anesthetized with pentobarbital, and 0.3 mL of 239Pu-hydroxide, Pu-Gd-H, or Pu-Gd-L was administered with a single and rapid instillation via an intratracheal cannula. It has been previously found that this dose of Pu-hydroxide colloidal solutions induces no significant inflammatory reactions in the lung (Sato et al. 1994; Takahashi and Sato 1991).

Four weeks after instillation, three rats of each group were sacrificed to determine the lung retention and translocation of 239Pu. The lung, liver, kidney, spleen, and right femur were dissected. Feces and urine were collected 4 d, 1 wk, 2 wk, 3 wk, and 4 wk after instillation. The activity of 239Pu was measured by liquid scintillation counting as described by Keough and Powers (1970). In brief, the tissue samples and feces were ashed at 550°C for 12 h, and the residues were dissolved in 0.2 mL of 7 M nitric acid containing a small amount of concentrated hydrogen fluoride. Then 3.6 mL of scintillation cocktail was added to the sample solution, and the activity was measured using a liquid scintillation counter. The activity of 239Pu in urine was measured directly with a liquid scintillation counter after filtering through a 0.22 μm membrane filter. The lung retention, excretion, and translocation of 239Pu were represented as a percent of the initial alveolar deposition. The initial alveolar deposition (IAD) was defined as the whole body retention of 239Pu on the third day after administration (i.e., the administered 239Pu minus that excreted up to the third day). This value did not exactly represent the actual retention of 239Pu in the lung, since the activity of 239Pu in organs other than the lung is also included. However, this value could be used for IAD, since the activity of 239Pu in organs other than the lung is assumed to be negligible (Ishigure et al. 1994; Oghiso et al. 1994).

To monitor the occurrence of inflammation in the lung, the remaining animals were serially killed, and bronchoalveolar lavage cells were collected by lung lavage with sterilized Dulbecco’s phosphate-buffered saline. The total number of cells collected was determined with a hemocytometer, and differential cell counts were performed on smeared cells after Giemsa staining.

Means of the groups were compared by single-tailed t-tests to determine the level of significance. An observed difference was considered statistically significant if \( p < 0.05 \).

**RESULTS**

As shown in Table 1, the ratios of IAD to instilled activity were 0.30±0.15, 0.38±0.13, and 0.41±0.19 in the rats administered 239Pu-hydroxide, Pu-Gd-L, and Pu-Gd-H, respectively. There was no statistically significant difference among the 3 groups.

Whole-body retention and total excretion of 239Pu are shown in Fig. 1. The retention of 239Pu in the lungs and the fecal and urinary excretions are shown in Fig. 2. In the rats administered 239Pu-hydroxide, the lung retention of 239Pu was 25.8±2.5% IAD 4 wk after administration. In contrast, the lung retention of 239Pu in the rats administered Pu-Gd-H was 43.2±4.4% IAD. The difference between the groups was statistically significant \( p < 0.05 \). The lung retention of 239Pu in the Pu-Gd-L group was similar to that in the 239Pu-hydroxide group. The fecal excretions of 239Pu decreased with the increase in gadolinium contents: 51.9±5.9, 40.9±6.4, and 28.2±0.6% IAD in the 239Pu-hydroxide, Pu-Gd-L, and Pu-Gd-H groups, respectively. The urinary excretion of 239Pu in the Pu-Gd-L group was significantly higher than in the other groups \( p < 0.005 \). Figs. 3 and 4 show the daily and cumulative excretion of 239Pu in feces and urine. The daily fecal excretion was significantly lower in the Pu-Gd-H group than the 239Pu-hydroxide group throughout the experimental period (4 d to 4 wk after the administration, \( p < 0.05 \)). In the Pu-Gd-L group, the daily fecal excretion was significantly lower than the 239Pu-hydroxide group only 3 wk after administration \( p < 0.01 \). Cumulative fecal excretion was similar to that in the 239Pu-hydroxide group. As shown in Fig. 4, the daily urinary excretion was significantly higher in the Pu-Gd-L group than the 239Pu-hydroxide group 4 d, 1 wk, 2 wk, and 4 wk after administration \( p < 0.05 \). Interestingly, there was no significant difference between Pu-hydroxide and Pu-Gd-H groups for the daily and cumulative excretion in urine.

Fig. 5 shows the translocations of 239Pu to the major organs, expressed as % IAD per organ. The amounts of 239Pu in the liver of rats administered 239Pu-hydroxide, Pu-Gd-L, and Pu-Gd-H were 3.16±0.29, 3.38±1.14, and 2.89±0.42%, respectively. The amounts of 239Pu translocated from lung to kidney, spleen, and femur were less than 1% in all groups. In all the organs examined, no
Fig. 1. Whole body retention and cumulative excretion of $^{239}$Pu in rats following intratracheal instillation of $^{239}$Pu-hydroxide, Pu-Gd-L, and Pu-Gd-H. Each point is the mean of three rats.

Fig. 2. Lung retention and cumulative excretion of $^{239}$Pu in feces and urine 4 wk after intratracheal instillation of $^{239}$Pu-hydroxide, Pu-Gd-L, and Pu-Gd-H. Vertical bar shows SD of three rats. * denotes significant difference from $^{239}$Pu-hydroxide group at $p < 0.05$; † $p < 0.01$; ‡ $p < 0.005$.

Fig. 3. Daily and cumulative excretion of $^{239}$Pu in feces following intratracheal instillation of $^{239}$Pu-hydroxide, Pu-Gd-L, and Pu-Gd-H. Vertical bar shows SD of three rats. * denotes significant difference from $^{239}$Pu-hydroxide group at $p < 0.05$; † $p < 0.01$.

Fig. 4. Daily and cumulative excretion of $^{239}$Pu in urine following intratracheal instillation of $^{239}$Pu-hydroxide, Pu-Gd-L, and Pu-Gd-H. Vertical bar shows SD of three rats. * denotes significant difference from $^{239}$Pu-hydroxide group at $p < 0.05$.

Statistically significant differences in the $^{239}$Pu translocation were observed between these groups.

Fig. 6 shows time-course changes in the numbers of alveolar macrophages and neutrophils in bronchoalveolar lavage cells of the rats administered Pu-Gd-H. Neutrophils appeared 1 d after administration, increased with time, and reached a maximum level 4 d after the instillation. The number of alveolar macrophages decreased during the first 2 d and then recovered to the control value. In the rats administered Pu-Gd-L, the number of neutrophils was low, but did not significantly increase during the first 2 d (data are not shown). Neutrophil number did not change in the rats administered $^{239}$Pu-hydroxide. No changes in the number of macrophages were observed in the rats administered Pu-Gd-L and $^{239}$Pu-hydroxide.

**DISCUSSION**

In the respiratory tract model, the clearance of $^{239}$Pu from the lung following acute intake of $^{239}$Pu-hydroxide is about 50% a month after intake. The daily excretion of plutonium into feces reaches a maximum 2 d after intake and then declines. The level of daily fecal excretion declines slowly from about 10 d after intake. The daily excretion of plutonium into urine reaches a maximum 1 d after intake and then declines continuously (ICRP 1988). In this study, the clearance of plutonium from the lung of rats was faster than the ICRP model and 75% IAD was cleared 4 wk after administration. The daily excretion of
Retention and translocation of $^{239}$Pu-hydroxide • H. SATO ET AL.

Fig. 5. Translocation of $^{239}$Pu from lung to other organs following intratracheal instillation of $^{239}$Pu-hydroxide, Pu-Gd-L, and Pu-Gd-H. Vertical bar shows SD of three rats.

Fig. 6. Time-course changes in the number of alveolar macrophages and neutrophils in the bronchoalveolar lavage cells following intratracheal instillation of Pu-Gd-H. Data represent mean±SD of three rats. The data at day 0 represent the number of cells in non-treated rats.

plutonium in feces from 2 to 4 wk after administration was a constant to very little in variation. This excretion pattern is very similar to the ICRP model. The differences in the daily excretion of plutonium into urine was small; that is, the level of daily excretion was similar throughout the experimental period.

The alveolar clearance of inhaled particles depends on many factors, including the physicochemical characteristics of particles such as size and solubility, as well as the physiological and pathological conditions of the animals (ICRP 1994). The present study demonstrated that the clearance of $^{239}$Pu from the lung was slower in the rats instilled with $^{239}$Pu-hydroxide containing a high level of gadolinium (Pu-Gd-H) than those instilled with pure $^{239}$Pu-hydroxide. It is well known that plutonium nitrate forms hydroxide colloids at near neutral pH, and the physicochemical properties of the resultant colloid particles are affected by reaction conditions including pH, concentration of plutonium, co-existing substances and ionic strength of the solution (Ockenden and Welch 1956; Lindenbaum and Westfall 1965). Therefore, it is likely that the physicochemical properties were different between the Pu-Gd-H and pure $^{239}$Pu-hydroxide and that this difference was responsible for the different clearance rate of plutonium from the lung.

Another possible cause of the slower clearance of $^{239}$Pu in the rats administered Pu-Gd-H colloids may be the gadolinium-induced inflammation in the lung. There are a few reports indicating that gadolinium is toxic for respiratory cells, especially for alveolar macrophages, and induces acute inflammatory pneumonia (Yoneda et al. 1995; Mizgerd et al. 1996). The number of alveolar macrophages decreased during the first 2 d after the instillation, and then gradually increased in the Pu-Gd-H group (Fig. 6). This pattern of change in the number of alveolar macrophages was a similar finding observed in the pulmonary inflammation after gadolinium administration (Yoneda et al. 1995). The increase in the number of neutrophils in bronchoalveolar lavage apparently indicated that an acute lung inflammation was induced in the Pu-Gd-H group in the present study.

A number of studies have demonstrated that inflammatory reactions in the lung retarded the alveolar clearance of inhaled particles (Sanders et al. 1975; Kubota et al. 1988; Slauson et al. 1989; Benson et al. 1995). For example, Sanders et al. (1975) have reported that the clearance of plutonium from the lungs of rats was decreased to 60% of the normal rate in animals with acute pneumonia induced by beryllium oxides. The authors have also shown that delayed type-hypersensitive pneumonia impaired the alveolar clearance mechanisms of inhaled $^{198}$Au-colloid particles in rats (Kubota et al. 1988). Initial pulmonary deposition of $^{198}$Au-colloid was decreased in DTH rats, but in this study IAD/instilled ratio was not different among the 3 groups; that is, the initial deposition of Pu-hydroxide was not affected by Gd. The clearance of particles is impaired not only in acute but also chronic inflammation. It has been reported that clearance of particles from the lung is delayed in chronic airway disease, chronic bronchitis or pulmonary fibrosis (Camner et al. 1973; Bohning et al. 1982).

In the human respiratory tract model, the clearance rate of particles from bronchial and bronchiolar regions of the lungs in asthma, chronic bronchitis, and mycoplasma pneumonia are assumed 70, 50, and 30% of normal, respectively (ICRP 1994). Therefore, it appears that inflammatory reactions induced in the lung by gadolinium may be, at least in part, responsible for the delayed clearance of $^{239}$Pu from the lung. Neither the lung retention of $^{239}$Pu nor the population of bronchoalveolar lavage cells changed significantly in the rats.
administered $^{239}$Pu-hydroxide containing a low level of gadolinium (Pu-Gd-L group). This may indirectly indicate the relationship between the inflammation and delayed alveolar clearance of plutonium by gadolinium.

The urinary excretion of $^{239}$Pu only increased in this Pu-Gd-L group. This may suggest the occurrence of some metabolic disturbance in the respiratory tract, but the detailed mechanisms of such a phenomenon are unclear.

The doses of gadolinium used here were 0.9 and 0.22 mg kg$^{-1}$ body weight for Pu-Gd-H and Pu-Gd-L groups, respectively. These doses are deemed to be relevant to those exposed in a practical setting under the following conditions. The solution in nuclear fuel reprocessing in the Japanese facility contains 0.7% of gadolinium, and it is supposed that this solution is released as aerosol into the environment, it would contain 5 g water m$^{-3}$ air, which corresponds to a dense fog (Green and Lane 1964). If the reference man breathing under a working physiological condition is exposed to the gadolinium aerosol, he will inhale 0.9 and 0.22 mg kg$^{-1}$ body weight in 40 min and 10 min, respectively. This indicates that in the case of accidental exposure, there is a possibility that workers may be exposed to levels of gadolinium used in this study.

**CONCLUSION**

The present study demonstrates that the coexistence of gadolinium may influence the alveolar clearance of plutonium administered as a hydroxide colloid, although the exact mechanisms remain unclear. This indicates that when plutonium is inhaled accidentally with gadolinium and the inhaled amount of gadolinium is so high that an inflammatory reaction is induced and the physicochemical properties of plutonium hydroxide is changed, the lung retention of plutonium and thus the radiation dose in lung may become larger than in ordinary cases.

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Research report

Different patterns of abnormal neuronal migration in the cerebral cortex of mice prenatally exposed to irradiation

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Abstract

A characteristic abnormal cortical architecture in the adult brain was produced in mice subjected to 1.5 Gy of X-irradiation on embryonic day 14. Neurons in the lateral regions were organized into an essentially six-layered structure, while neurons in the dorsal regions formed a unique four-layered cortex. The patterns of neuronal migration in these different cortical regions were examined with immunohistochemistry for anti-bromodeoxyuridine (BrdU), anti-midkine (MK), and anti-gial fibrillary acidic protein (GFAP) antibodies. In the cortical lateral region, BrdU-labeled cells in the upper layers were fewer, and those in lower layers more numerous in prenatally irradiated mice than in control, while in the dorsal region (four-layered region), BrdU-labeled cells were very few in layer 2, and a large number of labeled-cells remained in layer 4. These results indicated that some neuroblasts in the lateral cortical region could not migrate to the upper layers, and that most neuroblasts in the dorsal cortical region failed to pass through the earlier migration zone. MK- and GFAP-stained radial glial fibers showed that the radial fibers were consistently oriented in the direction of neuronal migration in the control brains. However, in the irradiated brain, such radial fibers were crumpled in the lateral region, or were reduced markedly in number in some parts of the dorsal region. These results revealed that neuronal migratory pathways (radial glial fibers) were destroyed differently in different regions, and that X-rays killed some cells including radial glial cells or their precursors during the embryonic stage. These effects of radiation on the developing brain may result from the possibility that neurogenetic time is different or there are cellular mechanisms involved in the radiosensitivity among different regions. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Bromodeoxyuridine; Disorder of neuronal migration; Glial fibrillary acidic protein; Midkine; Neuroblast; Radial glial fiber

1. Introduction

The developing brain is known to be one of the fetal structures most susceptible to radiation. This high vulnerability of the fetal brain is a distinctive teratological characteristic commonly recognized in all mammalian species including human beings [15,16]. A characteristic brain malformation may be the result of many developmental processes, depending on when the fetus was exposed to radiation, the amount of radiation, and the type of species. We have examined the patterns of brain malformation in 6-week old mice prenatally exposed to doses of X-irradiation ranging from 0.5 to 2.0 Gy on day 1 from embryonic days 12 to 19 (E12–E19) and found that different types of cortical malformation were induced at different doses or different periods of cortical neurogenesis. Among these different types of cortical malformation, a characteristic abnormal cortical architecture was found in mice subjected to 1.5 Gy on E14: (a) a four-layered cortex appeared in the dorsal region but not in the lateral cortex; (b) the cellular structure of the cortex in the lateral region showed minor changes but the lamination was essentially normal; (c) groups of ectopic cells forming ectopic gray matter were located below the subcortical white matter. These biologi-cal effects of irradiation on the developing brain might be responsible for cell death and abnormal neuronal migration.
We were interested to know the histogenesis of brain malformations of this type, especially for the four-layered cortical pattern, because a similar brain malformation can also be found in human lissencephaly type 1, in which it is suspected that neurons destined for the upper cortical layers fail to migrate to their normal position and accumulate below the external cellular layer [2,5,10,41].

In order to study the cytokinetics occurring in the course of histogenesis of the neocortex, 5-bromo-2-deoxyuridine (BrdU) was chosen as a marker to label migrating cells [14,38,46]. Like 3H thymidine, BrdU is incorporated into nuclear DNA, but it is neither radioactive or myelotoxic at the doses used [8,12,25,37]. To study the possibility that abnormalities in migration could be related to the abnormal guidance of neuroblasts, glial fibrillary acidic protein (GFAP) was chosen as a mark to analyze radial glial cells in the developing brain [3,31,39,48]. In view of the fact that the GFAP-immunoreactive glial fibers were observed markedly only after birth in the mouse cerebrum [44], radial glial fibers were also examined by means of midkine (MK) immunocytochemistry in the present study. MK is a new heparin-binding growth/differentiation factor specified by a retinoic acid-responsive gene [17,28]. It is the first number of a new protein family of developmentally regulated cytokines with diverse biological activities. MK is mitogenic for certain fibroblastic cell lines, and enhances both neurite outgrowth and the survival of various embryonic neuron types [26,27,43,49]. Increased expression of MK was detected on the processes of radial glial cells in the developing mouse and rat cerebral cortex [18,20,29]. Such radial glial fibers stained with anti-MK antibody were similar to those fibers stained with anti-vimentin (Vim) antibody in a distinct fashion during the embryonic stage [44]. Therefore, MK may be useful for the analysis of gliogenesis in the early stages of the developing central nervous system.

2. Materials and methods

2.1. Animals

A closed colony of Slc:ICR mice obtained from the Shizuoka Laboratory Animals Center (Shizuoka, Japan) was kept in a controlled atmosphere of 23 ± 3°C with 55 ± 5°C humidity under a 12-h dark/light cycle. They were given commercial laboratory food, CA-2, and tap water ad libitum. Eight-week old nulliparous females were paired with potent males in cages overnight and checked for vaginal plugs the next morning. The day on which a vaginal plug appeared was regarded as day 0 of pregnancy.

2.2. Treatment

Pregnant mice were subjected to a single whole-body exposure of X-irradiation at a dose of 1.5 Gy on E14. Pregnant control mice were sham-treated. The pregnant mice of both groups were then injected intraperitoneally (i.p.) with the thymidine analog BrdU (30 mg/kg b.wt; Becton Dickinson, USA) at E16 in order to label neurons normally committed to the upper cortical layers [22,32]. Some mice were allowed to give birth and rear their litters. Two mice were randomly selected for evaluation at each experimental stage when about five fetuses and pups were examined. Between 6 h after exposure and postnatal day 28 (P28), the brains from fetuses and pups were extracted and removed for study as follows.

2.3. Experimental techniques

For histological examination, brains were fixed in Bouin’s solution, dehydrated, embedded in paraffin, and

![Cerebral cortex of 1-week old mice. (a) In control cortex, a laminar pattern of six layers (from I to VI) can be observed clearly. (b) In the irradiated cerebral cortex, a basic cellular structure of six layers can be recognized in the lateral cortex, but not in the dorsal region. Layers II, III, and IV from the lateral cortical region to the dorsal cortical region become progressively thinner and unclear, and neurons in the dorsal cortex form a unique four-layered cortex. Between the two specific laminar patterns, no specific layers can be defined because of the disarranged cytoarchitecture. Exotic cell mass (asterisk) is situated beneath layer VI and is separated from layer VI by a thin band of fibers. L: Lateral, M: medial. HE stain. Scale bar = 150 μm.](image-url)
Fig. 2. Schematic drawings summarizing the laminar patterns of cerebral cortex in 4-week old mice.

L : lateral region
M : medial region

EGM : ectopic gray matter

Fig. 3. Cerebral cortex of 4-week old mice irradiated on E14. (a) The large pyramidal cell is aberrantly located below the molecular layer (arrow). (b) Higher magnification of the same photomicrograph showing the abnormal orientation of the large pyramidal cell (arrow). Luxol fast blue stain. Scale bar: a = 250 μm; b = 50 μm.
Fig. 4. Histogram showing the distribution of BrdU-labeled cells in the lateral regions of field 3 of cerebral cortex in 4-week old mice (mean ± S.D.; n = 5 in each group). **Significantly different from the control group at P < 0.01 (Student’s t-test).

serially sectioned in a frontal plane at 5 μm. The sections were stained with hematoxylin and eosin (HE) or Luxol fast blue. Because field 3 of Caviness [4] and Krieg [19] is the most completely stratified of all the cortical areas, the histological sections through field 3 in the adult brain or parietal areas in immature cerebral mantle from the fetuses and litters that corresponded to the adult field 3 were selected from each brain for the following examinations.

For BrdU immunohistochemical examination, samples were fixed with 5% acetic acid in 80% ethanol at 4°C overnight, then dehydrated in graded ethanol. Brains were embedded in paraffin and sectioned at 5 μm in the frontal plane. For each brain, three sections were selected and mounted on glass slides that had been previously coated with a mixture of equal amounts of glycerol and 10% albumin. These sections were deparaffinized with xylene, rehydrated in graded ethanol, and rinsed with 0.1 M phosphate buffered saline (PBS, pH 7.4). Following 8 min in 1 N HCl at 60°C to partially hydrolyze the double-stranded DNA into single strands, the sections were neutralized with 0.1 M sodium borate (pH 8.5) for 5 min, and washed with 0.1 M PBS containing 0.3% Triton X-100 for 30 min. The sections were incubated with anti-BrdU monoclonal antibody (diluted 1:100 in PBS with 1 mg/ml bovine serum albumin, pH 7.4; mouse IgG heavy chain and kappa light chain, Becton Dickinson) at 37°C for 60 min. These sections were sequentially incubated with goat

Fig. 5. Anti-BrdU staining in the dorsal region of the cerebral cortex of 1-week old mice. (a) In the control cortex, most labeled cells are located in the upper layers, whereas few labeled cells can be observed in the lower layers. (b) In the irradiated cortex (four-layered region), labeled cells are deficient in layer 2, but numerous in layer 4. Scale bar = 100 μm.
anti-mouse IgG (H + L) biotinylated secondary antibody (diluted 1:220, CALTAG) at 37°C for 30 min, and then in an avidin–biotin–horseradish peroxidase complex (diluted 1:800) for 60 min, followed by development of the reaction product with 3,3′-diaminobenzidine (DAB, Sigma) and hydrogen peroxide, resulting in the formation of a brown precipitate in the nuclei of BrdU-labeled cells. They were then counter-stained with HE and examined under a light microscope.

For MK and GFAP immunohistochemical examination, samples were fixed with Zamboni’s solution (4% formaldehyde and 0.2% picric acid in 0.1 M PMS, pH 7.4) at 4°C overnight. The brains were immersed in 20% sucrose at 4°C for 1 day and embedded in 10% gelatin at 4°C for 2 days, then snap-frozen by carbon dioxide gas, cut in the frontal plane (5–15 μm thickness) through the entire cerebrum by cryostat (CM 1800, Leica, Germany), and thaw-mounted onto poly-L-lysine-treated glass slides. The dissection and orientation of the tissue blocks were given special attention. The tissue blocks through the full thickness of the cerebral cortex were dissected as nearly as possible perpendicular to the longitudinal axis of the cerebral hemisphere. For each brain, six sections were selected for treatment with 0.6% hydrogen peroxide in 80% methanol for 5 min to block endogenous peroxide activity, then rinsed three times with 0.1 M PBS containing 0.3% Triton X-100. Three sections were individually incubated with affinity-purified polyclonal rabbit anti-MK antibody (diluted 1:200, containing 1% normal goat serum, 1% bovine serum albumin and 0.3% Triton-100) or with polyclonal rabbit GFAP (diluted 1:500, Dako) at 4°C overnight, and subsequently incubated with biotinated goat anti-rabbit IgG, then with a ABC kit (Vector Laboratories, USA) for 3 h at room temperature. Immunopositive structures were visualized with a freshly prepared solution of 0.02%, 3, 3′-DAB-4HCl and 0.005% hydrogen peroxide in 0.05 M Tris–HCl buffer (pH 7.6). After being rinsed with distilled water, the sections were dehydrated and mounted with resin.

The control mice were processed in the same manner as the treated animals.

2.4. Quantitative study

Histological examination showed that the cellular structure of the cortex in the lateral region was essentially normal. In order to know whether neuronal migration was affected by irradiation in this region, a quantitative study was performed in 4-week old mice. As mentioned above, histological sections which corresponded to field 3 of Caviness [4] and Krieg [19] were chosen to count the number of BrdU-labeled cells in the cortical layers of the
lateral region. BrdU-labeled cells were counted in the lateral regions bounded by two parallel lines of the same length, and the labeled cells in different layers were compared by Student's t-test between irradiated mice and controls. P-values of less than 0.05 were considered significant.

3. Results

Extensive cell death occurred 6 h after exposure (E14), and dead cells disappeared by 48 h after exposure. Following cellular remodelling, some bizarre cellular structures appeared temporarily in the irradiated brain during the embryonic period. The cortical abnormality could be defined in more detail as maturation processed. In postnatal mice, a laminar pattern of cortical neurons (six layers) was observed only in the lateral region of the brains irradiated at E14 (Figs. 1b and 2b). Layer I was readily detected, and the inner layers (layers V and VI) were recognized through the presence of large pyramidal cells. Layers II, III and IV formed bands of different densely packed cells. However, layers II, III and IV from the lateral cortical region to the dorsal cortical region became progressively thinner and unclear. In the mid-dorsal region, a four-layered cortical organization had formed (Figs. 1b and 2b). The four-layered sequence consisted of layer 1, corresponding to the molecular layer, layer 2 located in the upper cellular layer, layer 3 which was a sparsely cellular zone, and layer 4 which contained heterotopic neurons. Layer 2 harbored an external lamina of large cells that connected with layer V in the lateral cortex. The large pyramidal cells located just below the molecular layer could also be found in the mid-dorsal region. The primary dendrites of these large pyramidal cells appeared not to be normally oriented towards the cortical surface (Fig. 3). Another obvious malformation was the ectopic cell masses that formed beneath layer VI and were separated from layer VI by a thin band of fibers; no specific cell layers could be identified in these ectopic cell masses (Fig. 1b, Fig. 2b).

After 24 h of BrdU injection, BrdU-labeled cells were observed only in the ventricular zone of normal cortex or in the partially preserved ventricular zone of irradiated brain. Migration of labeled cells to the cortical plate, the future neocortex, started from here in a radially centrifugal fashion. From P7 onwards, almost all labeled cells seemed to finish complete their migration, and the distributive pattern of labeled cells was no longer changed. These

Fig. 7. Anti-GFAP staining in the cerebral cortex of P4. (a) In the control cortex, radial glial fibers are straight and perpendicular to the pial surface. (b) In the irradiated cortex, radial glial fibers are crumpled (arrows) and many astrocytes appear. Scale bar = 100 μm.
labeled cells in normal cortex were distributed widely in layers II and III, and continued to form an immunoreactive cell zone, with few labeled cells found in layers V and VI. A similar distribution of BrdU-labeled cells was observed in the lateral cortex region of irradiated mice, but quantitative study revealed significantly different distribution patterns in the lateral region between the irradiated animals and controls. The labeled cells in layers II to IV were fewer, and those in layers V and VI more numerous in irradiated mice than in controls (Fig. 4). In the region of the four-layered cortex, BrdU-labeled cells were so scarce in the upper layers that the immunoreactive cell zone was interrupted in layer 2, and a large number of labeled cells appeared in layer 4 (Fig. 5b).

MK-immunoreactive radial glial fibers in the brain mantle were detected during the embryonic period but could be not observed in the postnatal cerebral cortex. On E17, the radial glial fibers had radially traversed the entire distance from the ventricular zone to the pial surface in the control brain, and their courses were initially parallel to each other and perpendicular to the pial surface which was oriented in the direction of neuronal migration (Fig. 6a). In the irradiated brain, the MK-immunoreactive fibers formed several fascicles which changed their course in different directions, and could not be found in some parts of the dorsal region of the cerebral cortex (Fig. 6b). On E18, MK-immunoreactive fibers had decreased dramatically in number as had the intensity of staining for MK both in the control and the irradiated brain. At the same time, GFAP-immunoreactive fibers in the brain mantle first appeared. By P4, GFAP-stained fibers became more evident. In the control brain, these fibers were regularly distributed and perpendicular to the pial surface (Fig. 7a), while in the irradiated brain the fibers were crumpled and a number of fibrillary astrocytes appeared (Fig. 7b). In the following postnatal days, the radial glial fibers became progressively fewer, and disappeared completely about P21 both in control and irradiated groups.

4. Discussion

Prenatal exposure to several neuroteratogens during different embryonic stages, such as ionizing radiation, ethanol, and cytotoxic drugs (e.g., methylazoxymethanol acetate, MAM), can induce cortical laminar disorganization with varied patterns and formation of ectopic neurons. These biological effects of neuroteratogens have been presumed to result from cell death and interference with normal neuronal migration, presumably via effects on radial glia [23,24,45,50,51]. Numerous studies have revealed that a pattern of cortical laminar disorganization is decided by many developmental processes, but the time of exposure to neuroteratogens is one of the important key factors in the development of a lamina-specific pattern. Treatment of pregnant mice with MAM at E13 resulted in cortical hypoplasia that affected all but the first cortical layer, treatment at E15 caused a statistically significant reduction in cell density in the superficial layers, whereas treatment at E17 caused an alterable arrangement of the cortical layers, which were not easily separable, since MAM in perhaps limiting glial cell proliferation, modified the subsequent migration mechanisms [6,7]. Similar results also have been reported in the study of lamina-specific deletion of neurons in the monkey brain by irradiation at selected prenatal stages [1].

In our study, different distributive patterns of neurons were observed in different regions of the neocortex in mice prenatally irradiated at E14. Neurons in the lateral region were organized into an essential six-layered structure, while neurons in the dorsal regions formed a unique four-layered cortex.

In the lateral cortical region of irradiated brain, histological examination showed that the normal sequence of different layers was preserved, despite the thickness of each layer being less than that of control. However, BrdU-immunocytochemical study indicated that the migration of cortical neuroblasts in the lateral region of the animals irradiated was not really 'normal.' A high proportion of the labeled cells migrated to the upper cortical layers (layers II and III), but some remained in the lower cortical layers (layers V and VI). The quantitative study showed statistically significant differences in the distribution of the labeled cells between irradiated and sham-exposed mice. The labeled cells in the upper layers were fewer, and those in the lower layers more numerous in prenatally irradiated mice than in controls. These results suggested that some neuroblasts failed to migrate to the upper layers in the lateral cortical region.

On the other hand, in the dorsal region, a four-layered cytoarchitecture was identified; layer 1 was the molecular layer, layer 2 was the external cellular layer, layer 3 was a sparsely cellular zone, and layer 4 was the internal cellular layer. Because layer 2 was formed by an external lamina of large cells which continued to layer V and VI of the lateral region, layer 4 consisted of neurons which would have migrated to the upper layers in the normal cortex. BrdU-immunocytochemical study demonstrated that BrdU-labeled cells were very few in layer 2, whereas numerous labeled-cells appeared in layer 4. These results confirmed that the labeled cells in layer 4 were generated at the same time as those in layers II and III in the lateral cortex. These cells failed to pass through the earlier migration zone, so that large pyramidal neuron (a characteristic of layer V which formed earlier than layers II and III) appeared in layer 2.

The abnormal neuronal migration could be responsible for radial glial fibers injured by X-irradiation because it is well known that the migration of neuroblasts is guided from an early stage by a system of such fibers that span the width of the thickening telencephalon [30,33–35]. Therefore, the radial glial fibers were detected with a
technique combining MK- and GFAP-immunocytochemistry. MK-stained radial glial fibers were observed from the embryonic stage and disappeared after birth. The appearance of MK-stained radial glial fibers was consistent with the developmental stage of the brain when proliferated ventricular neuroblasts start to migrate to the cortical area [43]. During an earlier embryonic period (from E13 to E17), MK-stained radial glial fibers could be observed clearly. These fibers, spanning the cerebral wall from the ventricular zone to the pial surface, were consistently oriented in the direction of neuronal migration and traversed the cortical plate itself in a straight line in the control brains. However, such fibers were crumpled in the lateral region of brains following 1.5 Gy irradiation. Obviously, to travel such a disoriented pathway, some neuroblasts could not migrate to their proper position. Moreover, one notable finding of the present study was that MK-stained radial glial fibers were markedly reduced in number in some parts of the dorsal region. Radial glial fibers which ordinarily projected radially from the ventricular zone to the surface of the cerebral mantle were seldom found there. These results revealed that neuronal migratory pathways (radial glial fibers) were almost destroyed in some parts of the dorsal region and that X-rays might have killed some cells including radial glia cells or their precursors.

Similar feature of radial glia cells stained with anti-GFAP antibody were observed in the postnatal brains. About P4, clearly immunoreactive radial glial cells with anti-GFAP became evident in both control and irradiated brains. In the control brains, such fibers projected radially from the ventricular zone to the upper wall of the cerebral mantle, while in the irradiated brains they were disorganized, and numerous astrocytes appeared. The fact that numerous protoplasmic transitional forms were displaced by astrocytes indicated the presence of reactive gliosis as the response of a brain exposed to irradiation.

It was of importance to inquire why different types of cerebral malformations produced in the same cerebral cortex appeared after irradiation, and why a four-layered pattern was found only in the dorsal region but not in the lateral region. To answer such questions, the possibility of 'proliferative units' was considered. Studies of neurogenesis and neurogenetic gradients have indicated that neurons produced within individual proliferative units align themselves along a radial glial fascicle and enter the developing cortical plate where they form ontogenetic columns [36,47]. If the generation of cortical neuroblasts was not synchronous and uniform throughout the periventricular germlayer, irradiation could only destroy proliferative units which were susceptible at the time of exposure to X-irradiation. Therefore, some ontogenetic columns might become normally developing units, and the migratory pathway could be preserved, whereas others might be completely destroyed. Such destroyed proliferative units occurring in the dorsal region but not in the lateral region may demonstrate that the neurogenic time is different or that there are cellular mechanisms possibly involved in the radiosensitivity among different regions. Further studies are need to clarify these points.

A four-layered pattern of cortical malformation can also be found in human lissencephaly type I [9–11]. It has been identified as the eponymous, the Miller–Dieker and Norman–Roberts syndromes in recognition of the first authors [21]. A number of studies on human lissencephaly type I have shown that neurons destined for the upper cortical layers fail to migrate to their normal positions and remain below the external cellular layer [5,41]. Although the phenotype of brain malformation in humans was similar to animal models in this experiment, the agents inducing a four-layered cortex were different. Patients with Miller–Dieker syndrome often present combined with the anomaly of a short arm of chromosome 17 [9,11,42].

Another evident malformation in the neocortex of prenatally irradiated mice was the formation of groups of ectopic cells located below the subcortical white matter. Previous descriptions of the recovery of the geminal neuroepithelium from prenatal irradiation indicated that migrating cells detained along the migratory pathway (within the cortex) might result from an aberrant or partially broken migratory pathway, while the formation of the ectopic masses might result from a complete failure of migrating neuroblasts [45]. The neuroblasts which failed completely to migrate curled up and continued to proliferate to form the ectopic masses. Such ectopic masses may be produced at some time during cortical neurogenesis by irradiation, with larger masses forming at an earlier stage of brain development, and smaller ones at a later stage [13]. The size of these ectopic masses resulting from irradiation at different stages may depend on how severely the migratory pathways (radial glial fibers) were disturbed and how many neuroblasts failed to migrate. In the present study, the finding that a large number of MK-stained radial glial fibers appeared around E14 of mice may suggest that E14 is the time when neuronal migration waves reached their peak. Thus, ectopic masses in mice exposed at E14 caused the larger ectopic cell masses.

In addition, the present observations showed that the orientations of the apical dendrites of some large pyramidal neurons were changed so as not to be normally oriented towards the cortical surface in the irradiated animals. It is thus conceivable that the neuronal pathway might be affected due to an alteration in the dendrites of large pyramidal neurons. To study the structural disorders of neuronal pathways following irradiation may be a valuable subject for future study.

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CONCENTRATION OF GLOBAL FALLOUT $^{99}$Tc IN RICE PADDY SOILS COLLECTED IN JAPAN

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Abstract
Analysis of global fallout $^{99}$Tc in environmental samples should provide useful information for predicting the nuclide behaviour under natural conditions which is important from the viewpoint of radioecology. Concentrations of $^{99}$Tc in rice paddy soils collected in Japan have been studied. After chemical separation, $^{99}$Tc in the final solution was measured by ICP-MS. The activity ratio of $^{99}$Tc/$^{137}$Cs was used to understand the $^{99}$Tc behaviour in the environment because the fission yields of $^{99}$Tc and $^{137}$Cs from $^{235}$U or $^{239}$Pu are almost the same. The theoretical activity ratio from fission which is calculated now is about $3.0 \times 10^{-4}$. Our results showed that the range of activity ratios of $^{99}$Tc/$^{137}$Cs in the soil samples was (2.0–5.2) $\times 10^{-4}$; these ratios were one order of magnitude higher than the theoretical one. $^{99}$Tc has been accumulating in rice paddy soil like $^{137}$Cs has, although their mechanisms might differ. One of the reasons for the high ratio in the surface soil might be the ratios in the atmospheric samples, which have increased from the order of $10^{-3}$ to $10^{-2}$ (Garcia-León et al., 1993). © 1997 Elsevier Science Ltd. All rights reserved

Keywords: Technetium-99, paddy field soil, anaerobic condition, activity ratio, accumulation.

INTRODUCTION

Technetium-99 in the environment has been accumulating because of its long half-life of 2.1 $\times 10^{5}$ years. $^{99}$Tc is a pure beta emitter ($E_{\text{max}} = 294$ keV) and it is produced in the fission of $^{235}$U or $^{239}$Pu with a relatively high fission yield of ca 6% which is similar to the values for $^{137}$Cs and $^{90}$Sr. The radionuclide is released to the environment via nuclear weapons tests and nuclear industries. Nuclear medical use of $^{99m}$Tc (half-life: 6.01h) which decays directly to $^{99}$Tc is another source though the $^{99}$Tc introduced into the environment via this route is negligible. Generally, Tc is expected to be mobile in the surface soil environment because of its chemical form, $\text{TeOX}^{-}$. The negative surface charge of soil particles makes for this mobility and plants can absorb it easily. For this reason, a knowledge of Tc behaviour in the environment is important for dose assessment. However, clarification is not easy using results of laboratory tracer experiments because the behaviour of Tc is dependent upon its chemical form; the most stable chemical form in the surface soil is pertechnetate, $\text{TeOX}^{-}$, but, under reducing conditions, it could be an insoluble form (Bondietti & Garten, 1985; Sheppard et al., 1990; Yanagisawa & Muramatsu, 1995; Tagami & Uchida, 1996a).

Analysis data of global fallout $^{99}$Tc in environmental samples should give useful information for predicting the nuclide behaviour. But due to its very low concentration and analytical difficulties with environmental samples, there are not many data.

In this study, we focused on determination of $^{99}$Tc in paddy field soil samples. $^{137}$Cs concentration in the sample was also measured, then the activity ratio of $^{99}$Tc/$^{137}$Cs was calculated to understand Tc mobility in the paddy soil environment. Paddy field soil is one of the environmental samples in which the nuclide could be accumulated because of the relatively low redox conditions. Besides, the soil-rice plant system is an important path for transfer of $^{99}$Tc to humans in Japan and other Asian countries because rice is the main crop in these areas (The Times Atlas of the World, 1994).

MATERIALS AND METHODS

Soil preparation
Five soil samples were collected from the surface layer (< 20 cm) of paddy fields in Japan (Fig. 1). The soil properties are listed in Table 1. The samples were air-dried and passed through a 2 mm mesh sieve. They were inncinerated for 8 h at 450°C to decompose organic matter.

Chemical separation
The experimental method for soil sample was reported previously (Tagami & Uchida, 1993b, 1996b). The scheme is shown in Fig. 2 and only a brief explanation is given here.

1. Technetium was separated from the incinerated soil sample by volatilization, i.e. by combustion of the sample at 950°C and trapping of the gaseous element in a potassium carbonate solution.
2. The solution was shaken with cyclohexanone to extract its Tc and to remove ruthenium which has an isotopic abundance of 12.7% at mass 99 (Morita et al., 1991). Then Tc in the organic layer was back-extracted into deionized water by the addition of carbon tetrachloride.

3. The aqueous phase was adjusted to 2% nitric acid with super-analytical grade reagent (Tama Chemicals, AA-100). Then, the $^{99}$Tc concentration in the solution was measured by ICP-MS (Yokogawa, PMS-2000).

For the ICP-MS measurement, $^{99}$Tc standard solution (Japan Radioisotope Association, NH$_4$TcO$_4$ form in 0.1% ammonia solution) was used. Measurement time was approximately 10 min per solution. The detection limit for ICP-MS was 0.05 ppt (0.03 mBq ml$^{-1}$). During the measurement, mass numbers 101 and 102 were also detected for Ru in the samples.

Recovery of Tc was determined with $^{95m}$Tc (DuPont, NaTcO$_4$ in H$_2$O). A paddy field soil contaminated for one month was used for the determination of the yield. The area of the gamma peak of 204 keV of $^{95m}$Tc was measured with a NaI(Tl) well-type scintillation counting system (Aloka, ARC-300).

$^{137}$Cs measurement

The activities in the soil samples were measured by a Ge detector (Seiko EG&G Ortec) coupled with a multi channel analyzer (Seiko EG&G, Model 7800). 100 ml of each soil sample were transferred into a plastic vessel and measured for 80,000 s. The same volume standard solution (a mixed radionuclide $\gamma$-ray reference standard in solution, Amersham, QCY.46) was used for the $^{137}$Cs determination. The method was applicable because a preliminary experiment showed that there was no remarkable difference in efficiencies between the liquid standard and a reference soil (IAEA Soil-6).

RESULTS AND DISCUSSION

For the determination, 300 to 500 g of incinerated soil samples were used. The soil incineration was carried out at 450°C to decompose organic matter because its

<table>
<thead>
<tr>
<th>Sampling place</th>
<th>Ogata Village (Akita Prefecture)</th>
<th>Omagari City (Akita Prefecture)</th>
<th>Morioka City (Iwate Prefecture)</th>
<th>Imari City (Saga Prefecture)</th>
<th>Kawazoe Town (Saga Prefecture)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth (cm)</td>
<td>0–15</td>
<td>0–18</td>
<td>0–14</td>
<td>0–20</td>
<td>0–17</td>
</tr>
<tr>
<td>Classification</td>
<td>Gray lowland soils</td>
<td>Gray lowland soils</td>
<td>Andosols</td>
<td>Yellow soils</td>
<td>Gray lowland soils</td>
</tr>
<tr>
<td>Soil properties</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CEC (meq/100 g dry)</td>
<td>—</td>
<td>18.4</td>
<td>27.4</td>
<td>16.0</td>
<td>0.85</td>
</tr>
<tr>
<td>AEC (meq/100 g dry)</td>
<td>—</td>
<td>(&lt;0.05)</td>
<td>0.17</td>
<td>(&lt;0.05)</td>
<td>(&lt;0.05)</td>
</tr>
<tr>
<td>Active Al (mg/100 g dry)</td>
<td>—</td>
<td>223</td>
<td>6510</td>
<td>236</td>
<td>158</td>
</tr>
<tr>
<td>Active Fe (mg/100 g dry)</td>
<td>—</td>
<td>420</td>
<td>3600</td>
<td>741</td>
<td>600</td>
</tr>
<tr>
<td>Organic C (% dry)</td>
<td>1.84</td>
<td>6.64</td>
<td>0.35</td>
<td>2.06</td>
<td>0.00</td>
</tr>
<tr>
<td>Total N (% dry)</td>
<td>1.06</td>
<td>0.56</td>
<td>0.23</td>
<td>0.23</td>
<td>0.19</td>
</tr>
</tbody>
</table>
Table 2 shows the results of $^{99}$Tc and $^{137}$Cs measurements. The ranges of $^{99}$Tc and $^{137}$Cs concentrations are 0.02–0.11 Bq kg$^{-1}$ dry and 4.9–16.9 Bq kg$^{-1}$ dry, respectively. The activity ratios of $^{99}$Tc/$^{137}$Cs are in the last column, (2.0–5.2)$\times 10^{-3}$. The activity ratio of $^{99}$Tc/$^{137}$Cs from fission is now calculated as $3.0 \times 10^{-4}$ with correction of decay out. The measured ratios in the paddy field soil samples were one order of magnitude higher than the theoretical one from fission.

The higher $^{99}$Tc/$^{137}$Cs activity ratio in soil is presumably influenced by that of depositions containing rain and dry fallout. Ehrhardt and Attrep (1978) reported that the range of the activity ratio of $^{99}$Tc/$^{137}$Cs in rain samples which were collected in the USA was (0.11–2.5)$\times 10^{-2}$ during 1961–1974. In Spain, García-León et al. (1993) measured similar values of (0.3–12.3)$\times 10^{-2}$ in rain samples collected during 1984–1987. These ratios in rain samples were almost the same as those of soils. It seems that the $^{99}$Tc/$^{137}$Cs in soil was affected by that in deposition on the soil. However, it is still difficult to understand the $^{99}$Tc behaviour in the atmosphere and terrestrial environments because of the limited numbers of $^{99}$Tc data in deposition samples. Our results, at least, lead to a tentative conclusion that more Tc might be fixed on the soil we had expected before.

The mechanisms of Tc accumulation in paddy fields can be explained by the changes of Tc’s chemical form in soil under the waterlogged condition. Generally, during the planting period, the paddy field soils are waterlogged and subsequently the redox potentials decrease, so that relatively low redox conditions are generated in the soils. Although the nuclide is expected to be in a soluble form as pertechnetate (TcO$_4^-$) under an aerobic condition like that in surface soils, this changes through a combination of factors such as the redox condition and microbial activity (Tagami & Uchida, 1996a, 1996c). Probably, Tc is transformed from its soluble TcO$_4^-$ form to a lower oxidation form such as TcO$_2$, TcO(OH)$_2$ or TcS$_2$ under a relatively low redox condition (Lieser & Bauscher, 1987; Brookins, 1988). The sulfi de might occur because H$_2$S gas can be produced in waterlogged soil when rice plants are growing. These lower oxidation forms have lower solubility and Tc in these forms would be sorbed onto soil. The activity ratio of $^{99}$Tc/$^{137}$Cs in unploughed soil

Table 2. Concentrations of $^{99}$Tc and $^{137}$Cs in paddy field soils in Japan on a dry weight basis and activity ratios of $^{99}$Tc to $^{137}$Cs

<table>
<thead>
<tr>
<th>Collection place and type of the soil</th>
<th>Collection year</th>
<th>$^{99}$Tc (Bq kg$^{-1}$)</th>
<th>$^{137}$Cs (Bq kg$^{-1}$)</th>
<th>$^{99}$Tc/$^{137}$Cs ($\times 10^{-3}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ogata Village, Akita Prefecture Paddy soil</td>
<td>1991</td>
<td>0.11 ± 0.03</td>
<td>28.2 ± 0.8</td>
<td>3.9 ± 1.0</td>
</tr>
<tr>
<td>Omagari City, Akita Prefecture Paddy soil</td>
<td>1992</td>
<td>0.034 ± 0.005</td>
<td>16.92 ± 0.64</td>
<td>2.0 ± 0.3</td>
</tr>
<tr>
<td>Morioika City, Iwate Prefecture Paddy soil</td>
<td>1992</td>
<td>0.052 ± 0.01</td>
<td>10.13 ± 0.63</td>
<td>5.1 ± 0.1</td>
</tr>
<tr>
<td>Kawazoe Town, Saga Prefecture Paddy soil</td>
<td>1991</td>
<td>0.022 ± 0.003</td>
<td>4.94 ± 0.47</td>
<td>4.5 ± 0.8</td>
</tr>
<tr>
<td>Imari City, Saga Prefecture Paddy soil</td>
<td>1991</td>
<td>0.088 ± 0.015</td>
<td>16.84 ± 0.66</td>
<td>5.2 ± 0.9</td>
</tr>
</tbody>
</table>

±: Counting errors in the measurements or statistical errors in calculation.
in Japan was \((1.2-39)\times10^{-3}\) (Otsuji, 1989; Matsuoka et al., 1990; Morita et al., 1993). These values were one to two orders of magnitude higher than the theoretical one. Since these soil conditions were aerobic conditions, it is expected that the chemical form of Tc is \(\text{TcO}_4^-\) has only a small possibility of changing to a lower oxidation form. Although local reduction conditions could be seen even in the surface soil, the mechanisms of Tc sorption onto the soil should not be explained only by this. Further studies are needed to clarify the phenomena of \(^{99}\text{Tc}\) accumulation in the surface soil layer including upland field.

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Inhibition of Repair of Radiation-Induced DNA Double-Strand Breaks by Nickel and Arsenite

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The effect of arsenite or nickel on the repair of DNA double-strand breaks (DSBs) was studied in γ-irradiated Chinese hamster ovary cells using pulsed-field gel electrophoresis. After treatment with nickel chloride or arsenite for 2 h, cells were irradiated with γ rays at a dose of 40 Gy, and the numbers of DNA DSBs were measured immediately after irradiation as well as at 30 min postirradiation. Both arsenite and nickel(II) inhibited repair of DNA DSBs in a concentration-dependent manner; 0.08 mM arsenite significantly inhibited the rejoining of DSBs, while 76 mM nickel was necessary to observe a clear inhibition. The mean lethal concentrations for the arsenite and nickel(II) treatments were approximately 0.12 and 13 mM, respectively. This indicates that the inhibition of repair by arsenite occurred at a concentration at which appreciable cell survival occurred, but that nickel(II) inhibited repair only at cytotoxic concentrations at which the cells lost their proliferative ability. These novel observations provide insight into the mechanisms underlying the effects of combined exposure to arsenite and ionizing radiation in our environment. © 2000 by Radiation Research Society

INTRODUCTION

Recent advances in science and technology involve the potential release of various toxic agents into the environment that might have adverse effects on human health. Since simultaneous exposure to multiple toxicants frequently occurs in the environment, studies of the combined and/or competitive effects of toxicants are of great importance. In the field of radiation protection, the combined effects of radiation and other toxic agents are recognized as an important research area, but data for use in providing accurate risk assessment are limited. Thus elucidation of the basic mechanisms involved in combined effects is crucial.

It has been shown that exposure to some heavy metals or to arsenic results in supra- or subadditive effects when they are combined with ionizing or nonionizing radiation including X rays and UV light (for reviews, see refs. 1–3). Although the underlying mechanisms are not clearly understood, the interference with DNA repair as well as the enhancement of radical formation are possible explanations for the combined effects of these metals and radiation. Many metals or metalloids including nickel (1–14), arsenite (4, 5, 8, 15–19), cadmium (4, 6, 11, 12, 14, 17, 20), mercury (4, 5, 21, 22), cobalt (4, 23), lead (20), and copper (4, 5) have been shown to interfere with the process of repair of DNA damage induced by a variety of genotoxic substances.

The inhibitory potential of a specific metal may depend on the nature of the DNA damage. For instance, nickel(II) inhibited the repair of DNA damage induced by X rays and UV light but not the damage induced by methyl methanesulfonate (7, 8). In contrast, mercury(II) interfered with the repair of DNA damage induced by X rays but not the repair of damage induced by UV light (21, 22). There are many kinds of DNA damage, including double-strand breaks (DSBs), single-strand breaks (SSBs), and base modifications. Of these, the DNA DSB is known to be a very destructive lesion in which both strands of the DNA double helix are sheared at the same time by chemical or physical actions. The misrepaired DSBs or DSBs that remain when repair has been completed can lead to cell death, mutation or neoplastic transformation (24). Although an inhibitory effect on the repair of X-ray-induced DNA SSBS has been demonstrated (4, 5, 9, 21, 22), there has been no study of the effect of metals or metalloids on DNA DSB repair. Thus we investigated the effects of arsenite and nickel on the repair of DSBs induced by γ radiation. These compounds were selected because of the increased interest in their inhibitory effects on various forms of DNA repair (4–19). Our data demonstrate that arsenite at subtoxic concentrations has a strong inhibitory effect on the repair of radiation-induced DSBs.
MATERIALS AND METHODS

Chemicals

The medium, serum and antibiotics used for the cell culture are products of Gibco (Gaithersburg, MD). Nickel chloride and sodium arsenite are analytical-grade reagents produced by Wako Chemical (Tokyo, Japan), and chemicals used for electrophoresis are analytical-grade reagents from Sigma (St. Louis, MO) unless otherwise specified.

Cell Culture

Chinese hamster ovary cells (CHO-K1) were obtained from Riken Cell Bank (Ibaraki, Japan) and were maintained in Ham’s F-12 medium supplemented with 10% FBS, 100 µg/ml streptomycin, and 100 U/ml penicillin at 37°C in an atmosphere of 5% CO₂ and 95% air. Plateau-phase cells were obtained by growing 3-4 × 10⁴ cells in a 60-mm tissue culture dish (5 ml medium) for 4 days without a change of medium. Subsequently 1.5 × 10⁸ cells were inoculated in a 35-mm tissue culture dish in 2 ml of the above medium and labeled with 0.74 KBq/ml [3H]thyminde plus 5 µM cold thyminde added at the time of culture preparation. Cells were used for the experiment 24-28 h after incubation, when approximately 5 × 10⁷ cells had accumulated. Thus the experiments were performed with exponentially growing cells. This culture protocol gave consistent results regarding the amount of DNA DSBs measured, and the cell cycle distribution of the population used was similar in all of the experiments.

Exposure to Chemicals and γ Radiation

At the beginning of the experiments, the culture medium was replaced with 1 ml of fresh medium, and then 0.1 ml of chemical solution was added. The chemicals used were nickel chloride and sodium arsenite; they were dissolved in freshly prepared Hank’s balanced salt solution. The cells were exposed to various concentrations of chemicals for 2 h and then irradiated on ice using a 140Cs irradiator at a dose of 40 Gy (dose rate: 10.2 Gy/min). Subsequently, in some cultures (non-repair group), the cells were washed once with cold medium, trypsinized at room temperature for 1.5-2 min, and collected in a test tube on ice for further analysis of DNA DSBs. In other cultures (repair groups), cell incubation continued at 37°C for 30 min to allow DSB repair, and then the cells were prepared for analysis of DSBs with the same procedure used for the non-repair groups. In the non-repair group, there is a possibility that a few DSBs might be rejoined since the cells were maintained at room temperature during the trypsin-EDTA treatment. If we assume that DSBs were repaired during the trypsin-EDTA treatment at the same rate as at 37°C, about 7% of DSBs were repaired before the assay. However, our procedures were very consistent from experiment to experiment, so no serious deviation in the data was expected.

Analysis of DNA DSBs

The assay of DSBs was carried out by pulsed-field gel electrophoresis as described elsewhere (25, 26). In brief, the recovered cells were mixed with melted 0.5% agarose solution, pipetted into plastic molds, and cooled on ice until they had solidified. These agarose samples were immersed in an ice-cold lysis solution containing 0.5 M EDTA, 0.01 M Tris, 2% sarcosyl, and 0.2 mg/ml proteinase K for 1 h and then incubated overnight at 50°C. After lysis, samples were washed and treated with 0.1 mg/ml ribonuclease A for 1 h. Sample plugs were electrophoresed in 0.5X TBE buffer (45 mM Tris, 45 mM boric acid, 1.5 mM EDTA, pH 8.2) in a clamped homogeneous electric-field gel box (Bio-Rad, Tokyo, Japan). After a 108 pulse sequence (1.6%, grade, Bio-Rad) at 14°C. The voltage applied was 200 V with a 60-s pulse time for the first 9 h followed by a 120-s pulse time for the last 14 h (total run time, 23 h). After electrophoresis, the gels were stained with ethidium bromide, photographed under UV light, and cut to separate the plug from the lane of each sample. The [3H] activity of each piece was measured in a scintillation counter and the fraction of activity released (FAR) was calculated as the disintegrations per minute (dpm) for a lane divided by the total dpm (lane + plug) per sample. Background FAR values, obtained from the lanes containing unirradiated DNA, were typically between 2 and 5% and were subtracted from the FAR values of treated samples.

Cell Viability and Colony-Forming Ability

The proliferative ability of the cells was determined by a colony-forming assay. The cells were prepared and exposed to each chemical for 2.5 h, which corresponded to the sum of the preincubation time with the chemicals (2 h) and the repair incubation time (0.5 h). Then they were trypsinized, counted and replated onto 35-mm tissue culture dishes in triplicate to yield 100–500 colonies per dish 8–9 days later. The colonies were stained with Giemsa solution and counted. In some experiments, the toxicities of nickel and arsenite were also determined by trypan blue dye exclusion. After exposure to chemicals for 2.5 h, the culture medium was recovered, and the dish was rinsed to remove nonadherent cells. The recovered medium and rinsed solutions were centrifuged to collect the nonadherent cells. The number and viability of the nonadherent cells recovered and the adherent cells on the dish were determined by a dye exclusion test with 0.5% trypan blue. The cytotoxicity and DNA DSB assays were performed in duplicate or triplicate in at least three independent experiments. Statistical analysis was carried out with the Student’s t test (n = 6–12).

RESULTS

The Effect of Chemicals on Cell Adherence and Viability

Figure 1A and B shows the cell viability determined by trypan blue dye exclusion and the percentage of cells that adhered to the culture dish at the end of 2.5 h exposure. The viability was greater than 90% in cells exposed to arsenite at all the concentrations used. In cells exposed to Ni(II), the viability was greater than 90% for concentrations of 20 mM or less and decreased to 84 and 20% at 76 and 200 mM, respectively. The percentages of adherent cells were greater than 95% except for the cells exposed to the highest concentration of arsenite, where approximately 20% of the cells were detached from the culture dish (Fig. 1A). These results indicate that most cells adhered to culture dishes after exposure and were processed for the subsequent DNA DSB assay. The plating efficiencies of the cells after 2.5 h exposure to arsenite and Ni(II) are shown in Fig. 2A and B, respectively. The survival data fit a log-linear curve well, and the lack of a shoulder in the dose-response curves is consistent with previous reports (7, 27). The mean lethal concentrations for the arsenite and Ni(II) treatments were approximately 0.121 and 13.3 mM, respectively.

Induction of DNA DSBs by γ Radiation and their Repair

To examine the kinetics of DSB repair, the repair incubation was extended up to 120 min after irradiation, and DSB repair was assayed 30, 45, 60 and 120 min postirradiation. As shown in Fig. 3, there was a rapid decrease in the FAR in the first hour. By 120 min, the FAR value had almost reached the background level. This decrease in FAR is attributable to the rejoining of DSBs as reported in previous studies (28, 29). In the following study, the inhibitory effects of Ni(II) and arsenite on DSB rejoining were inves-
tigated at 0 (no repair) and 30 min postirradiation, since the most rapid and significant repair occurred during this period.

**Inhibition of DNA DSB Repair by Ni(II) and Arsenite**

Cells were preincubated with chemicals for 2 h and irradiated with 40 Gy γ rays. Some cultures were then assayed for DSBs immediately and the others were incubated for a further 30 min to allow repair. Figure 4A shows the FAR values for the cells exposed to arsenite. Up to 0.12 mM arsenite, the FAR for cells assayed immediately after irradiation (no repair) were at levels similar to those in untreated (0 mM) cells and then increased gradually with increasing arsenite concentration. The FAR values at 30 min postirradiation were significantly increased due to the exposure to arsenite at 0.08 mM or more, indicating a strong inhibitory effect on repair by arsenite in this concentration range. The arsenite concentration dependence of the inhibition of DSB repair is clearly shown in Fig. 4A. In this figure, the repair fraction (fraction of repaired DSBs) was calculated by the following equation:

\[
\text{Repair fraction} = \frac{[\text{FAR}(0) - \text{FAR}(\text{BL})] - [\text{FAR}(30) - \text{FAR}(\text{BL})]}{\text{FAR}(0) - \text{FAR}(\text{BL})},
\]

where FAR(0) and FAR(30) denote the FAR values at 0 and 30 min after irradiation, respectively, and FAR(BL) is the blank FAR values in the cells that received neither γ irradiation nor chemical exposure. Since it has been shown that FAR is proportional to the number of DSBs in a linear manner in these ranges (28, 30), the above equation indicates the actual fraction of DSBs repaired.

The data in Fig. 5A indicate that the repair inhibition occurred in the biologically significant concentration range of arsenite. For Ni(II), as shown in Fig. 4B, no significant changes in the FAR values were observed up to 20 mM for the initial induction of DSBs (0 min). Then the initial damage gradually increased and the FAR at 30 min also increased at concentrations higher than 40 mM Ni(II). These results indicate that Ni(II) also inhibits the repair of DSBs at concentrations of more than 40 mM, and that 76 mM nickel was necessary to observe a clear inhibition (Fig. 5B).

**FIG. 1.** Cell viability determined by the trypan blue dye exclusion test and the percentage of adherent cells after 2.5 h exposure to arsenite (panel A) or Ni(II) (panel B). Vertical bars indicate the SD for 6 to 12 cultures obtained from at least three independent experiments.

**FIG. 2.** Cell survival as determined by the colony-forming assay after 2.5 h exposure to arsenite (panel A) or Ni(II) (panel B). Vertical bars indicate the SD for six to nine cultures obtained from at least three independent experiments.
However, these concentrations are much higher than the biologically significant concentration range for Ni(II).

It has been reported that rejoining of DSBs has a fast and a slow component (25). To show how arsenite affects these phases, data for rejoining kinetics up to 120 min post-irradiation are presented in Fig. 6. The inhibition of repair by arsenite is very distinct in the first 60 min after irradiation, and this appears to lead to a substantial number of unjoined DSBs (~40% of initial breaks) at 2 h postirradiation. Such unjoined breaks could well be the cause of the sensitization by arsenite and could be more important than the repair effects on the rate of each component.

**DISCUSSION**

In this study, we have shown the inhibitory effects of arsenite and Ni(II) on the repair of DSBs induced by \( \gamma \) radiation. Although a high concentration of Ni(II) (>40 mM) was necessary to inhibit repair of DSBs, a low concentration of arsenite (0.08 mM) inhibited DSB repair. This arsenite concentration is significant since it allowed more than 50% of the cells to survive. To the best of our knowledge, this is the first direct evidence that arsenite inhibits the repair of DSBS. It has been reported that arsenite enhances the accumulation of DSBs in cells exposed to methyl methanesulfonate (I6). The presence of DSBs in that study may be the result of effects on the repair of the lesions induced by methyl methanesulfonate.

There are a number of reports demonstrating that both Ni(II) and arsenite inhibit the repair of X-ray- or UV-radiation-induced DNA damage measured using alkaline elution, alkaline unwinding, or the nucleoid sedimentation assay (4–11, 13–15). The concentrations of compounds used in those studies were relatively low and resulted in little or no cytotoxicity. In the present study, however, only arsenite interfered with the repair of DSBs at relatively low concentrations, while Ni(II) inhibited repair only at high concentrations. Interestingly, the repair of DNA damage induced by methyl methanesulfonate was inhibited by nontoxic levels of arsenite, but not by Ni(II) (8).

The initial yield of DSBs after irradiation was higher at greater concentrations of Ni(II) or arsenite (Fig. 4A and B). Since DSBs were induced after the exposure to high concentrations of nickel or arsenite alone (without radiation), the increase may be attributable to the direct induction of DNA damage by Ni(II) or arsenite. An interaction between metals and scavengers such as thiols may not be the case in this situation.

Although the mechanism of inhibition of DSB repair by these compounds is not fully understood, the ligation step may be affected by these compounds, as has been suggested in the inhibition of repair of other types of DNA damage (2, 8, 11, 18, 19). Unique effects of arsenite on mutagenesis have been reported and may be relevant to the inhibitory effects of arsenite on DSB repair demonstrated in the present study. Arsenite was believed to be a weak mutagen in standard mutation assays using cultured mammalian cells (for reviews, see refs. 2 and 31). However, Hei et al. (27) recently demonstrated that arsenite is a strong mutagen and that it induced mostly large deletion mutations. Not only the direct effect on DNA strands but also the inhibition of DSB repair by arsenite may be responsible for the large deletions observed.
As described above, 0.08 mM of arsenite inhibited the repair of DSBs. This concentration is not much higher than the levels of arsenic found in the environment (as reviewed in ref. 32). For example, in some regions, the concentration of arsenic in natural well water (not artificially contaminated) reaches 1 ppm (0.014 mM). In artificially contaminated areas, concentrations that were 10 to 100 times higher were observed. In human blood and soft tissues, the arsenic concentration is usually less than 0.3 ppm (0.004 mM), which is 1/30 of the effective concentration in the present study. However, in human tissues accidentally subjected to arsenic or exposed to a environment with a higher level of arsenic, the tissue concentrations could become more than 10 ppm (0.14 mM), which is greater than those found to inhibit DSB repair in the present study.

The International Agency for Research on Cancer (IARC) categorizes both nickel and arsenic as group 1 human carcinogens (33, 34). Nickel induces lung and sinonasal cancer, and its carcinogenic action has been demonstrated in human epidemiological studies as well as in animal experiments (33, 35, 36). Exposure to arsenic compounds leads to skin and lung cancer in humans, although animal studies failed to detect a carcinogenic potential (31, 33, 34, 36). Therefore, several studies indicated that the interaction with DNA repair processes might be the predominant mechanism in arsenic-induced carcinogenicity compared to direct DNA damage (as reviewed in refs. 1–3 and 36). The present results, which demonstrate that arsenite inhibits the repair of DSBs induced by γ radiation, may clarify and give new insight into the mechanism(s) underlying the carcinogenic potential of arsenic compounds.

In conclusion, it is apparent that arsenite inhibits the repair of DSBs induced by γ radiation. The observed inhibitory effect of arsenite on DSB repair may have important implications for the combined effects of arsenite and ionizing radiation, since this kind of combined exposure is possible in the environment. In the case of Ni(II), this metal ion interfered with the rejoicing of DSBs only at highly cytotoxic concentrations under the present experimental conditions. Since Ni(II) is known to cause inhibition of repair of other types of DNA damage at low concentrations (4–14), a longer incubation period (e.g., >4 h) may be needed to observe the effect (10, 12), and further studies may be necessary to elucidate a clear effect of nickel on DSB repair.

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REFERENCES


Comparative biokinetics of tritium in rats during continuous ingestion of tritiated water and tritium-labeled food

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Abstract.

Purpose: The biokinetics of tritium during continuous ingestion of tritiated water and tritiated wheat were investigated to estimate the radiation dose rates at the end of two modes of chronic exposure.

Materials and methods: Wistar strain male rats continuously ingested tritiated water as drinking water and tritiated wheat as food for 14 weeks. Urine and tissue samples were obtained and total tritium in the fresh wet samples and organically bound tritium (OBT) in the freeze-dried samples were determined.

Results: The biokinetics of tritium was different between the two modes of exposure. The concentration of total tritium in the tissues exposed to tritiated water attained a steady-state condition by 2–3 weeks. The steady-state condition in the case of exposure to tritiated wheat was not observed for 10 weeks after the start of exposure in the majority of tissues. The relatively efficient and prolonged OBT formation during chronic exposure to tritiated wheat resulted in relatively high incorporation and retention of tritium in the tissues compared with those for exposure to the same activity of tritiated water.

Conclusion: Radiation dose rates estimated at the end of continuous ingestion showed that tritiated wheat gave higher dose rates than tritiated water by a factor of 1.3 to 4.5, but the factors were within 2.0 in the majority of tissues except for small intestine and adipose tissue.

1. Introduction

A considerable amount of tritium is currently produced in fission reactors and in future a significant amount of this isotope will be used as fuel in fusion reactors. Tritium is released from these nuclear facilities mainly in the form of water. In this form, it is widely distributed in the biosphere and easily enters not only environmental water but also the bodies of plants and animals. This radionuclide is incorporated into the organic constituents of organisms, more efficiently in plants by photosynthetic processes. Thus, tritium transformed into organic form may be ingested by man as food as well as tritium in the form of water.

It is well known that tritium in organic form is more efficiently incorporated into mammalian tissues than tritium as water. Some investigators have already reported a greater tritium incorporation from tritiated organic molecules (Vennart 1969, Mewissen et al. 1977, Takeda 1982) and from tritiated foods (Kirchmann et al. 1977, Rochalska and Szot 1977, Pietrzak-Flis et al. 1978, Takeda et al. 1985). However, there are few experimental studies of tritium dynamics in mammals under the condition of chronic exposure to tritiated water or tritiated food. For chronic exposure to tritium, especially in the form of food, few long-term data are available on tritium dynamics in mammalian tissues. Rodgers (1992) reported on tritium dynamics in mice chronically exposed to tritiated water and diet, but the duration of chronic exposure (56 days) was not enough to ensure that a steady-state condition had been attained. Tritiated food used in the experiments was prepared by mixing tritiated amino acid mixture with powdered commercial diet.

In the present study, rats were continuously exposed to tritiated water as drinking water or tritiated wheat as food for about 100 days. During chronic exposure, the biokinetics of tritium was investigated to elucidate the dynamics into a steady-state condition and to estimate the radiation dose rates under the steady-state condition. The results will be useful for comparative evaluation of the potential risk between the two modes of exposure.

2. Materials and methods

Wistar male rats weighing about 450 g were used in this study. Sixty rats were separated into two groups receiving chronic exposure either to tritiated water or tritiated wheat. Throughout the experiments, rats were housed in metabolic cages (Metabolica, Sugiyama-gen Co.) in order to measure consumption of water and food, and to collect daily samples of urine from each animal. The daily ingested amounts of drinking water and food averaged over all the present experiments were 36 ml

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and 24 g, respectively. The daily excreted amount of urine averaged 17 ml.

Tritiated water (purchased from NEN) was diluted to an appropriate concentration with distilled water to prepare tritiated drinking water and tritiated food. The concentration of tritium in drinking water was adjusted to 6.3 kBq/ml. For preparation of tritiated food, wheat was grown in a 0.02 m² plastic Wagner's pot and after flowering the plant was sometimes irrigated with tritiated water in a phytotron with controlled temperature and natural light. At the time of ripening, the plants were harvested and the edible part was fed to the rats; it was first pulverized, freeze-dried and mixed with the powdered standard chow. The concentration of tritium in the food including tritiated wheat was 0.18 kBq/g.

Urine samples collected daily during these experiments were assayed for radioactivity. The rats were sacrificed by decapitation under ether anesthesia from weeks 1–14 after start of ingestion. Tissue samples were taken and the total tritium activity in the fresh wet tissues was determined. Part of each tissue sample was lyophilized for determination of organically bound tritium (OBT). Lyophilization was repeated twice with addition of distilled water to remove water-bound tritium and easily exchangeable OB T. Samples were combusted in an oxidizer (Model 306 Tri-Carb, Packard Instrument Co.) and tritium radioactivity in the combustion water was measured with a liquid scintillation counter.

Data were presented as a percentage of the concentration of tritium in the ingested water or food, and also as a percentage of tritium ingested per gram of wet or dry tissue. The former was expressed in terms of relative concentration and the latter was in terms of relative integrated activity. They were respectively calculated as follows

\[
\text{Relative concentration} = \frac{\text{Radioactivity per g of wet or dry sample}}{\text{Radioactivity per g of ingested drinking water or food}} \times 100
\]

\[
\text{Relative integrated activity} = \frac{\text{Radioactivity per g of wet or dry sample}}{\text{Radioactivity ingested per g of tissue}} \times 100
\]

Data values show the average of determinations on three or five animals.

The R-value, defined as the ratio of specific activity (T/H) of tritium in dry tissues to specific activity (T/H) of tritium in daily ingested drinking water or food, was also calculated for rat tissues at the end of 98 days of chronic exposure to tritiated water or tritiated wheat. For this calculation, the values used were for the hydrogen content for the organic component of various tissues given by Pietrzak-Flis et al. (1978). The hydrogen content of drinking water or tritiated food was calculated on the basis of their chemical composition to be 11.1% and 7.0%, respectively.

All experimental animals were treated and handled according to the 'Recommendations for handling of laboratory animals for biomedical research', compiled by the Committee on the Safety and Handling Regulations for Laboratory Animal Experiments, NIRS.

3. Results

Time variations in the relative concentration of tritium in urine collected daily during continuous ingestion of tritiated water or tritiated food are shown in figure 1. Concentrations of tritium in urine rapidly increased after the start of ingestion, and attained a steady-state condition by about day 10 after the start of tritiated water ingestion and day 20 after the start of tritiated food ingestion. The steady-state concentrations were approximately 64% of the concentration of tritium in drinking water and approximately 37% of that of tritium in the food. These values at steady-state seemed to be an index of contribution to body water tritium from drinking water and food, respectively. It was apparent in the preliminary experiments that the presence of OB T in urine could be ignored due to the low content of organic materials (about 3%), even after exposure to tritiated organic compounds including foods.

Time variations in the relative concentrations of total tritium and OB T in the selected tissues are shown in figure 2 and figure 3. In the case of exposure to tritiated water, the concentrations of total tritium increased rapidly and attained steady-state by 2–3 weeks. The steady-state concentrations of total tritium for the majority of tissues, including the tissues not shown in this figure, ranged from 40% to 50% of the concentration of tritium in drinking water. The steady-state concentration in blood was somewhat higher than that for other tissues, at about 60%. In adipose tissue, the steady-state condition was not clearly observed. The steady-state concentration of OB T in rats exposed to tritiated water was observed at about 4 weeks after the start of chronic exposure, although the steady-state concentrations for all the examined tissues were within 20% of the concentration of the ingested tritiated water. In the case of chronic exposure to tritiated food, a steady-state for both total tritium and OB T was not so clearly observed for 10 weeks after the start of chronic exposure for the majority of the examined tissues except for liver, in which the steady-state seemed to be attained at about 4 weeks. At the steady-state, the
Comparative biokinetics of tritium

Figure 1. Time variations in the relative concentration of total tritium in urine.

concentrations of total tritium ranged from 39% to 56%, and those of OBT ranged from 47% to 71% of the concentration of tritium in the ingested food.

Table 1 shows the amount of total tritium and OBT retained in the tissues of rat at the end of chronic exposure, expressed in terms of the relative integrated activity, which enables the comparison of the amount of tritium incorporated and retained in the tissues between the two modes of exposure. It was evident that the amount of total tritium or OBT in the tissues of rats exposed to tritiated wheat was higher than that in rats exposed to tritiated water, the difference being more significant for OBT than for total tritium. The ratios of the relative integrated activity of OBT after exposure to tritiated wheat to that after exposure to tritiated water ranged from 5.6 in adipose tissue to 10.4 in heart.

The R-value, as defined above, is a useful factor for evaluating the extent of tritium incorporation into organic constituents of tissues. The R-values calculated for different tissues of rats at the termination of 98 days of chronic exposure to tritiated water or tritiated wheat are shown in table 2: they ranged from 0.13 for adipose tissue to 0.24 for testis (for tritiated water ingestion), and for tritiated wheat exposure the range was 0.36 for brain and adipose tissue and 0.78 for lung.

On the basis of the biokinetics data, the radiation dose rates at the end of chronic exposures were calculated. The following formula was used

\[ D = 1.38 \times 10^{-8} (A \times E) \]

where \( D \) is the dose-rate in Gy/day, \( A \) is the tritium concentration in kBq/g, and \( E \) is the average energy of tritium \( \beta \)-rays (5.7 \times 10^{-3} \text{ MeV}). In this calculation, it was assumed that total radioactivity ingested during the chronic exposures was 3.7 MBq/g bodyweight in order to enable a comparative evaluation of the dose rates from the exposures to tritiated water and tritiated wheat. The dose calculation was performed not only for total tritium but also for OBT. For the calculation of the OBT contribution, the values of water content for individual tissues of rats determined in a previous study (Takeda and Kasida 1979) were used. The results are shown in table 3. In this table, the ratios of the dose rates from tritiated wheat ingestion to those from tritiated water ingestion are also given. The results show that the total dose rates from exposure to tritiated wheat were higher than those from exposure to tritiated water by a factor of 1.3 to 4.5, but the factors did not exceed 2.0 in the majority of tissues, except for small intestine and adipose tissue.

4. Discussion

The results of the present study showed a difference in tritium dynamics in rats chronically exposed to tritiated wheat as a food compared with rats exposed to tritiated water as a drinking water. The time taken to attain a steady-state concentration of total tritium in urine was longer in rats exposed to tritiated food than in rats exposed to tritiated water. With regard to the contribution of tritium in drinking water to body water tritium, the result was similar to that previously reported (Thompson and Ballou 1956, Hatch and Mazrimas 1972, Takeda 1991).

The steady-state condition for total tritium in the tissues was observed at 2–3 weeks in rats exposed to tritiated water, while that in rats exposed to tritiated wheat was not observed for 10 weeks in the majority of tissues. The differences in the dynamics of tritiated
water and tritiated wheat were mostly due to the rate and duration of OBT formation. Because of the relatively efficient and prolonged OBT formation during the exposure to tritiated wheat, the relative integrated activity of tritium incorporated and retained in the tissues at the end of exposure was greater in rats exposed to tritiated wheat than those in rats exposed to tritiated water (table 1). As described previously, there are a few comparative studies on tritium incorporation into the tissue organic constituents from tritiated food and from tritiated water (Kirchmann et al. 1977, Rochalska and Szot 1977, Pietrzak-Flis et al. 1978). Comparing the results of these reports with those of present study, the relative integrated activity of OBT after the chronic exposure to tritiated water was almost the same level irrespective of the different duration of chronic exposure; in contrast, the integrated activity after exposure to tritiated food showed differences that may have been due to differences in the types of food used in individual experiments.

R-values estimated at the end of chronic exposure were higher for tritiated wheat than for tritiated water. In a previous study (Takeda et al. 1985), the R-values in tissues of rats continuously exposed for 22 days were also estimated. Compared with the
Figure 3. Relative concentration of (a) total tritium and (b) OBT in tissues during chronic exposure to tritiated wheat.

previous result, the present R-values for the exposure to tritiated water did not differ significantly, but those for the exposure to tritiated wheat were greater in tissues other than liver. Nevertheless, values never exceeded 1.0, indicating that the specific activity of tritium in the tissue organic constituents did not exceed the specific activity of tritium in the food as well as that in the drinking water.

Estimation of radiation dose rates at the end of chronic exposure showed that the total dose rates from exposure to tritiated wheat were 1.3–4.5 times higher than those from exposure to tritiated water. In the previous study, in which tritiated water and tritiated wheat were continuously ingested for about 3 weeks, it was reported that the dose rates from tritiated food were higher than those from tritiated water by a factor of 2.1 to 4.8. The results of the present study therefore indicate that the difference in the dose rates between tritiated water and tritiated food would not increase by lengthening the period of chronic exposure.

The differences in the tritium biokinetics observed
Table 1. Relative integrated activity of total tritium and OBT in the tissues of rats at the end of chronic exposure to tritiated water or tritiated wheat.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Tritiated water</th>
<th>Tritiated wheat</th>
<th>Tritiated water</th>
<th>Tritiated wheat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>6.3 ± 0.2</td>
<td>12.2 ± 1.3</td>
<td>2.3 ± 0.2</td>
<td>19.4 ± 0.7</td>
</tr>
<tr>
<td>Kidney</td>
<td>6.7 ± 0.3</td>
<td>11.6 ± 1.1</td>
<td>1.9 ± 0.2</td>
<td>17.1 ± 0.8</td>
</tr>
<tr>
<td>Testis</td>
<td>7.7 ± 0.3</td>
<td>9.7 ± 0.5</td>
<td>2.2 ± 0.2</td>
<td>15.7 ± 0.7</td>
</tr>
<tr>
<td>Spleen</td>
<td>6.7 ± 0.4</td>
<td>10.6 ± 0.4</td>
<td>1.7 ± 0.3</td>
<td>15.3 ± 1.1</td>
</tr>
<tr>
<td>Brain</td>
<td>5.9 ± 0.3</td>
<td>10.2 ± 0.3</td>
<td>2.1 ± 0.2</td>
<td>12.0 ± 1.7</td>
</tr>
<tr>
<td>Muscle</td>
<td>6.4 ± 0.4</td>
<td>10.3 ± 0.3</td>
<td>1.6 ± 0.2</td>
<td>13.4 ± 1.0</td>
</tr>
<tr>
<td>Small intestine</td>
<td>5.9 ± 0.6</td>
<td>15.1 ± 1.1</td>
<td>1.8 ± 0.3</td>
<td>16.2 ± 1.5</td>
</tr>
<tr>
<td>Lung</td>
<td>6.4 ± 0.3</td>
<td>10.2 ± 0.4</td>
<td>1.8 ± 0.2</td>
<td>15.7 ± 2.1</td>
</tr>
<tr>
<td>Heart</td>
<td>6.6 ± 0.5</td>
<td>10.5 ± 0.6</td>
<td>1.7 ± 0.3</td>
<td>17.6 ± 2.5</td>
</tr>
<tr>
<td>Adipose tissue</td>
<td>3.2 ± 0.2</td>
<td>14.4 ± 0.9</td>
<td>2.5 ± 0.3</td>
<td>13.9 ± 0.7</td>
</tr>
<tr>
<td>Blood</td>
<td>9.4 ± 0.4</td>
<td>13.4 ± 0.7</td>
<td>1.7 ± 0.2</td>
<td>16.7 ± 1.2</td>
</tr>
</tbody>
</table>

*Each value is the mean ± SD of five rats for tritiated water and three rats for tritiated wheat.

Table 2. Ratio of specific activity of tritium in dry tissue to specific activity in the daily ingested tritiated water or tritiated food, estimated at the end of chronic exposure.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>R-value Hydrogen content (%)</th>
<th>Tritiated water</th>
<th>Tritiated food</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>7.11</td>
<td>0.23</td>
<td>0.70</td>
</tr>
<tr>
<td>Kidney</td>
<td>7.62</td>
<td>0.20</td>
<td>0.62</td>
</tr>
<tr>
<td>Testis</td>
<td>6.32</td>
<td>0.24</td>
<td>0.68</td>
</tr>
<tr>
<td>Spleen</td>
<td>6.12</td>
<td>0.19</td>
<td>0.69</td>
</tr>
<tr>
<td>Brain</td>
<td>9.26</td>
<td>0.16</td>
<td>0.36</td>
</tr>
<tr>
<td>Muscle</td>
<td>5.93</td>
<td>0.21</td>
<td>0.67</td>
</tr>
<tr>
<td>Small intestine</td>
<td>7.95</td>
<td>0.17</td>
<td>0.56</td>
</tr>
<tr>
<td>Lung</td>
<td>5.61</td>
<td>0.23</td>
<td>0.78</td>
</tr>
<tr>
<td>Heart</td>
<td>8.15</td>
<td>0.15</td>
<td>0.59</td>
</tr>
<tr>
<td>Adipose tissue</td>
<td>11.69</td>
<td>0.13</td>
<td>0.36</td>
</tr>
</tbody>
</table>

Table 3. Radiation dose rates from total tritium and OBT contribution to dose rates at the end of chronic exposure.

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Dose rate (mGy/day)* and OBT contributionb</th>
<th>Rate of dose rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>18 (11.3) 36 (49.3)</td>
<td>2.0</td>
</tr>
<tr>
<td>Kidney</td>
<td>20 (7.7) 34 (39.6)</td>
<td>1.7</td>
</tr>
<tr>
<td>Testis</td>
<td>22 (4.8) 28 (24.3)</td>
<td>1.3</td>
</tr>
<tr>
<td>Spleen</td>
<td>20 (6.6) 31 (37.5)</td>
<td>1.6</td>
</tr>
<tr>
<td>Brain</td>
<td>17 (8.9) 30 (29.4)</td>
<td>1.8</td>
</tr>
<tr>
<td>Muscle</td>
<td>19 (7.0) 30 (36.8)</td>
<td>1.6</td>
</tr>
<tr>
<td>Small intestine</td>
<td>17 (8.5) 44 (30.0)</td>
<td>2.6</td>
</tr>
<tr>
<td>Lung</td>
<td>19 (6.8) 30 (36.9)</td>
<td>1.6</td>
</tr>
<tr>
<td>Heart</td>
<td>19 (7.0) 31 (45.3)</td>
<td>1.6</td>
</tr>
<tr>
<td>Adipose tissue</td>
<td>9 (66.4) 42 (82.0)</td>
<td>4.5</td>
</tr>
<tr>
<td>Blood</td>
<td>27 (3.6) 39 (24.9)</td>
<td>1.4</td>
</tr>
</tbody>
</table>

*The dose rates were calculated assuming tritium exposure to be 3.7 MBq/g body weight.

b OBT contribution is expressed as a percentage of total dose rate in parentheses.

at the chronic exposures to tritiated drinking water and tritiated food may be explained ultimately on the basis of the physiology and metabolism of water and organic materials in the body of mammals, although it is too intricate to permit an explanation of the detailed mechanism.

The dose coefficients (Sv/Bq) for organic tritium, currently presented by ICRP (1995), are higher than those for tritiated water by a factor of 1.9 to 2.5. The results of the present study support the ICRP recommendation as an average value. In a previous study (Takeda 1995), it was shown that the cumulative doses from a single intake of various tritiated organic compounds were 1.3–5.2 times higher than that from the intake of tritiated water, and it was proposed that the annual limit on intake (ALI) for organic tritium should be five times smaller than that for tritiated water. The results of present study support this proposal.

Acknowledgements

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References


DETERMINATION OF $^{99}$Tc DEPOSITED ON THE GROUND WITHIN THE 30-km ZONE AROUND THE CHERNOBYL REACTOR AND ESTIMATION OF $^{99}$Tc RELEASED INTO ATMOSPHERE BY THE ACCIDENT

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ABSTRACT - Technetium-99 was determined in samples from the 30-km zone around the Chernobyl reactor. Concentrations of $^{99}$Tc in soil samples taken from three forest sites ranged from 1.1 to 14.1 Bq kg$^{-1}$ dry weight for the organic soil layers, and from 0.13 to 0.83 Bq kg$^{-1}$ dry weight for the mineral soil layers. In particular, for the organic layers, the measured $^{99}$Tc concentrations were one or two orders of magnitude higher than those due to global fallout $^{99}$Tc. The $^{99}$Tc depositions (Bq m$^{-2}$), based on the sum of the depositions measured in organic and mineral layers, ranged from 130 Bq m$^{-2}$ within the 10-km zone to about 20 Bq m$^{-2}$ close to the border of the 30-km zone. Taking the corresponding measured $^{137}$Cs depositions into account, it was found that the activity ratio of $^{99}$Tc/$^{137}$Cs ranged from 6 x 10$^2$ to 1.2 x 10$^4$. It was estimated that about 970 GBq of $^{99}$Tc had been released by the Chernobyl accident. This figure corresponded to 2% - 3% of the total $^{99}$Tc inventory in the core.

Key words: Technetium-99; Chernobyl accident; Forest soil; Deposition; Cesium-137; Migration

INTRODUCTION

There have been many reports concerning radionuclides' concentrations in environmental samples due to the accident in Unit 4 of the Chernobyl Nuclear Power Station (CNPS). Some reports have shown $^{99}$Mo concentrations in environmental samples$^{1-6}$. This radioisotope decays to $^{99}$Tc ($^{99}$Mo (t$_{1/2}$: 65.9 h) --> $^{99m}$Tc (t$_{1/2}$: 6.0 h) --> $^{99}$Tc), but the amount may be negligible compared to the deposited $^{99}$Tc released directly into the atmosphere with other radionuclides at the time of the accident. Because $^{99}$Tc has a high
fission yield of about 6% and a long half-life of $2.1 \times 10^5$ y and Tc has a high absorbability by plants\textsuperscript{74}, a survey of the contamination levels in environmental samples is of interest to estimate the long term $^{99}$Tc exposure to humans. At present, however, there are almost no data on $^{99}$Tc contamination of environmental samples and consequently, limited information on the $^{99}$Tc activity released by the Chernobyl accident is available.

In this paper, results of $^{99}$Tc and $^{137}$Cs measurements in samples taken from three forest sites within the 30-km zone around the CNPS are presented. $^{99}$Tc and $^{137}$Cs concentrations [Bq kg$^{-1}$ dry weight (DW)] and depositions [Bq m$^{-2}$] are given for the organic soil layers and for the first 6 to 9 cm of mineral soil. The results are discussed with respect to expected values and different characteristics of $^{99}$Tc and $^{137}$Cs. In addition, the amount of $^{99}$Tc released from the reactor into the environment is estimated using data from this paper, and results reported previously by Aarkrog and others\textsuperscript{9,10}.

**MATERIALS AND METHODS**

**Soil Samples**

For the present analysis, soil samples collected within three forest sites around Chernobyl were used. The sites D1 (mixed forest: 50% oak, 30% pine, 20% birch; 55-60 years old) and D3 (mixed forest: 50% alder, 40% birch, 10% pine; 50-75 years old) are located 28.5 km and 26.0 km to the south of the CNPS, respectively, while K2 (pine forest; about 50 years old) is located 6.0 km to the southeast of the CNPS. Soils at D1 and K2 are podsol on sandy fluvioglacial deposits and at D3, peat-ogley on sandy fluvioglacial deposits. D1 and K2 are so-called dry forests, whereas D3 can be characterized as wet forest, since the groundwater level at this site is at a depth of about 45 cm from the top of the soil surface\textsuperscript{11}. Samples were collected by the Federal Office for Radiation Protection, Germany, in 1994 and 1995, assisted by the Moscow State University, Russia, and the Research and Industrial Association 'Pripyat', Ukraine. Nine profiles of undisturbed soil per site and year were randomly collected within an area of about 100 x 100 m$^2$.

In order to produce representative organic soil samples, materials from the organic layers (L, Of, Oh horizons) gathered at the same site and in the same year, were combined to one mixed sample. The same procedure was used in order to get representative mixed mineral samples using materials from the Ah horizon and from the first 6 cm of the B horizon. At D3, no Ah-horizon could be identified and the first 9 cm of the B horizon were used. For the present analysis, D1 samples from 1994 and 1995 were combined.

**Reagents**

Prepacked columns of Tc-selective chromatographic resin (ELChroM Industries Inc., TEVA · Spec resin) were used for the chemical separation. Nitric acid was ultrapure-analytical grade (Tama Chemicals, AA-100). Deionized water (>17MO) was used throughout the work. A radiotracer of $^{95m}$Tc, which was obtained from a Nb foil using the reaction $^{95}$Nb (α, 2n) $^{95m}$Tc$^{12}$, was applied to determine the recovery of $^{95}$Tc in the samples during the chemical separation procedure.
Chemical Separation and Measurements

In all samples, $^{99}\text{Tc}$ and $^{137}\text{Cs}$ concentrations were determined. The $^{137}\text{Cs}$ activities were measured by use of a Ge detector system (Seiko EG&G, Ortec) without any chemical separations. For the $^{99}\text{Tc}$ determination, chemical separation was necessary. The separation method used was a revised form of the method reported previously$^{13,14}$. Only a brief explanation is given here.

1) The soil sample was incinerated at 450°C in an electrical oven for one night. 2) After addition of $^{99m}\text{Tc}$, the sample was placed in a combustion apparatus. 3) Tc was volatilized from the sample at 1000°C under O$_2$ gas stream and the element was collected in a trap solution (deionized water). 4) After adjusting to 0.1 M HNO$_3$, the trap solution was introduced into a TEVA·Spec resin column to extract Tc onto the resin. Then 40 mL of 2 M HNO$_3$ solution were passed through the column to elute co-existing elements. 5) In order to desorb the Tc from the resin, 5 mL of 8 M HNO$_3$ solution were pipetted onto the column. 6) The strip solution of 8 M HNO$_3$ from the resin was put in a glass beaker and evaporated to dryness at 70°C on a hot plate. 7) The residue was dissolved with 5 mL of 2% HNO$_3$ solution. 8) The $^{99m}\text{Tc}$ activity in each 2% HNO$_3$ solution was measured with a NaI (Tl) scintillation counter (Aloka, ARC-380) to obtain chemical recoveries. Total recoveries of $^{99m}\text{Tc}$ during the chemical separation process ranged from 39 to 93%.

After the chemical separation, $^{99}\text{Tc}$ concentrations in each 2% HNO$_3$ solution were determined by inductively coupled plasma mass spectrometry (ICP-MS; Yokogawa PMS-2000). Counting time at mass 99 was 10 min, or 200 s for three times. The concentrations of stable Mo and stable Ru in the solution were also measured. Because Ru, which has an isotopic abundance of 12.7% at mass of 99, interferes with $^{99}\text{Tc}$ measurement by ICP-MS and there is a possibility of a molecular peak from $^{99}\text{MoF}$ at mass of 99. For the ICP-MS measurement, $^{99}\text{Tc}$ standard solution (Japan Isotopes Association, NH$_4$TcO$_4$ form in 0.1% ammonia solution) was used.

For concentrations (Bq kg$^{-1}$ DW), uncertainties are given with respect to statistical one-sigma errors of the measurements, throughout the paper. For given depositions (Bq m$^2$), standard errors for the area-related densities (kg DW m$^2$) are also taken into account. It is not possible to give standard errors arising due to the heterogeneous horizontal deposition pattern in natural ecosystems directly, since only mixed samples were analyzed in this study. From earlier measurements of $^{137}\text{Cs}$ at the investigated sites it is estimated, however, that this contribution to the overall one-sigma standard error of mean concentration values is less than 10% for the combined L/Ot/Oh samples, and less than 20% for the combined Ah/B samples.

RESULTS AND DISCUSSION

Deposition of $^{99}\text{Tc}$ and $^{137}\text{Cs}$ within the 30-km Zone

Table 1 shows the results of $^{99}\text{Tc}$ and $^{137}\text{Cs}$ concentrations in the investigated soil samples. In the case of $^{137}\text{Cs}$, the concentrations range from (0.84 ± 0.02) kBq kg$^{-1}$ DW (D1, '94 & '95, Ah/B) to (230 ±
Table 1  Concentrations of $^{99}$Tc and $^{137}$Cs in Bq kg$^{-1}$ DW with statistical one-sigma measurement error; corresponding area-related deposition in Bq m$^{-2}$, and $^{99}$Tc/$^{137}$Cs ratios on 26 April 1986, in soil samples collected in the 30-km zone around Chernobyl. Area-related densities are based on 5 soil profiles, taken in 1993 at each site. The corresponding errors are one-sigma standard errors. Additional uncertainties to mean concentration values due to the inhomogenous deposition are estimated to be less than 10% (L/Of/Oh) and less than 20% (Ah/B).

<table>
<thead>
<tr>
<th>Soil sample</th>
<th>Tc recovery</th>
<th>$^{99}$Tc</th>
<th>$^{137}$Cs</th>
<th>Area-related density</th>
<th>$^{99}$Tc</th>
<th>$^{137}$Cs</th>
<th>Activity Ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$^{99}$Tc (%)</td>
<td>Bq kg$^{-1}$ DW</td>
<td>Bq kg$^{-1}$ DW</td>
<td>kg DW m$^{-2}$</td>
<td>Bq m$^{-2}$</td>
<td>kBq m$^{-2}$</td>
<td>$^{99}$Tc/$^{137}$Cs x 10$^{-4}$</td>
</tr>
<tr>
<td>D1 (94 &amp; 95), L/Of/Oh</td>
<td>87</td>
<td>1.11 ± 0.02</td>
<td>19 ± 0.02</td>
<td>4.4 ± 0.4</td>
<td>4.9 ± 0.4</td>
<td>83.6 ± 7.3</td>
<td>0.58 ± 0.01</td>
</tr>
<tr>
<td>D1 (94 &amp; 95), Ah/B</td>
<td>73</td>
<td>0.13 ± 0.03</td>
<td>0.84 ± 0.02</td>
<td>86.9 ± 1.9</td>
<td>11.6 ± 0.3</td>
<td>72.4 ± 2.5</td>
<td>1.60 ± 0.05</td>
</tr>
<tr>
<td>D3 (94), L/Of/Oh</td>
<td>93</td>
<td>2.43 ± 0.01</td>
<td>19.5 ± 0.04</td>
<td>4.4 ± 0.7</td>
<td>10.6 ± 1.6</td>
<td>85.2 ± 12.8</td>
<td>1.24 ± 0.01</td>
</tr>
<tr>
<td>D3 (95), L/Of/Oh</td>
<td>88</td>
<td>1.72 ± 0.05</td>
<td>19.9 ± 0.4</td>
<td>4.4 ± 0.7</td>
<td>7.5 ± 1.1</td>
<td>86.9 ± 13.2</td>
<td>0.86 ± 0.03</td>
</tr>
<tr>
<td>D3 (94 &amp; 95), B</td>
<td>62</td>
<td>0.36 ± 0.01</td>
<td>1.8 ± 0.03</td>
<td>37.1 ± 2</td>
<td>13.4 ± 0.8</td>
<td>68.2 ± 3.8</td>
<td>1.97 ± 0.04</td>
</tr>
<tr>
<td>K2 (94), L/Of/Oh</td>
<td>77</td>
<td>8.58 ± 0.26</td>
<td>230 ± 2.1</td>
<td>5.2 ± 0.5</td>
<td>44.8 ± 4.3</td>
<td>1200 ± 111</td>
<td>0.37 ± 0.01</td>
</tr>
<tr>
<td>K2 (95), L/Of/Oh</td>
<td>73</td>
<td>13.4 ± 0.16</td>
<td>178 ± 2.7</td>
<td>5.2 ± 0.5</td>
<td>70.1 ± 6.5</td>
<td>928 ± 87</td>
<td>0.76 ± 0.01</td>
</tr>
<tr>
<td>K2 (94 &amp; 95), Ah/B</td>
<td>39</td>
<td>0.83 ± 0.02</td>
<td>11.4 ± 0.1</td>
<td>86.5 ± 1.7</td>
<td>72.1 ± 2.1</td>
<td>982 ± 23</td>
<td>0.72 ± 0.02</td>
</tr>
</tbody>
</table>
2.1) kBq kg\(^{-1}\) DW (K2, '94, L/Of/Oh). Expressed on a kBq m\(^2\) basis, \(^{137}\)Cs contamination levels of the organic samples and the mineral samples are similar at each site, i.e. between 70 and 90 kBq m\(^2\) for D1 and D3, and about 1000 kBq m\(^2\) for K2. A total deposition was calculated for each site by adding the depositions of the corresponding organic and mineral layers, averaged over 1994 and 1995. The results are (156 ± 8) kBq m\(^2\), (154 ± 10) kBq m\(^2\) and (2046 ± 74) kBq m\(^2\), for D1, D3 and K2, respectively (Table 2). These results are in close agreement to values published previously for the same sites\(^{11}\). This shows that the samples chosen for this study are representative with respect to the measured \(^{137}\)Cs deposition, and it is therefore assumed that the same is true for the measured \(^{99}\)Tc deposition.

The \(^{99}\)Tc concentrations range from (1.11 ± 0.02) to (13.4 ± 0.2) Bq kg\(^{-1}\) DW for the organic samples, and from (0.13 ± 0.03) to (0.83 ± 0.02) Bq kg\(^{-1}\) DW for the mineral samples. Expressed on a Bq m\(^2\) basis, the contamination levels for \(^{99}\)Tc range from 4.9 ± 0.4 Bq m\(^2\) (D1, '94 & '95, L/Of/Oh) to 72.1 ± 2.1 Bq m\(^2\) (K2, '94 & '95, Ah/B). In particular, the results for the organic samples are one or two orders of magnitude higher compared to global fallout \(^{99}\)Tc concentrations, which have been reported to range from 0.01 to 0.1 Bq kg\(^{-1}\) DW\(^{15,16}\). From this result it is evident that most of the \(^{99}\)Tc measured in Chernobyl samples originates from the Chernobyl accident. This conclusion is also supported by the fact that the total \(^{99}\)Tc deposition at K2 (organic + mineral: 130 ± 4 Bq m\(^2\)), which is located much closer to the CNPS than D1 and D3, is 6 to 8 times larger compared to the total \(^{99}\)Tc deposition at D1 ((16.5 ± 0.6) Bq m\(^2\)) and D3 ((22.5 ± 1.2) Bq m\(^2\)) (Table 2). Similar differences in the contamination of K2 compared to D1 and D3 have been observed for many other radionuclides released by the CNPS (see Table 2 for \(^{137}\)Cs, and reference\(^{11}\) for other radionuclides).

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**Table 2** Lower limits for the deposition of \(^{99}\)Tc and \(^{137}\)Cs at the investigated sites.

Uncertainties include statistical one-sigma measurement errors and one-sigma standard errors for the area-related densities. Additional uncertainties due to the inhomogenous deposition are estimated to be less than 10% (L/Of/Oh) and less than 20% (Ah/B).

<table>
<thead>
<tr>
<th>Site</th>
<th>(^{99})Tc [Bq m(^2)]</th>
<th>(^{137})Cs [kBq m(^2)]</th>
<th>(^{99})Tc/(^{137})Cs</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>16.5 ± 0.6</td>
<td>156 ± 8</td>
<td>(1.06 ± 0.06) x 10(^4)</td>
</tr>
<tr>
<td>D3</td>
<td>22.5 ± 1.2</td>
<td>154 ± 10</td>
<td>(1.46 ± 0.12) x 10(^4)</td>
</tr>
<tr>
<td>K2</td>
<td>130 ± 4</td>
<td>2046 ± 74</td>
<td>(6.4 ± 0.3) x 10(^4)</td>
</tr>
</tbody>
</table>
99\textsuperscript{Tc}/\textsuperscript{137}Cs Activity Ratios and Their Mobilities in the Soils

The investigated samples were taken 8 and 9 years after the accident. During this time, some 99\textsuperscript{Tc} and 137\textsuperscript{Cs} might have reached deeper mineral layers than the layers investigated in this study. At present, no information on ecological half-lives for 137\textsuperscript{Cs} is available for the different soil layers at the investigated sites. However, ecological half-lives at a forest in Bavaria, Germany ranged from 2.8 ± 0.5 years in the L horizon to 7.7 ± 4.9 years in the Ah horizon\textsuperscript{17}, so it is expected that less than half of the initially deposited cesium had migrated to mineral layers by 1995. Since we have included, in this study, even the first 6 to 9 cm of mineral soil, loss of 137\textsuperscript{Cs} is expected to be of minor importance. Since mobility for 99\textsuperscript{Tc} might be higher compared to 137\textsuperscript{Cs} (see below), loss of 99\textsuperscript{Tc} to deeper layers might be larger than for 137\textsuperscript{Cs}. It should be bare in mind, therefore, that the results for the total 99\textsuperscript{Tc} deposition released by the Chernobyl accident might represent only lower limits.

In order to compare the mobility of 99\textsuperscript{Tc} and 137\textsuperscript{Cs}, 99\textsuperscript{Tc}/\textsuperscript{137}Cs ratios were separately calculated for the organic and mineral layers, and decay-corrected back to the accident date of 26 April 1986 (Table 1). With one exception (sample K2 '95, L/Oh/Oh), all organic samples showed significantly lower 99\textsuperscript{Tc}/\textsuperscript{137}Cs ratios than those of the mineral samples in the corresponding sites. This can be interpreted in terms of a higher mobility of 99\textsuperscript{Tc} compared to 137\textsuperscript{Cs}, allowing 99\textsuperscript{Tc} to penetrate to deeper layers within the given time, and increasing the 99\textsuperscript{Tc}/\textsuperscript{137}Cs ratios in deeper layers while possibly decreasing these ratios in the organic layers. This is in line with results from experiments on undisturbed forest soil columns, where it was shown that after simulating artificial rain showers, 95\textsuperscript{m}Tc applied as pertechnetate was more mobile compared to 137\textsuperscript{Cs}\textsuperscript{18,19}.

Since the Chernobyl accident, only one publication has appeared reporting on the simultaneous measurement of 137\textsuperscript{Cs} and 99\textsuperscript{Tc} originating from the Chernobyl accident in the same sample: Aarkrog \textit{et al.}\textsuperscript{9} determined the ratio of 99\textsuperscript{Tc}/\textsuperscript{137}Cs in a heavily contaminated motor filter of a fishing boat in the western Baltic Sea, which had passed through the Chernobyl cloud at the end of April 1986. These authors found a ratio of 1.01 \times 10^5. For comparison, we calculated 99\textsuperscript{Tc}/\textsuperscript{137}Cs ratios from the total depositions deduced for each site, again decay-corrected back to the accident date of 26 April 1986 (Table 2). Our results range from (0.6 ± 0.03) \times 10^4 (K2) to (1.46 ± 0.12) \times 10^4 (D3). These values are higher than the result reported by Aarkrog \textit{et al.}\textsuperscript{9}, but close to the theoretical ratio of 1.4 \times 10^5 estimated from the corresponding fission yields and half-lives.

It should be noted that Aarkrog's data and our data are difficult to compare, since 99\textsuperscript{Tc} and 137\textsuperscript{Cs} are expected to behave differently in the environment with respect to volatility, deposition velocities, and migration rates, due to their differences in the actual physicochemical forms in which both nuclides appear. For example, 99\textsuperscript{Tc}/\textsuperscript{137}Cs ratios are expected to be influenced by the different mobilities of 99\textsuperscript{Tc} and 137\textsuperscript{Cs} in our soil samples (see above), but not in Aarkrog's filter sample. Atmospheric transport is also expected to influence ratios calculated for different radionuclides. For example, it was reported that 103\textsuperscript{Ru}/\textsuperscript{137}Cs ratios decreased by a factor of 20 over a distance of approximately 1000 km\textsuperscript{10}. Since the characteristics of Tc are expected to be similar to those of Ru\textsuperscript{8,20}, it is possible that in comparison to
$^{137}\text{Cs}$, $^{99}\text{Tc}$ would be apt to deposit close to the Chernobyl region, and subsequently, the corresponding $^{99}\text{Tc}/^{137}\text{Cs}$ ratios could be higher close to the CNPS than those obtained from other areas far from it.

$^{99}\text{Tc} \text{ Released from the Chernobyl Accident}$

Recently a procedure was suggested to estimate the release of $^{137}\text{Cs}$ from the Chernobyl accident\textsuperscript{10}. We followed this procedure and plotted our data for $^{137}\text{Cs}$ in addition to the data given in Aarkrog's report (1988)\textsuperscript{10} versus distance from the CNPS. A least squares fit of these data gives a regression function of $D_{\text{Cs}} = 5.14 \times 10^8 \times R^{-1.477}$, where $D_{\text{Cs}}$ is the $^{137}\text{Cs}$ deposition on the ground (GBq km\textsuperscript{-2}) and $R$, the distance from the CNPS (km) (see Fig. 1). Assuming the same horizontal distribution of the $^{137}\text{Cs}$

![Graph showing the deposition of $^{99}\text{Tc}$ and $^{137}\text{Cs}$ as a function of distance from Chernobyl.](image)

Fig. 1 $^{137}\text{Cs}$ and $^{99}\text{Tc}$ deposition as function of distance from Chernobyl. $^{137}\text{Cs}$ deposition data are from this study and from (10). $^{99}\text{Tc}$ deposition data are from this study and from (9) which was estimated for a distance of 1000 km (see text).
deposition in all directions from the CNPS, we calculate the $^{137}$Cs release as

$$\int_{1}^{1000} 2\pi R \times 5.14 \times 10^{-2} \times R^{-1.477} \, dR \approx 76 \text{ PBq}$$

This is close to the $^{137}$Cs release estimated for the Chernobyl accident$^{20,21}$. We applied a similar procedure in order to estimate the release of $^{99}$Tc due to the Chernobyl accident, using our $^{99}$Tc depositions (Table 2) for distances of 6 km (K2), 26 km (D3), and 28.5 km (D1). For a distance of 1000 km, we assumed that the $^{99}$Tc/$^{137}$Cs ratio of $1.01 \times 10^{-5}$ given in the report of Aarkrog et al.$^9$, and the $^{137}$Cs deposition of about 1 GBq km$^{-2}$ given in Fig. 1, produce a representative $^{99}$Tc deposition of about $1 \times 10^{-5}$ GBq km$^{-2}$. The resulting regression function is $D_{\text{Te}} = 4.88 \times R^{-1.771}$, where $D_{\text{Te}}$ is the $^{99}$Tc deposition on the ground (GBq km$^{-2}$) and R, the distance from the CNPS (km) (see Fig. 1). Again, the release may be calculated by integration over R as

$$\int_{1}^{1000} 2\pi R \times 4.88 \times R^{-1.771} \, dR \approx 970 \text{ GBq}$$

The total core inventory of $^{137}$Cs is estimated to range from 210 to 290 PBq$^{20,21}$. A $^{137}$Cs release of 76 PBq, as estimated in this paper, corresponds therefore to a fraction of 26% to 36% of the total $^{137}$Cs core inventory. From the inventory of $^{137}$Cs and from the theoretical ratio of $^{99}$Tc to $^{137}$Cs of $1.4 \times 10^4$, the total inventory of $^{99}$Tc can be calculated to range from 30 to 40 TBq. Consequently, the percentage of $^{99}$Tc released into the atmosphere by the accident could be estimated to range from 2% to 3%. This result is close to the estimate given by Aarkrog et al. who suggested the release percentage of $^{99}$Tc would be an order of magnitude less than that of $^{137}$Cs. However, our result is higher by a factor of 3 to 4 compared to the estimate of the release percentage of $^{103}$Ru and $^{106}$Ru given in the report of Gudiksen et al.$^{20}$, which is believed to be similar to the release percentage of $^{99}$Tc$^9$. At this point we emphasize that all estimates of the $^{99}$Tc release of the Chernobyl accident still have a large uncertainty, since data on $^{99}$Tc concentrations in environmental samples are quite limited. Further investigations are required for a precise estimate of the amount of $^{99}$Tc released by the Chernobyl accident.

ACKNOWLEDGMENT

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95Sr


Transfer of technetium from paddy soil to rice seedling

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Tracer experiments on the chemical transformation of technetium in paddy soil and the transfer to rice seedlings have been carried out using $^{99m}$Tc as a tracer. Two common Japanese soils, Andosol and Gray lowland soil were used in the soil incubation experiments. The chemical form of soluble Tc in soil water was a mixture of Tc-organic matter complex, Tc-iron complex and pertechnetate. An uptake experiment with rice seedlings using nutrient solution showed that the Tc-organic matter complex was less available than pertechnetate or the Tc-iron complex. These chemical forms of Tc were also observed in the root bleeding sap of rice seedlings when introduced to the nutrient solution containing soluble Tc. These results suggested that the transfer of technetium from soil to rice would depend on the chemical form of Tc and they would transport from the root to the leaf without chemical transformation.

Introduction

Technetium-99 is one of the important radionuclides for radiation protection because of its long half-life (2.1·$10^5$ y) and relatively high fission yield. In the chemical form of TcO$_4$$^-$. This radionuclide is well dissolved in soil water and easily available to plants. $^1$ The transfer factor of technetium recommended by the IAEA is 5 for food crops. $^2$ This value is higher than other radionuclides such as $^{137}$Cs (0.03) or $^{90}$Sr (0.3). For nuclear safety assessment, it is necessary to obtain information on the behavior of this nuclide in the soil-plant system. In Japan, rice is one of the most important agricultural products and its consumption is very high. Studies on this plant are important for dose assessment through the intake of foodstuffs. Since the transfer factor is expected to vary with different crops, we have performed laboratory experiments on the transfer of technetium from soil to various Japanese crops using $^{95m}$Tc as a tracer. $^{3-5}$ In this, we found the transfer factors for brown rice (hulled rice grain) grown in a flooded condition were markedly lower than those for other cereals grown in a non-flooded condition. It was suggested that TcO$_4$$^-$ added to the soil rapidly transformed to an insoluble form due to the reducing conditions caused by the flooding. The chemical form of Tc fixed with soil is thought to be TcO$_2$ and/or the Tc-humic acid complex as suggested by VAN LOON et al. $^6$ and SEKINE et al. $^7$ VAN LOON et al. found from their incubation experiment that minor quantities of Tc were present in an aqueous extract with distilled water at pH 6. They suggested that the Tc was associated with organic matter dissolved in the water. We found that the chemical form of the soluble Tc in flooded soils was not pertechnetate since it was associated with soluble organic matter. $^8$ The availability of this Tc-organic matter complex to a rice seedling was lower than pertechnetate. However, a more detailed study was necessary to know whether the Tc-organic matter complex is the only chemical form that was generated in the flooded soil. In addition, the chemical transformations of Tc in the plant root are still unknown. Therefore, we have carried out following three experiments. In the first experiment, we determined the precise chemical form of soluble Tc in the flooded soil. In the second one, we examined the influence of the chemical form of Tc on the plant availability, and in the third, determined the chemical form of Tc in the root bleeding sap of a rice seedling.

Experimental

Soil incubation and determination of chemical form of soluble Tc

Andosol and Gray lowland soil were used in the experiments. Andosol was collected in Tokai Village, Ibaraki, and Gray lowland soil was collected in Mito City, Ibaraki. The chemical properties of these soils are listed in Table 1. Both soils were air-dried and sieved to pass a 1 mm mesh. Soil (5 g), deionized water (10 ml) containing 3.7 MBq of $^{95m}$TcO$_4$$^-$, and glucose (10 mg) to enhance microbial activity, were mixed thoroughly and placed in a 50 vial. The soil mixtures were incubated at room temperature (about 20 °C) for 40 days and the soluble Tc was extracted as follows. After mixing thoroughly, the samples were centrifuged at 5000 rpm for 20 minutes. The supernatant was filtered with a 0.4 μm pore diameter filter.

The chemical form of Tc was examined by gel filtration chromatography (Sephadex G-15, column diameter = 1 cm; column length = 30 cm; eluents = 0.01M CaCl$_2$; flow rate = 12/h; injection volume = 2; fraction size = 1). To detect soluble organic matter in the solution, the optical density of the effluent was measured at 254 nm (Tokyo Rikakikai UV-9000).
Table 1. Chemical properties of soil used in the experiments

<table>
<thead>
<tr>
<th></th>
<th>Gray lowland soil</th>
<th>Andosol</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEC, * meq/100 g of dry soil</td>
<td>10.2</td>
<td>16.6</td>
</tr>
<tr>
<td>Total carbon, %</td>
<td>2.4</td>
<td>4.3</td>
</tr>
<tr>
<td>pH</td>
<td>5.7</td>
<td>5.3</td>
</tr>
</tbody>
</table>

* Cation exchange capacity.

Samples of the fractionated effluents were placed in polyethylene vials and $^{95m}$Tc concentration was measured with a scintillation counter (Aloka ARC-300). Decay corrections were made to the beginning of the experiment.

**Determination of availability to rice seedlings**

Since the concentration of soluble $^{95m}$Tc in Andosol was low, the soluble fraction of Gray lowland soil was used in this experiment. Fractions obtained from Gel filtration chromatography, were diluted with nutrient solution (Kasugai solution: $N = 40$ mg/l; $P_2O_5 = 20$ mg/l; $K_2O = 30$ mg/l; $CaO = 4$ mg/l; $MgO = 6$ mg/l) to a volume of 200. As a control, 200 of nutrient solution containing 2 kBq of $^{95m}$TcO$_4^-$ was prepared. These solutions were placed in glass beakers and shaded with black polyethylene film. Rice seedlings (Oryza sativa L. C.V. Kosihikari) were pre-cultivated on a floating mesh (nylon mesh: 2 mm) using nutrient solution in a growth chamber for 20 days, before they were exposed to the nutrient solutions mentioned above. Five plants were used for determination of the availability of Tc for each solution. After a 24 hours exposure, the concentrations of $^{95m}$Tc in the leaves and roots of each rice plant were measured. During the exposure experiments, the temperature in the chamber was maintained at 30°C during the daylight period (14 h) and 25°C during the night. The light intensity at the plant level in the chamber was about 20000 lux. At the harvest, each plant was separated into leaves and roots. Samples of the plant parts (0.5–0.8 g) or the nutrient solutions (2) were placed in polyethylene vials and measured with an automatic well type sodium iodide scintillation counter. Values of the concentration ratio between plant parts and the nutrient solution were calculated as "the activity per unit weight of plant (on a fresh weight basis)" divided by "the activity per unit weight of the nutrient solution".

**Determination of the chemical form of Tc in the root bleeding sap**

Soluble Tc obtained from two kinds of soil incubation (about 5 ml) was diluted with nutrient solution to a volume of 30 ml. These solutions were placed in polyethylene vials and shaded with black polyethylene film. Immediately after the rice seedling was transplanted to the vial, the stem of the seedling was cut near the root and bleeding sap was collected from rice seedling. For the collection, absorbent cotton was placed on the cutting surface of the rice seedling and covered by polyethylene film to prevent evaporation. After 12-hour collection, bleeding sap was extracted from the absorbent cotton with water and analyzed by gel filtration chromatography as mentioned above.

**Results and discussions**

**Determination of chemical form of soluble Tc**

Table 2 shows the distribution of $^{95m}$Tc between the soil solid phase (insoluble form) and the soil solution (soluble form). More than 98% of the $^{95m}$Tc in both soils was distributed in the soil-solid phase.

As we observed in the cultivation experiment with rice, the transformation of TcO$_4^-$ to the insoluble form occurred due to the reducing condition generated by soil micro-organisms. Between the two soils, the concentration of the soluble $^{95m}$Tc in Gray lowland soil was markedly higher than that of Andosol. This tendency was consistent with the results obtained in our previous experiments. The chromatograms of soluble $^{95m}$Tc in the two soils are shown in Fig. 1 (Gray lowland soil) and Fig. 2 (Andosol). In the two soils, three peaks were observed in the elution pattern of $^{95m}$Tc. The first peak corresponded to the peak of the optical density, which represents the elution of soluble organic matter. This result indicates that $^{95m}$Tc eluted in the first peak of both soils were associated with soluble organic matter. The peak of $^{95m}$Tc-organic matter complex and the optical density of Gray lowland soil (Fig. 1) were higher than those of Andosol (Fig. 2). This difference would depend on the amount of soluble organic matter released from each soil to water.

In the second and third peak, there was no distinct difference between the two soils. Concerning the second peak, we assumed that the chemical form of $^{95m}$Tc eluted in this position might be formed by the reaction of Fe$^{2+}$ ion and $^{95m}$TcO$_4^-$. To confirm this assumption, we mixed 10 ml of 2% FeSO$_4$ solution and 1 kBq of $^{95m}$TcO$_4^-$ in a 50 ml vial and analyzed the solution by gel filtration chromatography. The results (Fig. 3) showed that a small amount of the $^{95m}$Tc was eluted in the same position of the second peak. This suggested that the $^{95m}$Tc eluting in the second peak would be associated with iron ion or iron hydroxide.

Table 2. Distributions of $^{95m}$Tc in the soil solid phase and soil solution

<table>
<thead>
<tr>
<th>Phase</th>
<th>Percent of total, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil solid phase (insoluble)</td>
<td>98.3</td>
</tr>
<tr>
<td>Water phase (soluble)</td>
<td>1.7</td>
</tr>
</tbody>
</table>

*Percent of total*
Fig. 1. Chromatogram of soluble $^{99m}$Tc isolated from Gray lowland soil; $^{99m}$Tc; optical density

Fig. 2. Chromatogram of soluble $^{99m}$Tc isolated from Andosol; $^{99m}$Tc; optical density
Table 3. Distribution of $^{95m}$Tc (in Bq) in various chemical forms isolated from the soil solution

<table>
<thead>
<tr>
<th>Chemical form</th>
<th>Gray lowland soil</th>
<th>Andosol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tc-organic matter complex</td>
<td>861</td>
<td>53</td>
</tr>
<tr>
<td>Tc-iron complex</td>
<td>58</td>
<td>76</td>
</tr>
<tr>
<td>Pertechnetate</td>
<td>12</td>
<td>13</td>
</tr>
</tbody>
</table>

Note: 3.7 MBq of $^{95m}$TcO$_4^-$ added in the incubation of two soils.

Since this chemical form of Tc is soluble in water and can filter through the column, it would differ from Tc-iron hydroxide aggregates. Details of this form of Tc remain to be investigated. In this paper, we describe this chemical form as Tc-iron complex for convenience. The third peak was pertechnetate, which was confirmed by the analysis of $^{95m}$TcO$_4^-$.$^7$ The amount of soluble $^{95m}$Tc in each chemical form is shown in Table 3. The amounts of pertechnetate and Tc-iron complex were similar for the two soils. The production of soluble Tc-organic matter complex in the soils might relate to the soil nature, e.g., quantity and quality of soil organic matter.

Availability of soluble Tc to rice seedling

Plant availability of different chemical forms of Tc is shown in Table 4. The concentrations of $^{95m}$Tc in the plant samples were not less than 100 Bq g$^{-1}$. The concentration ratios observed in the leaves of the rice plant were as follows: 2.7 for Tc-organic matter complex, 23 for Tc-iron complex and 43 for pertechnetate. The concentration ratio observed in Tc-organic matter complex was markedly lower than that for other two chemical forms of Tc, suggesting that Tc-organic matter complex is less available than Tc-iron complex or pertechnetate. For the root, the concentration ratio was opposite to that of the leaves: 15 for Tc-organic matter complex, 9.4 for Tc-iron complex and 5.9 for pertechnetate. The higher concentration ratio of the Tc-organic matter complex observed at the root of the rice seedling can be explained by its lower efficiency of transportation from root to leaf than those of other form of Tc.

Chemical form of Tc in the root bleeding sap

Chromatograms of the root bleeding sap collected from rice seedling transplanted in the nutrient solution containing the soluble fraction of $^{95m}$Tc isolated from Gray lowland soil and Andosol are shown in Figs 4 and 5. Compared with the soluble Tc (Figs 1 and 2), the chemical forms of Tc does not seem to be changed.

Table 4. Concentration ratios for rice plant seedling and nutrient solution after a 24 hour exposure

<table>
<thead>
<tr>
<th>Chemical form</th>
<th>Leaves*</th>
<th>Root*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tc-organic matter complex</td>
<td>2.7 ± 0.2</td>
<td>14 ± 0.6</td>
</tr>
<tr>
<td>Tc-iron complex</td>
<td>23 ± 1.3</td>
<td>9.4 ± 0.5</td>
</tr>
<tr>
<td>Pertechnetate</td>
<td>43 ± 2.9</td>
<td>5.9 ± 0.4</td>
</tr>
</tbody>
</table>

* ± Standard deviation for 5 samples.

Table 5. Concentration ratios of each chemical form of Tc between root bleeding sap and nutrient solution containing soluble technetium from two soils

<table>
<thead>
<tr>
<th>Chemical form</th>
<th>Andosol</th>
<th>Gray lowland soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tc-organic matter complex</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Tc-iron complex</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Pertechnetate</td>
<td>8.0</td>
<td>5.2</td>
</tr>
</tbody>
</table>
The concentration ratios between the root bleeding sap and nutrient solutions in each chemical form are shown in Table 5. The concentration ratio of the pertechnetate was higher than that of Tc-organic matter complex and Tc-iron complex. This suggested that the uptake and transport of $\text{TcO}_4^-$ was more efficient than the other two chemical forms and that the chemical transformation of Tc into other chemical forms would not occur in the root. Technetium absorbed at the root is supposed to transport to the leaves without chemical transformation.

**Conclusions**

Most of the $^{95m}\text{Tc}$ added as pertechnetate to flooded soil was transformed into an insoluble form due to the reducing condition generated by soil micro-organisms and subsequently it was fixed in the soil. Although the concentration was very low, a soluble form of $^{95m}\text{Tc}$ was present in the soil solution. The concentrations of the soluble $^{95m}\text{Tc}$ in Gray lowland soil were higher than in Andosol. From the gel filtration chromatographic
determination, three chemical forms of the $^{95m}$Tc were found in the soluble fraction: Tc-organic matter complex, Tc-iron complex and pertechnetate. An uptake experiment with rice seedlings using nutrient solution showed that the Tc-organic matter complex was less available than pertechnetate or the Tc-iron complex. Chromatograms of the root bleeding sap collected from rice seedlings showed that the chemical form of $^{95m}$Tc was not different from the nutrient solution containing soluble $^{95m}$Tc from the two soils. These results suggested that the transfer of technetium from soil to rice depends on the chemical form in the soil solution and there was no chemical transformation in the transfer process.

References

Concentrations of Alkali and Alkaline Earth Elements in Mushrooms and Plants Collected in a Japanese Pine Forest, and Their Relationship with $^{137}\text{Cs}$

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ABSTRACT

$^{137}\text{Cs}$ and stable Na, K, Rb, Cs, Mg, Ca, Sr, Ba and Al were determined in 29 mushrooms and 8 plants collected from a Japanese pine forest. Concentrations of $^{137}\text{Cs}$, Cs and Rb in mushrooms were one order of magnitude higher than those in plants growing in the same forest. On the other hand, concentrations of Ca and Sr in mushrooms were obviously lower than those in plants. A good correlation ($r = 0.99, p < 0.01$) between $^{137}\text{Cs}$ and stable Cs concentrations was observed for mushrooms. The $^{137}\text{Cs}/\text{Cs}$ ratios were almost constant ($^{137}\text{Cs}/\text{Cs} = 134 \pm 36 \text{ Bq mg}^{-1}$ in 1990) and were significantly higher than that in the surface soil ($27 \text{ Bq mg}^{-1}$). The ratios for plants were almost the same as those for mushrooms. In plant samples, good correlations were observed among the concentrations of K, Rb, Cs and Mg. Correlation between those of Ca and Sr was also good. In contrast, Cs was not correlated with K in mushrooms, indicating that the mechanism of Cs uptake was different from that for K. Rb showed a correlation with Cs ($r = 0.82$).

INTRODUCTION

Radiocesium discharged to the environment through nuclear weapons testing and nuclear accidents is accumulated in forest ecosystems, mainly due to the wide surface areas of tree canopies and the ability of forest soils to hold radiocesium. Even 10 years after the Chernobyl accident, radiocesium contamination of forest products is high in contrast to agricultural products. Since removal of radiocesium from a contaminated forest is difficult,
studies on the distribution and transfer of radiocesium in forest ecosystems are important in order to predict the future contamination of forest product. Many studies were carried out before and after the Chernobyl accident (e.g. Waller and Olsen, 1967; Yamagata et al., 1969; Heinrich et al., 1989; Schimmack et al., 1993; Wirth et al., 1994). Accumulation and migration of radiocesium in the soil profiles have been discussed and many studies indicate that radiocesium deposited in the forests is accumulating at the surface of the forest soils (e.g. Thiry and Myttenaere, 1993; Yoshida and Muramatsu, 1994a; Bunzl et al., 1995).

Concentrations of radiocesium in biological samples were also measured. A great deal of effort has gone into the measurement of radiocesium in mushrooms, because mushrooms accumulate radiocesium. High concentrations of radiocesium in mushrooms were reported in European forests (e.g. Haselwandter et al., 1988; Baldini et al., 1989; Römmelt et al., 1990) and also in Japanese forests (Muramatsu et al., 1991; Yoshida and Muramatsu, 1994a, b; Yoshida et al., 1994; Sugiyama et al., 1994). Many factors controlling the radiocesium concentration in mushrooms, e.g. type of forest (Heinrich, 1992), soil pH (Eckl et al., 1986; Sugiyama et al., 1994) and species of mushrooms (Fraiture et al., 1990; Dighton et al., 1991), were discussed. The relationship between the habitat of the mycelium and the radiocesium concentration in the fruit body has also been studied (Giovani et al., 1990; Guillitte et al., 1990; Yoshida and Muramatsu, 1994a; Yoshida et al., 1994; Rühm et al., 1997). Recent studies (Olsen et al., 1990; Guillitte et al., 1994; Brückman and Wolters, 1994) indicated the importance of mycelium on the accumulation of radiocesium in surface soils. Many of them demonstrated that more than 30% of $^{137}$Cs existing in the surface soil is accumulated in the mycelia of fungi.

These experimental measurements in forest ecosystems have provided information for the development of models which explain radiocesium transport through several compartments in a forest ecosystem. Some different types of models were developed as summarized by Schell et al. (1994, 1996). However, the prediction of radiocesium behaviour in forest ecosystems is still difficult.

Analytical data of stable elements must be useful to understand the long-term behaviour of radionuclides. Chemical behaviour of radiocesium is expected to be similar to that of stable Cs and the other alkali elements. Myttenaere et al. (1993) summarized the relationship between radiocesium and K in forests, and suggested the possible use of K behaviour for the prediction of radiocesium behaviour.

There are many studies on the behaviour of major nutrient elements such as K, Mg and Ca in forests (e.g. Eaton et al., 1973; Likens et al., 1977) because these elements are directly related to the forest growth. Effect of
Concentrations of alkali and alkaline earth elements

acid deposition on the behaviour of these elements was also studied in the last two decades (e.g. Mayer and Ulrich, 1977; Yoshida and Ichikuni, 1989; Liechty et al., 1993). However, information on the relationship between radionuclides and the major nutrient elements in forest ecosystems is still limited. In addition, analytical data about the nutrient elements in wild mushrooms are very much limited, although some studies such as Tyler (1980) are available. The data for trace alkali and alkaline earth elements such as Rb, Cs, Sr and Ba in forest ecosystems are limited because of the lack of simple analytical methods. Recently, inductively coupled plasma-mass spectrometry (ICP-MS) has been used for accurate and precise determination of trace elements in environmental samples for more than 50 elements including Rb, Cs, Sr and Ba (Casetta et al., 1990; Yamasaki and Tamura, 1990; Alaimo and Censi, 1992; Schönberg, 1993; Yoshida et al., 1996).

In the previous papers (Yoshida and Muramatsu, 1994a, b; Yoshida et al., 1994), the authors studied the behaviour of radiocesium in a Japanese pine forest on sandy soil near the coast in Tokai-mura, Ibaraki Prefecture. Radiocesium in plants, mushrooms and soils were determined and accumulation of it in mushrooms was discussed. However, data for stable elements, which can be discussed with the radiocesium concentrations are limited, except for some plant and soil samples (Yoshida and Muramatsu, 1997).

In this paper, the stable alkali (Na, K, Rb and Cs), alkaline earth (Ca, Sr and Ba) elements and Mg in mushrooms collected in the pine forest were determined by ICP-MS or inductively coupled plasma-atomic emission spectrometry (ICP-AES), and the data of stable elements for mushrooms, plants and soils were summarized together with those of $^{137}$Cs. Al was also measured to check the contamination due to soil particles adhering on the surface of the samples. Concentration ratio between mushroom or plant and surface soil was estimated for each stable element and $^{137}$Cs. Relationships among the concentrations of $^{137}$Cs and the above-mentioned stable elements were discussed.

MATERIALS AND METHODS

Sampling of mushrooms and plants

Samples were collected from a pine forest where the behaviour of radiocesium has been studied by the authors (Yoshida and Muramatsu, 1994a, b; Yoshida et al., 1994). The forest is situated in lat. 36° 26' N. and long. 140° 36' E. on sandy soil near the coast in Tokai-mura, Ibaraki Prefecture. Mushroom samples were collected in 1989–1991 and plant
samples (leaves for trees and leaves and stems for shrubs and grasses) were collected in November 1990. The samples were cleaned by using a brush or cellulose wiper, gently removing attached soil and humus. They were not rinsed with water to avoid possible loss of a part of the elements by leaching. After cleaning, samples were freeze-dried and pulverized with a cooking blender. Soils from different depths were also sampled in the forest. The organic layer of the soil was thin in this forest. Therefore, after removing the litter layer, the soil was separately sampled at four depths (0–2, 2–5, 5–10 cm and deeper). The organic layers except litter layer were included in the first 0–2 cm. Analyses of the soils were described in Yoshida et al. (1994) and Yoshida and Muramatsu (1997). The soils were sampled at one place in the forest. Therefore, the analytical data for soils might be less representative than those for mushrooms and plants.

**Determination of $^{137}$Cs**

Each dried sample (usually 20–40 g) was placed in a plastic bottle (diameter: 50 mm) and concentration of $^{137}$Cs was determined by counting with a Ge-detector for 40 000–80 000 s. The decay correction was made as to October 1990. The approximate detection limit for $^{137}$Cs by the counting system for a 30 g dry sample was 3 Bq kg$^{-1}$. The results of the radioesium determination were already reported in the previous papers (Muramatsu et al., 1991; Yoshida et al., 1994; Yoshida and Muramatsu, 1994a, b). The concentrations of $^{137}$Cs were under the detection limit in some mushroom samples. One of the most important objectives of the present study is to get information about the relationship between radioesium and stable elements in mushrooms. Therefore, the 29 mushrooms in which $^{137}$Cs was detected were selected and analyzed for stable elements. Samples used in the present study are listed in Table 1. They were mycorrhizal or saprophytic fungi growing on the soil. Saprophytic fungi growing on dead trees were not included. The concentrations of $^{137}$Cs in this type of mushrooms were below the detection limits because of relatively low concentrations of $^{137}$Cs in their substrata (dead trees).

**Determination of stable elements**

A mushroom sample (0.4 g) was digested in Teflon™ PFA pressure decomposition vessel with HNO$_3$, HF and HClO$_4$. After leaving the vessels on a hot plate (80°C) overnight for pre-decomposition, the samples were heated in a microwave digester (CEM, MDS-2000) for 1–2 h. After digestion, the samples were evaporated to dryness. Then, the residues were dissolved in 1–2% HNO$_3$. 

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### Concentrations of alkali and alkaline earth elements

**TABLE 1**
List of Samples Collected from a Pine Forest in Tokai-mura, Ibaraki

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Species</th>
<th>Sample type</th>
<th>Sampling date</th>
</tr>
</thead>
<tbody>
<tr>
<td>MR-075</td>
<td><em>Russula mariae</em></td>
<td>M</td>
<td>1 July 1990</td>
</tr>
<tr>
<td>MR-089</td>
<td><em>Astraeus hygrometricus</em></td>
<td>M/S</td>
<td>13 July 1990</td>
</tr>
<tr>
<td>MR-093</td>
<td><em>Russula mariae</em></td>
<td>M</td>
<td>18 July 1990</td>
</tr>
<tr>
<td>MR-097</td>
<td><em>Russula mariae</em></td>
<td>M</td>
<td>18 July 1990</td>
</tr>
<tr>
<td>MR-146</td>
<td><em>Suillus luteus</em></td>
<td>M</td>
<td>9 Oct. 1990</td>
</tr>
<tr>
<td>MR-149</td>
<td><em>Armillariella tabescens</em></td>
<td>S</td>
<td>9 Oct. 1990</td>
</tr>
<tr>
<td>MR-152</td>
<td><em>Amanita pantherina</em></td>
<td>M</td>
<td>9 Oct. 1990</td>
</tr>
<tr>
<td>MR-175</td>
<td><em>Suillus granulatus</em></td>
<td>M</td>
<td>Oct. 1990</td>
</tr>
<tr>
<td>MR-185</td>
<td><em>Gymnopilus aeruginosus</em></td>
<td>S</td>
<td>18 Oct. 1990</td>
</tr>
<tr>
<td>MR-211</td>
<td><em>Tricholoma flavovirens</em></td>
<td>M</td>
<td>6 Nov. 1990</td>
</tr>
</tbody>
</table>

**Plants**

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Species</th>
<th>Sample type</th>
<th>Sampling date</th>
</tr>
</thead>
<tbody>
<tr>
<td>TL-2a</td>
<td><em>Pinus thunbergii</em></td>
<td>TL</td>
<td>6 Nov. 1990</td>
</tr>
<tr>
<td>TL-2b</td>
<td><em>Pinus thunbergii</em></td>
<td>TL</td>
<td>6 Nov. 1990</td>
</tr>
<tr>
<td>TL-4</td>
<td><em>Morus bombycis</em></td>
<td>TL</td>
<td>6 Nov. 1990</td>
</tr>
<tr>
<td>GR-5</td>
<td><em>Indigofera pseudo-tintoria</em></td>
<td>GR</td>
<td>6 Nov. 1990</td>
</tr>
<tr>
<td>GR-6</td>
<td><em>Vitex rotundifolia</em></td>
<td>GR</td>
<td>6 Nov. 1990</td>
</tr>
<tr>
<td>GR-8</td>
<td><em>Oenothera lamarckiana</em></td>
<td>GR</td>
<td>6 Nov. 1990</td>
</tr>
<tr>
<td>GR-9</td>
<td><em>Miscanthus sinensis</em></td>
<td>GR</td>
<td>6 Nov. 1990</td>
</tr>
<tr>
<td>GR-10</td>
<td><em>Ophiopogon japonicus</em></td>
<td>GR</td>
<td>6 Nov. 1990</td>
</tr>
</tbody>
</table>

---

a Sample type: (M) mycorrhizal fungi, (S) saprophytic fungi, (M/S) unknown fungi, (TL) tree leaves, (GR) leaves and stems of shrubs and grasses.

b Current needles.

c 1 and 2 year old needles.
Trace elements (Rb, Cs, Sr and Ba) were measured by ICP-MS (Yokogawa Analytical Systems, PMS-2000). Rh was used for internal standard to compensate for changes in analytical signals during the operation. Major elements, Na, K, Mg, Ca and Al were determined by ICP-AES (Seiko Instruments, SPS7700). Standard solutions were prepared from SPEX Multi-Element Plasma Standards (SPEX Industries Inc.) and used to get calibration curves. Tomato Leaves (1573a) and Orchard Leaves (1571) supplied by the National Institute of Standards and Technology were used to validate the analytical procedure. Details for the analyses have been described by Yoshida and Muramatsu (1997).

RESULTS AND DISCUSSION

Validation of analytical procedure

Good agreements between the certified and measured values were observed for standard reference materials (see Table 2). In the case of Tomato Leaves, errors of measured values were less than 10% of the certified values for Na, K, Ca, Rb and Cs, and 10–20% for Mg, Al, Sr and Ba. Precisions calculated using 6 independent runs of Tomato Leaves were better than 5% relative

<table>
<thead>
<tr>
<th>Elements</th>
<th>Observed values(^a) (mg kg(^{-1}))</th>
<th>RSD (%)</th>
<th>Certified values (mg kg(^{-1}))</th>
<th>Obs./Cer.</th>
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</thead>
<tbody>
<tr>
<td>ICP-AES</td>
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<tr>
<td>Na</td>
<td>130</td>
<td>3.0</td>
<td>136</td>
<td>0.96</td>
</tr>
<tr>
<td>Mg</td>
<td>10700</td>
<td>3.8</td>
<td>12000(^b)</td>
<td>0.89</td>
</tr>
<tr>
<td>Al</td>
<td>515</td>
<td>4.5</td>
<td>598</td>
<td>0.86</td>
</tr>
<tr>
<td>K</td>
<td>25900</td>
<td>4.7</td>
<td>27000</td>
<td>0.96</td>
</tr>
<tr>
<td>Ca</td>
<td>49200</td>
<td>6.0</td>
<td>50500</td>
<td>0.97</td>
</tr>
<tr>
<td>ICP-MS</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rb</td>
<td>14.8</td>
<td>7.1</td>
<td>14.89</td>
<td>0.99</td>
</tr>
<tr>
<td>Sr</td>
<td>67.9</td>
<td>5.1</td>
<td>85(^b)</td>
<td>0.80</td>
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<tr>
<td>Cs</td>
<td>0.053</td>
<td>14</td>
<td>0.053</td>
<td>1.00</td>
</tr>
<tr>
<td>Ba</td>
<td>51.7</td>
<td>5.8</td>
<td>63(^b)</td>
<td>0.82</td>
</tr>
</tbody>
</table>

\(^a\)Mean values of six independent runs.

\(^b\)Not certified but provided as an additional information value.
standard deviation (RSD) for Na, Mg, Al and K, 5–10% RSD for Ca, Rb, Sr and Ba, and 14% RSD for Cs.

Concentrations of $^{137}\text{Cs}$ and stable elements in mushrooms and plants

Analytical results of alkali and alkaline earth elements in 29 mushrooms collected from a pine forest are shown in Table 3 together with the data for plants and soils collected in the same forest (Yoshida and Muramatsu, 1997). The data for $^{137}\text{Cs}$ (Muramatsu et al., 1991; Yoshida et al., 1994; Yoshida and Muramatsu, 1994a, b) are also listed in the table. The mean and median concentrations are summarized in Table 4. The median values seemed to be more representative than the mean values for $^{137}\text{Cs}$ and most stable elements in mushrooms and plants, because of the high variations of their concentrations.

The concentrations of $^{137}\text{Cs}$ in mushrooms varied very widely, ranging from 5.4 to 3110 Bq kg$^{-1}$ (dry wt). The median value for all the mushroom species was 135 Bq kg$^{-1}$ (dry wt). In comparison with the median concentration in 124 species of mushrooms collected from all over Japan of 53 Bq kg$^{-1}$ (dry wt), reported by Yoshida and Muramatsu (1994b), the present result is more than two times higher, because mushrooms with relatively high concentration of $^{137}\text{Cs}$ were used in the present study as mentioned before. In comparison with the values reported in Europe after the Chernobyl accident (e.g., Haselwandter et al., 1988; Baldini et al., 1989; Römmelt et al., 1990), the present results are generally 1–2 orders of magnitude lower.

The highest median concentration in mushrooms was found for K followed by Mg, Na, Ca, Rb, Ba, Sr and Cs. For plants (leaves and stems), the highest median value was found for Ca followed by K, Mg, Na, Sr, Ba, Rb and Cs. In comparison with the element composition of plants, the mushroom composition can be characterized by high Rb and Cs concentrations and low Ca and Sr concentrations. Tyler (1980) analyzed 200 mushrooms belonging to 130 species, which were rinsed with water, for several elements including Na, K, Rb, Mg, Ca and Al. Takahashi (1974) summarized the concentrations of many elements in plants (angiosperms). These values are listed in Table 4 for comparison. The present results agree with these previous studies, except for Na and Al in mushrooms. Higher Al concentrations in mushrooms observed in the present study might be attributable to the effect of soils adhering on the surface of mushrooms as mentioned below. Seeger and Schweinshaut (1981) determined stable Cs in 433 mushroom species and obtained the mean value, 7 mg kg$^{-1}$ (dry wt). The present result of stable Cs in mushrooms is comparable with this previous study.
### TABLE 3
Concentration of Alkali and Alkaline Earth Elements and $^{137}$Cs in Mushrooms, Plants and Soils Collected in a Pine Forest in Tokai, Ibaraki

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Dry/wet ratio</th>
<th>$^{137}$Cs (Bq kg$^{-1}$, dry wt)</th>
<th>Na</th>
<th>K</th>
<th>Rb</th>
<th>Cs</th>
<th>Mg</th>
<th>Ca</th>
<th>Sr</th>
<th>Ba</th>
<th>Al</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(mg kg$^{-1}$, dry wt)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Mushrooms</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MR-006</td>
<td>0.149</td>
<td>42.4</td>
<td>1630</td>
<td>35500</td>
<td>75.4</td>
<td>0.304</td>
<td>863</td>
<td>201</td>
<td>1.74</td>
<td>2.03</td>
<td>228</td>
</tr>
<tr>
<td>MR-007</td>
<td>0.139</td>
<td>449</td>
<td>319</td>
<td>30300</td>
<td>115</td>
<td>2.32</td>
<td>1100</td>
<td>199</td>
<td>2.05</td>
<td>5.12</td>
<td>415</td>
</tr>
<tr>
<td>MR-101</td>
<td>0.108</td>
<td>179</td>
<td>1000</td>
<td>33800</td>
<td>88.2</td>
<td>1.02</td>
<td>1200</td>
<td>174</td>
<td>1.89</td>
<td>3.31</td>
<td>352</td>
</tr>
<tr>
<td>MR-023</td>
<td>0.096</td>
<td>182</td>
<td>208</td>
<td>26800</td>
<td>110</td>
<td>2.55</td>
<td>1340</td>
<td>450</td>
<td>2.22</td>
<td>2.86</td>
<td>332</td>
</tr>
<tr>
<td>MR-024</td>
<td>0.085</td>
<td>230</td>
<td>191</td>
<td>23000</td>
<td>105</td>
<td>2.19</td>
<td>1050</td>
<td>124</td>
<td>0.93</td>
<td>1.48</td>
<td>169</td>
</tr>
<tr>
<td>MR-075</td>
<td>0.224</td>
<td>28.7</td>
<td>1200</td>
<td>28600</td>
<td>56.5</td>
<td>0.212</td>
<td>777</td>
<td>356</td>
<td>2.88</td>
<td>3.13</td>
<td>347</td>
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<tr>
<td>MR-089</td>
<td>0.202</td>
<td>8.2</td>
<td>1290</td>
<td>25000</td>
<td>31.0</td>
<td>0.163</td>
<td>1410</td>
<td>1910</td>
<td>17.3</td>
<td>19.3</td>
<td>2640</td>
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<tr>
<td>MR-093</td>
<td>0.121</td>
<td>36.0</td>
<td>1430</td>
<td>34200</td>
<td>47.7</td>
<td>0.351</td>
<td>963</td>
<td>203</td>
<td>2.27</td>
<td>3.36</td>
<td>377</td>
</tr>
<tr>
<td>MR-097</td>
<td>0.120</td>
<td>34.0</td>
<td>1520</td>
<td>32100</td>
<td>49.8</td>
<td>0.285</td>
<td>874</td>
<td>202</td>
<td>2.68</td>
<td>6.75</td>
<td>454</td>
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<tr>
<td>MR-128</td>
<td>0.211</td>
<td>5.4</td>
<td>1090</td>
<td>27100</td>
<td>59.8</td>
<td>0.044</td>
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<td>432</td>
<td>4.61</td>
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<tr>
<td>MR-132</td>
<td>0.063</td>
<td>55.6</td>
<td>614</td>
<td>40900</td>
<td>93.1</td>
<td>0.364</td>
<td>976</td>
<td>469</td>
<td>4.80</td>
<td>7.96</td>
<td>1040</td>
</tr>
<tr>
<td>MR-133</td>
<td>0.078</td>
<td>127</td>
<td>1780</td>
<td>33800</td>
<td>85.4</td>
<td>1.01</td>
<td>1100</td>
<td>870</td>
<td>11.7</td>
<td>28.1</td>
<td>3040</td>
</tr>
<tr>
<td>MR-145</td>
<td>0.052</td>
<td>65.2</td>
<td>1820</td>
<td>38400</td>
<td>87.6</td>
<td>0.552</td>
<td>1070</td>
<td>1010</td>
<td>13.2</td>
<td>26.0</td>
<td>3190</td>
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<tr>
<td>MR-146</td>
<td>0.051</td>
<td>317</td>
<td>1030</td>
<td>30800</td>
<td>137</td>
<td>2.75</td>
<td>976</td>
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<td>7.68</td>
<td>22.4</td>
<td>1790</td>
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<tr>
<td>MR-147</td>
<td>0.062</td>
<td>675</td>
<td>809</td>
<td>27200</td>
<td>133</td>
<td>3.79</td>
<td>948</td>
<td>368</td>
<td>4.79</td>
<td>9.63</td>
<td>1030</td>
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<td>MR-149</td>
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<td>1410</td>
<td>18800</td>
<td>29.5</td>
<td>0.347</td>
<td>1080</td>
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<td>3.69</td>
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<td>568</td>
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<td>0.080</td>
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<td>165</td>
<td>27400</td>
<td>76.5</td>
<td>0.328</td>
<td>683</td>
<td>81</td>
<td>0.93</td>
<td>1.77</td>
<td>185</td>
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<tr>
<td>MR-152</td>
<td>0.083</td>
<td>27.4</td>
<td>197</td>
<td>26800</td>
<td>73.9</td>
<td>0.239</td>
<td>721</td>
<td>254</td>
<td>2.45</td>
<td>3.90</td>
<td>556</td>
</tr>
<tr>
<td>MR-171</td>
<td>0.055</td>
<td>1150</td>
<td>557</td>
<td>25200</td>
<td>159</td>
<td>6.52</td>
<td>934</td>
<td>99</td>
<td>0.77</td>
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<td>102</td>
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<td>MR-175</td>
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<td>246</td>
<td>75</td>
<td>25400</td>
<td>98.4</td>
<td>2.56</td>
<td>876</td>
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<td>0.50</td>
<td>0.39</td>
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<td>MR-180</td>
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<td>356</td>
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<td>MR-211</td>
<td>MR-274</td>
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*Soils*

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<td>Mean and Median Concentration of Alkali and Alkaline Earth Elements and $^{137}$Cs in Mushrooms, Plants and Soils</td>
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<tr>
<td>$^{137}$Cs (Bq kg$^{-1}$, dry wt)</td>
<td>Na</td>
<td>K</td>
<td>Rb</td>
</tr>
<tr>
<td>(mg kg$^{-1}$, dry wt)</td>
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<td></td>
<td></td>
</tr>
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<td>321</td>
<td>957</td>
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<tr>
<td></td>
<td>Median</td>
<td>135</td>
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<td>Tyler (1980)</td>
<td>(100)*</td>
<td>32000</td>
<td>(140)*</td>
</tr>
<tr>
<td><strong>Plants (n = 8)</strong></td>
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<td>11300</td>
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<td></td>
<td>Median</td>
<td>802</td>
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<td>Takahashi (1974)</td>
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<td><strong>Soils (0–5 cm)</strong></td>
<td>Mean</td>
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*a* Values in parentheses are not given in the paper but estimated from the figures.

*b* In the calculation of the mean and median values of $^{137}$Cs, values described as 'detection limit' were also included.
Concentrations of alkali and alkaline earth elements

In the case of wild mushrooms, surfaces were often contaminated by nearby soils, and removing all particles was quite difficult. Therefore, the analytical results of some specific elements might be affected by the adhering soils. Al, which is one of the major elements in soils, is not accumulated in biological samples except certain Al accumulating plants such as Thea sinensis and Hydrangea macrophylla. Worldwide average concentrations of Al in soils and plants are 71000 mg kg⁻¹ (dry wt) and 550 mg kg⁻¹ (dry wt), respectively (Takahashi, 1974). Al is often used as the indicator of the contribution of soils to the analytical data of environmental samples. Figure 1a and b summarize the binary plots of Al and alkali and alkaline earth elements including Mg for mushrooms and plants. The ratio of each element to Al in surface (0–5 cm) soils were calculated from the data in Table 4 and shown in the figures as the solid lines. For Ca, Sr and Ba, plots for mushrooms were distributed near the solid lines although the ratios in mushrooms were slightly higher than those in surface soil, indicating that the concentration of these elements in mushrooms was strongly affected by soils adhering on the surface of the samples. Concentration of Na in mushrooms with high Al concentration (>1000 mg kg⁻¹) might also be affected by the soil, because the plots were closer to the line in these samples. For the other elements, the plots for mushrooms were clearly far from the lines, indicating that the effect of adhering soil was negligible. In the case of plants, the plots were far from the lines for all measured elements, showing that the effect of adhering soil is negligible for these elements.

Plant surfaces can act as a filter for aerosols because of their wide surface area (e.g. Aylor, 1975; Lindberg and Lovett, 1985; Yoshida and Ichikuni, 1989). Therefore, the concentration of the elements in plant materials can be influenced by particulate materials deposited on the surface. The effect of soil particles is negligible for plants as already mentioned above. The effect of sea spray is also negligible, because there is no correlation between Na and the other elements as shown later in Table 6a and b. There is no other local source for the alkali and alkaline earth elements around the forest. Therefore, the contribution of the aerial particulate materials is negligible in the present study.

In order to compare the concentrations in mushrooms or plants with those in soil, the concentration ratio defined as ‘median concentration in mushrooms or plants on a dry weight basis’ divided by ‘concentration in the surface 0–5 cm soil on a dry weight basis’ was calculated for each element and ¹³⁷Cs as shown in Table 5. The concentration ratios of higher than 1 were observed for ¹³⁷Cs, K and Rb in mushrooms and for Ca in plants. The ratios between 0·1 and 1 were observed for Cs and Mg in mushrooms and ¹³⁷Cs, K, Rb, Mg and Sr in plants. The ratios for the others were lower than 0·1.
Fig. 1(a). Binary plots of Al and alkali elements for mushrooms (●) and plants (□) collected in a pine forest. Solid line indicates the ratio of each element to Al in surface 0–5 cm soil.
Fig. 1(b). Binary plots of Al and alkaline earth elements for mushrooms (○) and plants (□) collected in a pine forest. Solid line indicates the ratio of each element to Al in surface 0–5 cm soil.
TABLE 5
Concentration Ratio of Alkali and Alkaline Earth Elements and $^{137}$Cs for Mushrooms and Plants

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<th>K</th>
<th>Rb</th>
<th>Cs</th>
<th>Mg</th>
<th>Ca</th>
<th>Sr</th>
<th>Ba</th>
<th>Al</th>
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<tbody>
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<td>Mushrooms</td>
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<td>0.03</td>
<td>0.02</td>
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<td>1.40</td>
<td>0.60</td>
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The concentration ratios of $^{137}$Cs, Cs and Rb for mushrooms were one order of magnitude higher than those for plants growing in the same forest. High concentrations of $^{137}$Cs discharged through nuclear weapons testing and nuclear accidents were reported for mushrooms in many countries as described in the introduction. The high concentration ratio for stable Cs obtained in this study indicates that mushrooms are important Cs accumulators and $^{137}$Cs is taken up from soils together with stable Cs. Accumulations of Cs and Rb in mushrooms were also observed by Ban-nai et al. (1994, 1997) from cultivation experiments in flasks using radiotracers. High transfer of Rb from substrata to mushrooms was also reported by Tyler (1982). The concentration ratios of $^{137}$Cs were different from those of stable Cs for both plants and mushrooms. The surface 0–5 cm soil is a mixture of organic materials and minerals (sand). Stable Cs is originally contained in the mineral components and this stable Cs is difficult for plant and mushrooms to take up. Therefore, the concentration ratio for stable Cs estimated by using 0–5 cm soil layer was lower than that for $^{137}$Cs. Oughton et al. (1992) applied a sequential extraction procedure to study the speciation of Chernobyl-derived radionuclides ($^{137}$Cs and $^{90}$Sr), stable Cs and Sr in contaminated 0–4 cm soils and found that higher fraction of $^{137}$Cs was extractable into the exchangeable fraction compared with stable Cs.

The low concentration ratios for Ca, Sr and Ba observed in mushrooms suggest no appreciable accumulation of these elements in mushrooms. As demonstrated in Fig. 1b, most part of these elements were derived from soils adhering on the surface of the samples. This result was consistent with the low concentrations of $^{90}$Sr in mushrooms after the Chernobyl accident reported by Mascanzoni (1990). The activity ratio of $^{90}$Sr to $^{137}$Cs in mushrooms given by Mascanzoni ranged in 0.001–0.021%, while the ratio in fallout from the accident was about 1%. Lower concentration ratios for Sr in mushrooms than those in plant were also observed by radiotracer experiments in a laboratory (Ban-nai et al., 1994). Tyler (1982) reported the low transfer of Ca from substrata to mushrooms.

Concentrations of major nutrient elements in plants can fluctuate due to many factors such as differences of sampling season and age of plants. As
Concentrations of alkali and alkaline earth elements

shown in Table 3, concentrations of K, Rb and Cs in young pine needles (TL-2a) were higher than those in older needles (TL-2b), while concentrations of Ca, Sr and Ba were higher in older needles. The higher concentration of K and the lower concentration of Ca in younger leaves and needles than older ones were also reported in Yoshida and Ichikuni (1988). K migrates easily in the plant body and tends to be translocated from old tissue to new tissue (Bukovac and Witter, 1957; Johnson et al., 1982). Ca exists in an insoluble form such as calcium oxalate and does not migrate easily in the plant body (Biddulph et al., 1959). The higher concentration of $^{90}$Sr in mugwort (Artemisia vulgaris) in autumn than in spring was observed by Fujinami et al. (1996). In the present study, plants were sampled only in autumn. Further studies may be required to discuss the concentrations of elements in plants sampled in other seasons. In the case of mushrooms used in the present study, they grow quickly and are decomposed within 1 or 2 weeks. Therefore, the effect of age is probably much smaller.

Relationship among $^{137}$Cs and alkali and alkaline earth elements

Correlation coefficients among alkaline and alkaline earth elements and $^{137}$Cs were calculated for mushrooms and plants (Table 6a and b).

As shown in Fig. 2, a good correlation ($r = 0.99$, significance level: $p < 0.01$) between $^{137}$Cs and stable Cs was observed for mushroom samples, although the species of mushrooms differed. The $^{137}$Cs/Cs ratios were almost constant. The mean value for autumn in 1990 was $134 \pm 36$ Bq mg$^{-1}$. The ratios for 2 plant samples, in which $^{137}$Cs was detected, were almost the same as those for mushrooms. The similar ratio, 166 Bq mg$^{-1}$, was observed also in the litter layer of the forest floor. These findings suggest that

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TABLE 6b
Correlation Coefficients among Alkali and Alkaline Earth Elements in Plants

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<td>-0.19</td>
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Fig. 2. Relationship between Cs and $^{137}$Cs in mushrooms (●) and plants (□) collected in a pine forest. Solid line indicates the mean value of the $^{137}$Cs/Cs ratios for mushrooms ($^{137}$Cs/Cs = 134 ± 36 Bq mg$^{-1}$ in 1990).

The $^{137}$Cs/Cs ratios are almost constant in the biological samples in this pine forest. Mushrooms and plants can take up the available part of total $^{137}$Cs and total stable Cs in soil. The $^{137}$Cs/Cs ratio in surface 0–5 cm soils in the forest was calculated to be 27 Bq mg$^{-1}$ by using the data in Table 4. This value is clearly smaller than that for mushrooms. This is due to the fact that stable Cs is originally contained in the mineral components of the sandy soil and this stable Cs is difficult for plants and mushrooms to take up as mentioned before in the discussion of concentration ratio. Yoshida and Muramatsu (1994b) calculated the proportions of $^{137}$Cs originating
from the Chernobyl accident in Japanese mushrooms by using $^{134}\text{Cs}/^{137}\text{Cs}$ ratio, and demonstrated that $^{137}\text{Cs}$ in Japanese mushrooms originates mainly from the fallout of nuclear weapons testing, particularly in the 1960s. These findings suggest that $^{137}\text{Cs}$, mainly deposited to the forest ecosystem in the 1960s, has become equilibrated with stable Cs biologically, and available Cs for mushrooms and plants is recycling in the pine forest with the constant $^{137}\text{Cs}/\text{Cs}$ ratio. The $^{137}\text{Cs}/\text{Cs}$ ratio might be a useful criterion for judging the equilibrium of deposited $^{137}\text{Cs}$ to stable Cs in a forest ecosystem.

Correlations among alkali elements are shown in Fig. 3. In plant samples, good correlations were observed among K, Rb and Cs. Correlations between these elements and Mg in plant samples were also good (Table 6b).

![Graphs showing relationships between K, Rb, and Cs concentrations in mushrooms and plants](image)

Fig. 3. Relationship among K, Rb and Cs in mushrooms (●) and plants (□) collected in a pine forest.
Ronneau et al. (1991) observed the good correlation between $^{137}$Cs and K in throughfall waters collected under spruce trees in a forest in Belgium. Myttenaere et al. (1993) measured the seasonal change of stable Cs and K in spruce needles, and demonstrated good correlation between them. These findings suggest that K could be used as an indicator of the behaviour of $^{137}$Cs in the soil-plant systems in forests. Correlations between Ca and Sr in plants were also good as shown in Table 6b, suggesting the possibility of using the behaviour of Ca to predict the behaviour of $^{90}$Sr. No correlations were observed between Na and the other elements studied.

In mushroom samples, good correlations were observed among Ca, Sr, Ba and Al. These elements were not accumulated in mushrooms and most parts in mushrooms were derived from soils adhering on the surface of the samples as already discussed in Fig. 1b. Cs was not correlated with K in mushrooms in contrast with plant samples. The K concentration seemed to be controlled within a narrow range regardless of the mushroom species, while the concentrations of Cs in mushrooms varied very widely. These findings suggested that the mechanism of Cs uptake was completely different from that of K. Rb showed intermediate behaviour between K and Cs. There was a correlation between Rb and Cs ($r = 0.82$). However, the Rb/Cs ratio was not constant as shown in Fig. 3. The ratio decreased with increasing Cs concentration in mushrooms, indicating that Rb might be partly taken up by mushrooms by the same mechanism as Cs is.

**CONCLUSIONS**

The stable alkali (Na, K, Rb and Cs), alkaline earth (Ca, Sr and Ba) elements and Mg in mushrooms collected in a Japanese pine forest on sandy soil were determined, and the data of the stable elements for mushrooms, plants and soils were summarized together with the data of $^{137}$Cs. The following conclusions have been drawn.

1. The element composition of mushrooms could be characterized by the high $^{137}$Cs, Cs and Rb concentrations and low Ca and Sr concentrations in comparison with the composition of plants. Concentrations of $^{137}$Cs, Cs and Rb for mushrooms were one order of magnitude higher than those for plants growing in the same forest. Ca, Sr and Ba were not accumulated in mushrooms, and their concentrations were affected by soils adhering on the surface of the samples.

2. A good correlation ($r = 0.99$, $p < 0.01$) between $^{137}$Cs and stable Cs concentrations was observed for mushrooms. The $^{137}$Cs/Cs ratios were almost constant ($^{137}$Cs/Cs = 134 ± 36 Bq mg$^{-1}$ in 1990) and were
significantly higher than that in the surface soil (27 Bq mg\(^{-1}\)). In the case of the 2 plant samples in which \(^{137}\)Cs was detected, the ratios are almost the same as those for mushrooms. These findings suggested that \(^{137}\)Cs deposited to this pine forest due to nuclear weapons testings has been equilibrated with stable Cs biologically, and available Cs for mushrooms and plants is recycling in the pine forest with the constant \(^{137}\)Cs/Cs ratio.

(3) In the plant samples, good correlations were observed among the concentrations of K, Rb, Cs and Mg. Correlation between those of Ca and Sr was also good. These findings suggested that the possibility of using the behaviour of alkali and alkaline earth elements to predict the \(^{137}\)Cs and \(^{90}\)Sr behaviour in soil-plant systems in forests. In contrast, Cs was not correlated with K in mushrooms, indicating that the mechanism of Cs uptake was different from that for K.

Analyses for stable elements have provided much information on the behaviour of elements which are related to radionuclides in a pine forest on sandy soil with a thin organic layer. Such studies must be useful in predicting the migration of radionuclides in forests contaminated by accidental releases such as the Chernobyl accident. Further studies on other forests with other types of soil, vegetation, source of contamination and in other seasons are earnestly desired.

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Concentrations of alkali and alkaline earth elements


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