

表8-1 食品群別放射性核種の放射能濃度(平成20年度、北九州ブロック)

福岡市	試料名	放射能濃度 (Bq/kg)											
		^{137}Cs	σ	^{134}Cs	σ	^{40}K	σ	^{214}Pb	σ				
I	米・米加工品類	<0.004	+	<0.005	+	7.9	+	0.1	<0.011	+			
II	穀類・雑実類・芋類	<0.014	+	<0.020	+	52.2	+	0.4	<0.038	+			
III	砂糖類・菓子類	<0.015	+	<0.024	+	64.6	+	0.4	0.052	+	0.014		
IV	油脂類	<0.014	+	<0.016	+	0.6	+	0.1	<0.073	+			
V	豆類	<0.024	+	<0.039	+	71.7	+	0.7	0.109	+	0.020		
VI	果実類	<0.011	+	<0.019	+	47.9	+	0.3	0.039	+	0.011		
VII	緑黄色野菜	<0.026	+	<0.041	+	87.0	+	0.7	<0.062	+			
VIII	その他野菜きのこ・海藻類	<0.015	+	<0.022	+	47.2	+	0.4	<0.052	+			
IX	嗜好飲料類	<0.008	+	<0.010	+	8.5	+	0.1	<0.023	+			
X	魚介類	0.071	+	0.011		<0.042	+	77.3	+	0.8	0.140	+	0.035
XI	肉類・卵類	<0.021	+	<0.030	+	63.7	+	0.6	0.113	+	0.022		
XII	乳類	0.017	+	0.005		<0.019	+	42.8	+	0.3	<0.045	+	
XIII	調味料・香辛料類	<0.139	+	<0.163	+	64.2	+	1.7	0.479	+	0.131		
XIV	飲料水	<0.00035	+	<0.00050	+	0.030	+	0.003	<0.00087	+			

福岡市	試料名	放射能濃度 (Bq/kg)									
		^{214}Bi	σ	^{228}Ac	σ	^{212}Pb	σ	^{208}Tl	σ		
I	米・米加工品類	<0.012	+	<0.019	+	<0.009	+	<0.006	+		
II	穀類・雑実類・芋類	<0.042	+	<0.072	+	0.035	+	0.011	<0.020	+	
III	砂糖類・菓子類	0.049	+	0.014		<0.094	+	<0.032	+	<0.022	+
IV	油脂類	<0.065	+	<0.100	+	<0.060	+	<0.030	+		
V	豆類	<0.065	+	<0.127	+	<0.050	+	<0.028	+		
VI	果実類	0.050	+	0.012		<0.065	+	<0.027	+	<0.018	+
VII	緑黄色野菜	<0.064	+	<0.121	+	<0.047	+	<0.033	+		
VIII	その他野菜きのこ・海藻類	<0.056	+	<0.083	+	<0.038	+	<0.025	+		
IX	嗜好飲料類	<0.035	+	<0.037	+	<0.015	+	<0.011	+		
X	魚介類	0.136	+	0.033		<0.156	+	<0.067	+	<0.045	+
XI	肉類・卵類	0.116	+	0.023		<0.121	+	<0.050	+	<0.029	+
XII	乳類	<0.046	+	<0.073	+	<0.033	+	<0.023	+		
XIII	調味料・香辛料類	0.599	+	0.126		<0.546	+	<0.269	+	<0.166	+
XIV	飲料水	<0.00100	+	<0.00143	+	<0.00076	+	<0.00048	+		

注1) 食品の調理等を施した状態での摂取量、注2) σ は計数誤差にともなう数値

表8-2 食品群別放射性核種の1日摂取量(平成20年度、北九州ブロック)

福岡市	試料名	1日摂取量 (mBq/day)										
		^{137}Cs	σ	^{134}Cs	σ	^{40}K	σ	^{214}Pb	σ			
I	米・米加工品類	<1.3	+	<1.9	+	2829	+	27	<4.1	+		
II	穀類・種実類・芋類	<2.3	+	<3.3	+	8617	+	62	<6.2	+		
III	砂糖類・菓子類	<0.5	+	<0.8	+	2139	+	14	1.7	+	0.5	
IV	油脂類	<0.1	+	<0.2	+	5.8	+	1.3	<0.8	+		
V	豆類	<1.5	+	<2.5	+	4459	+	42	6.8	+	1.2	
VI	果実類	<1.1	+	<2.0	+	5087	+	31	4.1	+	1.1	
VII	緑黄色野菜	<2.2	+	<3.4	+	7374	+	61	<5.3	+		
VIII	その他野菜きのこ・海藻類	<2.7	+	<4.0	+	8778	+	73	<9.7	+		
IX	嗜好飲料類	<4.6	+	<6.0	+	4967	+	83	<13.2	+		
X	魚介類	5.4	+	0.8	<3.2	+	5919	+	61	10.7	+	2.6
XI	肉類・卵類	<2.2	+	<3.1	+	6577	+	62	11.7	+	2.3	
XII	乳類	2.4	+	0.7	<2.7	+	6185	+	46	<6.5	+	
XIII	調味料・香辛料類	<12.0	+	<14.1	+	5534	+	145	41.3	+	11.3	
XIV	飲料水	<0.208	+	<0.302	+	18.1	+	1.5	<0.523	+		
	合計値:T(全14食品群)	7.9< T	<38.6	0.0< T	<47.5	T= 68489			76.3< T	<122.4		

福岡市	試料名	1日摂取量 (mBq/day)								
		^{214}Bi	σ	^{228}Ac	σ	^{212}Pb	σ	^{208}Tl	σ	
I	米・米加工品類	<4.2	+	<6.8	+	<3.1	+	<2.3	+	
II	穀類・種実類・芋類	<7.0	+	<11.9	+	5.8	+	5.8	<3.3	+
III	砂糖類・菓子類	1.6	+	0.5	<3.1	+	<1.1	+	<0.7	+
IV	油脂類	<0.7	+	<1.0	+	<0.6	+	<0.3	+	
V	豆類	<4.0	+	<7.9	+	<3.1	+	<1.7	+	
VI	果実類	5.3	+	1.2	<6.9	+	<2.8	+	<1.9	+
VII	緑黄色野菜	<5.4	+	<10.3	+	<4.0	+	<2.8	+	
VIII	その他野菜きのこ・海藻類	<10.3	+	<15.5	+	<7.1	+	<4.7	+	
IX	嗜好飲料類	<20.5	+	<21.5	+	<8.5	+	<6.3	+	
X	魚介類	10.4	+	2.5	<11.9	+	<5.1	+	<3.4	+
XI	肉類・卵類	12.0	+	2.4	<12.5	+	<5.1	+	<3.0	+
XII	乳類	<6.6	+	<10.5	+	<4.8	+	<3.3	+	
XIII	調味料・香辛料類	51.6	+	10.9	<47.1	+	<23.2	+	<14.3	+
XIV	飲料水	<0.600	+	<0.858	+	<0.454	+	<0.289	+	
	合計値:T(全14食品群)	81.0< T	<140.3	0.0< T	<167.7	5.8< T	<74.9	0.0< T	<48.4	

注1) 食品の調理等を施した状態での摂取量。 注2) σ は計数誤差にともなう数値

表8-3 食品群別放射性核種の預託実効線量(平成20年度、北九州ブロック)

福岡市	試料名	預託実効線量 (μSv)														
		^{137}Cs	σ	^{134}Cs	σ	^{40}K	σ	^{214}Pb	σ							
I	米・米加工品類	<0.006	+	<0.013	+	6.4	+	0.1	<0.00021	+						
II	穀類・種実類・芋類	<0.011	+	<0.023	+	19.5	+	0.1	<0.00032	+						
III	砂糖類・菓子類	<0.002	+	<0.006	+	4.8	+	0.0	0.00009	+	0.00002					
IV	油脂類	<0.001	+	<0.001	+	0.01	+	0.00	<0.00004	+						
V	豆類	<0.007	+	<0.017	+	10.1	+	0.1	0.00035	+	0.00006					
VI	果実類	<0.005	+	<0.014	+	11.5	+	0.1	0.00021	+	0.00006					
VII	緑黄色野菜	<0.010	+	<0.024	+	16.7	+	0.1	<0.00027	+						
VIII	その他野菜きのこ・海藻類	<0.013	+	<0.028	+	19.9	+	0.2	<0.00049	+						
IX	嗜好飲料類	<0.022	+	<0.042	+	11.2	+	0.2	<0.00067	+						
X	魚介類	0.026	+	0.004		<0.022	+		13.4	+	0.1	0.00055	+	0.00013		
XI	肉類・卵類	<0.010	+			<0.021	+		14.9	+	0.1	0.00060	+	0.00012		
XII	乳類	0.011	+	0.003		<0.019	+		14.0	+	0.1	<0.00033	+			
XIII	調味料・香辛料類	<0.057	+			<0.097	+		12.5	+	0.3	0.00211	+	0.00058		
XIV	飲料水	<0.00099	+			<0.00210	+		0.041	+	0.004	<0.00003	+			
	合計値;T(全14食品群)	0.037	<	T	<	0.183			0.000	<	T	<	0.329			
									T= 155.0			0.0039	<	T	<	0.0063

福岡市	試料名	預託実効線量 (μSv)												
		^{214}Bi	σ	^{228}Ac	σ	^{212}Pb	σ	^{208}Tl	σ					
I	米・米加工品類	<0.00017	+			<0.0011	+		<0.0069	+		NA	+	
II	穀類・種実類・芋類	<0.00028	+			<0.0019	+		0.0126	+	0.0040	NA	+	
III	砂糖類・菓子類	0.00007	+	0.00002		<0.0005	+		<0.0023	+		NA	+	
IV	油脂類	<0.00003	+			<0.0002	+		<0.0014	+		NA	+	
V	豆類	<0.00016	+			<0.0012	+		<0.0068	+		NA	+	
VI	果実類	0.00021	+	0.00005		<0.0011	+		<0.0082	+		NA	+	
VII	緑黄色野菜	<0.00022	+			<0.0016	+		<0.0088	+		NA	+	
VIII	その他野菜きのこ・海藻類	<0.00042	+			<0.0024	+		<0.0156	+		NA	+	
IX	嗜好飲料類	<0.00082	+			<0.0034	+		<0.0186	+		NA	+	
X	魚介類	0.00042	+	0.00010		<0.0019	+		<0.0113	+		NA	+	
XI	肉類・卵類	0.00048	+	0.00010		<0.0020	+		<0.0112	+		NA	+	
XII	乳類	<0.00027	+			<0.0016	+		<0.0106	+		NA	+	
XIII	調味料・香辛料類	0.00207	+	0.00044		<0.0074	+		<0.0508	+		NA	+	
XIV	飲料水	<0.00002	+			<0.00013	+		<0.00099	+		NA	+	
	合計値;T(全14食品群)	0.0033	<	T	<	0.0056			0.000	<	T	<	0.164	
														T

注1) 食品の調理等を施した状態での摂取量、 注2) σ は計数誤差にともなう数値

表9. 混合試料中の²¹⁰Po

都市名	濃度 (Bq/kg)*	σ	1日摂取量 (Bq/day)	σ	年実効線量 (mSv)	σ
札幌(平成20年度)	0.196	± 0.021	0.379	± 0.040	0.166	± 0.018
仙台(平成20年度)	0.287	± 0.026	0.577	± 0.053	0.253	± 0.023
大阪(平成20年度)	0.217	± 0.023	0.445	± 0.047	0.195	± 0.021
福岡(平成20年度)	0.172	± 0.021	0.344	± 0.041	0.151	± 0.018
横浜市(平成19年度)	0.187	± 0.023	0.371	± 0.046	0.162	± 0.020
新潟市(平成19年度)	0.345	± 0.032	0.694	± 0.065	0.304	± 0.028
高知市(平成19年度)	0.918	± 0.072	1.841	± 0.144	0.806	± 0.063
大阪8群(その他野菜きのこ・海藻類)	0.120	± 0.022	0.021	± 0.004	0.009	± 0.002
大阪10群(魚類)	5.431	± 0.245	0.418	± 0.019	0.183	± 0.008

* 濃度は調理後重量ベース

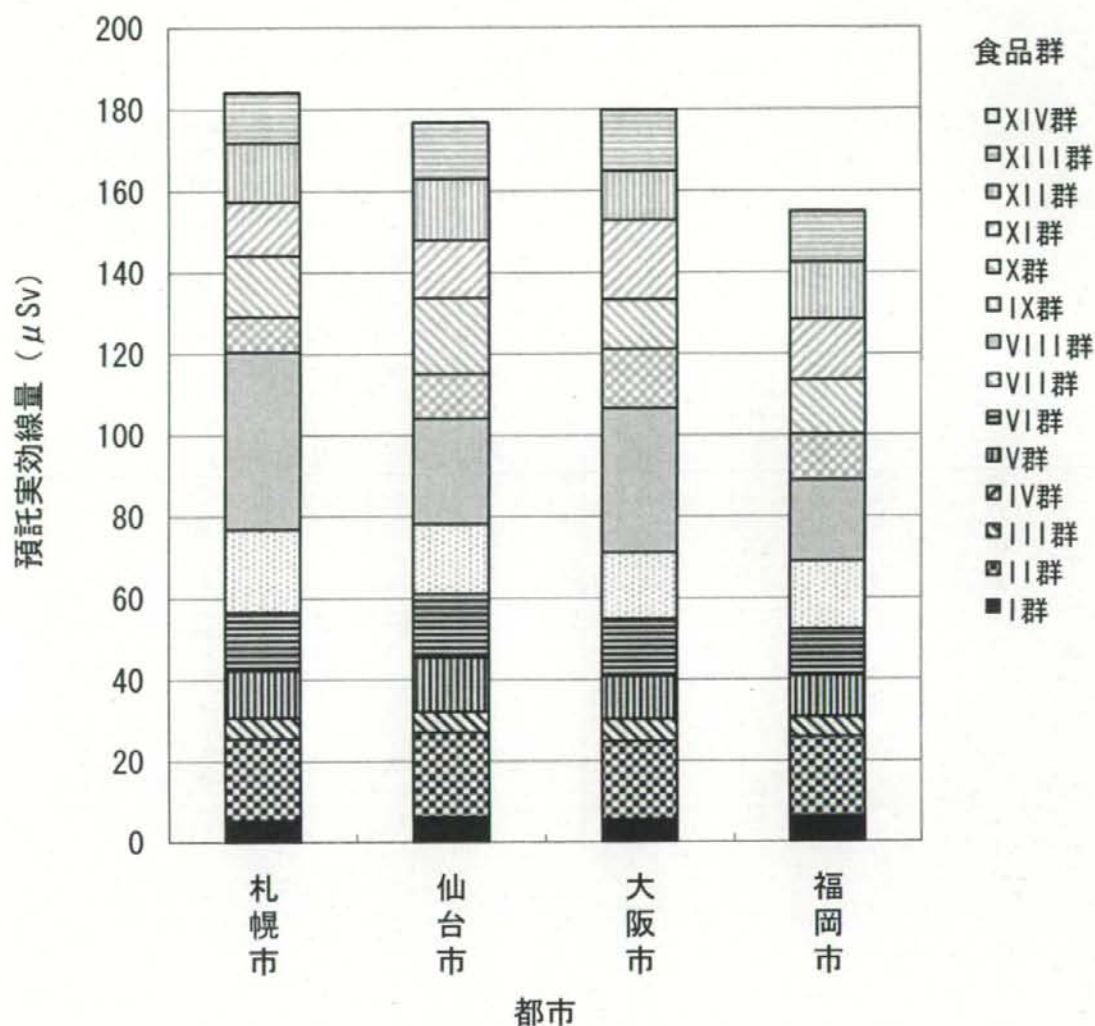
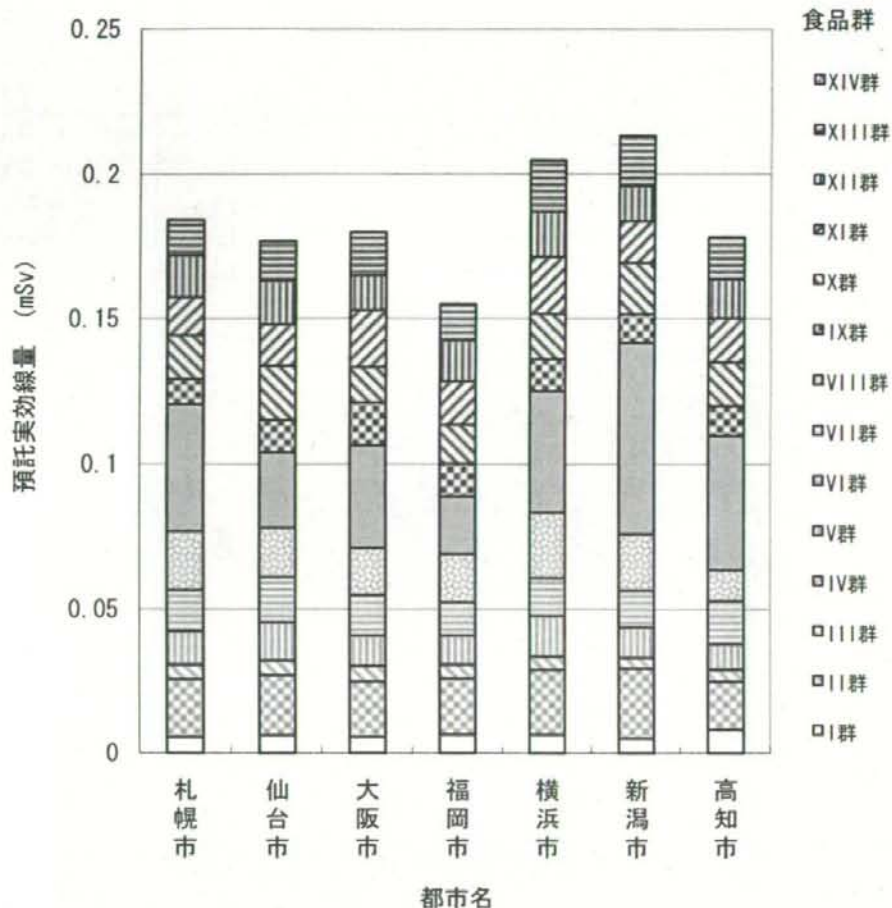


図1. 4都市におけるγ線放出核種の
預託実効線量

I群：米・米加工品、II群：穀類・いも類・種実類、III群：砂糖・甘味料・菓子類、IV群：油脂類、V群：豆類、VI群：果実類、VII群：緑黄色野菜類、VIII群：その他野菜・きのこ・海草類、IX群：嗜好飲料類、X群：魚介類、XI群：肉類・卵類、XII群：乳類、XIII群：調味料・香辛料類、XIV群：飲料水

* γ線放出核種の預託実効線量は大部分⁴⁰K由来である。



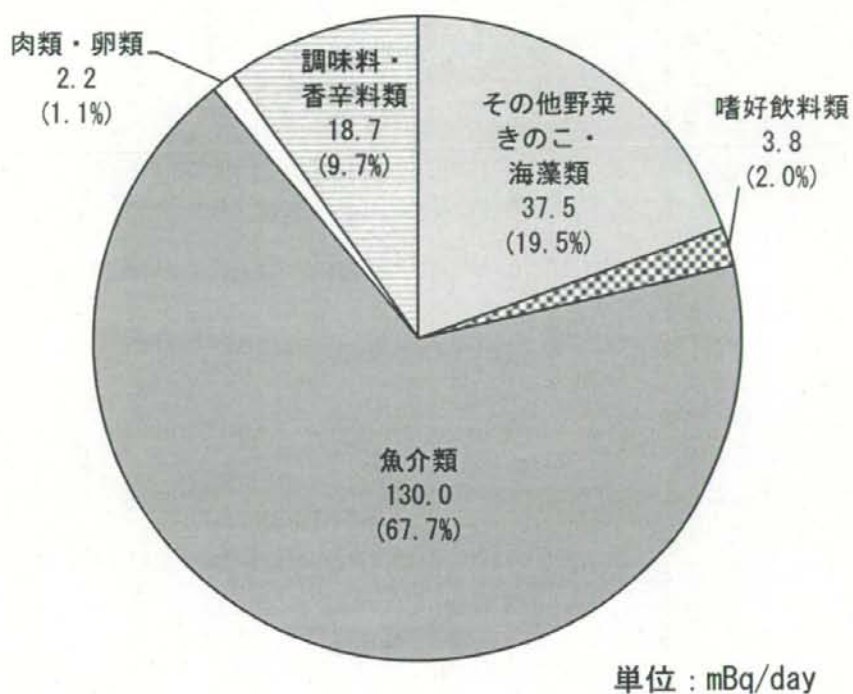
I群：米・米加工品類、II群：穀類・種実類・芋類、III群：砂糖類・菓子類、IV群：油脂類、V群：豆類、VI群：果実類、VII群：緑黄色野菜、VIII群：その他野菜きのこ・海藻類、IX群：嗜好飲料類、X群：魚介類、XI群：肉類・卵類、XII群：乳類、XIII群：調味料・香辛料類、XIV群：飲料水

注1 γ 線放出核種由来の預託実効線量の大部分は ^{40}K に由来する。

注2 横浜市、新潟市、高知市は平成19年度結果である。

注3 都市毎の預託実効線量の差はVIII群（その他野菜きのこ・海藻類）からの線量の差に起因する。

図2. 都市別 γ 線放出核種由来の預託実効線量



(参考) 図1. 食品群別²¹⁰Po 1日摂取量(平成19年度横浜市結果)

(参考) 表1. 食品中の²¹⁰Po濃度

地域	国	濃度 (mBq/kg)						
		乳製品	肉製品	穀物製品	葉菜	根菜と果実	魚製品	飲料水
北アメリカ	米国	-	-	-	-	-	150-5500	-
	中国	13	120	42	430	29	4900	-
アジア	インド	15	440	15-120	320	16-140	-	-
	日本	-	-	-	-	-	-	-
ヨーロッパ	イタリア	-	-	-	-	-	-	-
	ドイツ	2-80	37-4000	37-1900	4-7400	22-5200	50-5200	0.1-200
(以上、UNSCEAR 2000)	ポーランド	16	99-102	90-140	40-67	28-210	3100-3800	0.5
	ルーマニア	13-140	38-110	20-360	-	12-140	-	7-44
アジア	英国	20-220	62-67000	27-260	37-3300	-	60-5300	-
	日本	22	13	<50	190	<30	1600	7
*調理、調整品		(乳類)	(肉類・卵類)	(穀類・種実類・芋類)	(その他野菜・きのこ・海藻類)	(果実類)	(魚介類)	(嗜好飲料類)

(参考) 表2. 食品からの²¹⁰Poと⁴⁰Kの摂取量と実効線量の比較

試料	²¹⁰ Po 1日摂取量 (Bq/day)	²¹⁰ Po実効線量 (mSv)	⁴⁰ K 1日摂取量 (Bq/day)	⁴⁰ K実効線量 (mSv)	地域	文献
日常食	0.38	0.17	81.4	0.18	札幌市	平成20年度結果
	0.58	0.25	78.2	0.18	仙台市	平成20年度結果
	0.45	0.19	79.4	0.18	大阪市	平成20年度結果
	0.34	0.15	68.5	0.16	福岡市	平成20年度結果
	0.37	0.16	90.5	0.2	横浜市	平成19年度結果
	0.69	0.3	94.2	0.21	新潟市	平成19年度結果
	1.84	0.81	78.7	0.18	高知市	平成19年度結果
	0.11, 0.22	0.06, 0.11	—	—	インド	Avadhani et al. (2001)
	0.08, 0.19	—	—	—	インド	Narayana et al. (1995)
	0.05-0.06	—	—	—	米国	Holtzman et al. (1980)
食品	1.67 (610 Bq/year)	0.73	—	—	日本	Ota et al. (2009)
	—	—	39.41	0.1	韓国	Choi et al. (2008)
海産物	0.48-0.69	—	—	—	日本	Yamamoto et al. (1994)
	1.2	—	—	—	ポルトガル	Carvalho et al. (1995)
日常食	0.14 (52 Bq/year)	—	—	—	International	Arkrog et al. (1997)
	0.05-0.60 (18-220/year)	0.12 (mainly from ²¹⁰ Po)	—	0.17	International	UNSCEAR (2000)

研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル	発表誌名	巻号	ページ	出版年
松田りえ子, 渡邊敬浩, 五十嵐敦子, 白政優子, 米谷民雄	トータルダイエット試料の 分析による硝酸塩の摂取量 推定	食品衛生学雑誌	50	29-33	2009
Sugiyama H., Takahasi MN, Terada H., Kuwahara C., Maeda C., Anzai Y., Kato F.	Accumulation and localization of cesium in edible mushroom (<i>Pleurotus ostreatus</i>) mycelia	J. Agric. Food Chem.	56	9641-9646	2008
Iijima I., Takagi H., Tomura K., Watanuki T., Sugiyama H.	Evaluation of cesium-137 (¹³⁷ Cs) and elements intake from daily diets in residents of Kanagawa prefecture, Japan.	J. Health Sci.	55	192-205	2009

調査・資料

トータルダイエツト試料の分析による硝酸塩の摂取量推定

(2008年6月30日受理)

松田りえ子* 渡邊 敬浩 五十嵐敦子 白政優子 米谷民雄

Estimation of the Daily Intake of Nitrate Based on Analysis of Total Diet Samples

Rieko MATSUDA*, Takahiro WATANABE, Atsuko IKARASHI,
Yuko SHIRAMASA and Tamio MAITANINational Institute of Health Sciences: 1-18-1 Kamiyoga, Setagaya-ku,
Tokyo 158-8501, Japan; * Corresponding author

The concentrations of nitrates in total diet samples prepared at 11 places were determined according to the market basket method, and the daily intake of nitrates was estimated based on the analysis results. The mean estimated daily intake of nitrates was 200 mg/man/day (4.0 mg/kg/day) and exceeded the ADI (3.7 mg/kg/day) set by the JECFA by 8%. The principal sources of the nitrates were colored vegetables (7th food group) and other vegetables, seaweeds and mushrooms (8th food group), and the intake from these food groups accounted for more than 80% of the total intake. The reported concentrations of nitrates in individual foods and the estimated daily intake of these foods indicate that the intake of nitrates from spinach accounts for 38% of the ADI, that from Chinese cabbage for 10%, and that from Japanese radish for 20%.

(Received June 30, 2008)

Key words: 硝酸塩 nitrate; 一日摂取量 daily intake; トータルダイエツト試料 total diet sample

緒 言

硝酸塩は植物中に普遍的に含まれる成分であるとともに、発酵調製・発色の目的で食品添加物としても使用されている。硝酸塩自体の毒性は特に高いわけではないが、体内で亜硝酸塩に変化すると、メトヘモグロビン血症を引き起こすなどの作用があることが知られている^{1,2)}。さらに、亜硝酸塩はメチルグアニンなどと反応し、発がん性の高いニトロソアミンを生じることも指摘されている^{3,4)}。これらのことから、欧州連合(EU)は、収穫時期や冷凍であるか否かによって変わるが、レタスとホウレンソウに関する硝酸塩の最大基準値を2,000~4,500 mg/kgと設定している。また、FAO/WHO 合同食品添加物専門家会議(FAO/WHO Joint Expert Committee on Food Additives; JECFA)は硝酸塩の一日許容摂取量(ADI)として3.7 mg/kg bw/dayを定めている。

わが国における食品中の硝酸塩に関する調査としては、辻ら⁵⁾による199種類の生鮮食品および加工食品の実態調査がある。また、昭和51~60年にわたって実施された、「日本人の食品添加物一日摂取量実態調査研究」⁶⁾の一環として、硝酸塩の摂取量が調査されており、さらに平成11年には「食品添加物一日摂取量総点検調査」⁷⁾にお

いて硝酸塩摂取量が評価されている。これらの調査の目的は、主として添加物として使用されている硝酸塩の摂取量調査であるが、天然由来の硝酸塩と区別できないことから、総摂取量を把握するために個別の生鮮食品についても調査を行っている。しかし、これらの生鮮食品については調理加工などの処理を行っていない。そこでより実際の喫食内容を反映し、総摂取量に対して寄与率の高い食品群を推定するための調査が必要であると考え、平成15年には、国内3か所で作製したトータルダイエツト試料(TD試料)を用いて、硝酸塩の摂取量調査を行った^{*1)}。この調査結果から得られた硝酸塩総摂取量は184~227 mg/man/day、体重当たりでは3.7~4.5 mg/kg bw/dayであり、JECFAの定めたADIを超えていた。

上記調査では分析に供された試料検体数が少なかったこと、さらに調査後、国民健康・栄養調査^{*2)}の方式が変更され、対象となる食品などが増加したことから、本研究では国立医薬品食品衛生研究所、全国9か所の衛生研究所および大学1か所で調製した計11セット(総試料検体数143)のTD試料中の硝酸塩濃度を測定し、現時点での硝酸塩摂取量の実態把握を試みた。

* 連絡先
国立医薬品食品衛生研究所: 〒158-8501 東京都世田谷区上野買1-18-1

*1) 平成15年度食品等試験検査費報告書「食品中の汚染物質等の一日摂取量調査の実施」

*2) <http://www.mhlw.go.jp/bunya/kenkou/eiyoub.html>

実験方法

1. 試料調製

国内11か所（北海道，新潟，千葉，東京，神奈川，愛知，滋賀，大阪，香川，大分，沖縄）においてTD試料を調製した。

TD試料調製に含める食品の量は，平成13年から15年度の国民健康・栄養調査の結果を地域別に集計して求めた摂取量の平均値に従った。国民健康・栄養調査では食品は17の群に分類されているが，本研究では国立医薬品食品衛生研究所において実施されている汚染物摂取量調査で使用されている13群の分類（飲料水を除く）に従って試料を調製した。すべての食品を13の群に分類し，各食品群に含まれる食品を小売店等で購入し，一般的に生食されるものはそのまま，調理後喫食される食品は簡単な調理を行った後，決定した重量に従って秤量し，必要であれば加水して十分に混合し，試料とした。

2. 硝酸塩分析

試料5gを量り，0.5 mol/L水酸化ナトリウム溶液16

mL，80℃の水30 mL，9%酢酸亜鉛溶液16 mLを加えて，攪拌しながら80℃で20分間加熱後，室温まで冷却し水を加えて100 mLに定容した。2,000 rpmで遠心分離した上清を，シリンジフィルター（孔径0.45 μm）でろ過し，高速液体クロマトグラフ（HPLC）で分析した。1群試料は遠心分離により澄明な上清が得られなかったため，エタノールで2倍に希釈し沈殿を凝集させた後に遠心分離した。

HPLC条件は下記のとおりである。

カラム：Shodex Asahipak NH2P-50 4E，4.6 mm × 254 cm + 4.6 mm × 1 cm（ガードカラム）

カラム温度：50℃

移動相：0.1 mol/L過塩素酸ナトリウムを含む10 mmol/Lリン酸塩緩衝液

流量：1.0 mL/min

測定波長：210 nm

Table 1. Repeatability and reproducibility of analysis of spiked samples

Food group No. 5		Days									
		1st					2nd				
		1st	2nd	3rd	4th	5th	1st	2nd	3rd	4th	5th
		Low level spiked samples ^{a)}					High level spiked samples ^{b)}				
Concentration ^{c)} (μg/g)	Portion 1	1.538	1.554	1.540	1.578	1.605	4.998	4.992	4.945	5.129	5.112
	Portion 2	1.524	1.549	1.529	1.623	1.553	5.012	4.979	4.919	5.036	5.093
Recovery ^{d)} (μg/g)	Portion 1	1.190	1.206	1.192	1.230	1.257	4.650	4.644	4.597	4.781	4.764
	Portion 2	1.176	1.201	1.181	1.275	1.205	4.664	4.631	4.571	4.688	4.745

Food group No. 12		Days									
		1st					2nd				
		1st	2nd	3rd	4th	5th	1st	2nd	3rd	4th	5th
		Low level spiked samples ^{a)}					High level spiked samples ^{b)}				
Concentration ^{c)} (μg/g)	Portion 1	1.471	1.416	1.456	1.520	1.485	5.062	4.866	4.940	4.960	4.948
	Portion 2	1.492	1.406	1.453	1.493	1.456	4.938	4.812	4.979	5.052	5.052
Recovery ^{d)} (μg/g)	Portion 1	1.123	1.068	1.108	1.172	1.137	4.714	4.518	4.592	4.612	4.600
	Portion 2	1.144	1.058	1.105	1.145	1.108	4.590	4.464	4.631	4.704	4.704

^{a)} Low-level spiked samples contain 1.25 μg/g of nitrate.

^{b)} High-level spiked samples contain 5.0 μg/g of nitrate.

^{c)} To calculate the concentration, the measured values in the analytical blank was subtracted from the result for each spiked sample.

^{d)} To calculate the recovery, the mean of the measured values obtained by analysis of 5 non-spiked samples was subtracted from the result for each spiked sample.

Table 2. Recovery, repeatability and reproducibility of the method for food samples

Food groups	Spiked concentration (μg/g)	Mean of the analytical results (μg/g)	Recovery rate (%)	Repeatability		Reproducibility within laboratory	
				SD	RSD (%)	SD	RSD (%)
No. 5	1.25	1.21	96.9	0.023	1.87	0.034	2.79
	5	4.67	93.5	0.032	0.69	0.074	1.58
No. 12	1.25	1.12	89.4	0.015	1.30	0.037	3.31
	5	4.61	92.3	0.062	1.35	0.083	1.81

RSD: Relative standard deviation

Table 3. Nitrate concentrations in the total diet samples prepared at 11 laboratories located in different regions of Japan

Food group No.	Food group	Representative food	Sample weight (g)	No. of foods included	Codes for each laboratory where the samples were prepared											Average	
					A	B	C	D	E	F	G	H	I	J	K		
1	Rice	Rice, rice noodle	354.9	4	1.9	0.5	2.5	5.5	5.5	1.6	3.6	9.1	3.3	2.0	3.3	1.4	3.2
2	Cereals, potatoes and nuts	Flour, bread, sweet potato, nuts	169.8	20	6.9	2.3	2.5	11.3	9.7	30.0	49.9	68.9	10.7	38.2	43.1	24.9	24.9
3	Sugar and confectionery	Sugar, cookies, rice cracker	33.4	12	25.1	24.8	43.9	28.6	27.9	21.1	9.6	28.0	20.3	5.1	24.1	23.5	23.5
4	Fat and oils	Margarine, vegetable oils, animal oils	10.7	5	ND	1.7	0.2	0.3	0.1	0.3	0.1	0.3	0.1	0.5	0.2	0.8	0.4
5	Pulses and its processed	Soy, tofu, miso	58.5	8	0.7	1.3	1.0	3.4	5.1	3.8	8.8	1.5	2.6	5.7	3.7	3.4	3.4
6	Fruits	Apple, orange, melon, strawberry	119.7	12	9.5	19.4	31.3	23.9	13.2	19.3	12.0	36.2	38.2	25.9	25.2	23.1	23.1
7	Colored vegetables	Carrot, spinach, tomato, pumpkin	96.9	8	791.5	640.3	702.6	928.6	776.4	575.0	782.1	552.5	321.7	836.2	659.2	396.7	691.4
8	Vegetables, seaweeds and mushrooms	Radish, cabbage, hijiki, shimeji	205.6	16	537.6	457.1	253.9	837.8	567.6	697.4	403.4	821.7	836.2	659.2	396.7	588.1	588.1
9	Preference beverages	Carbonated drink, beer, Japanese sake	562.2	12	0.7	5.9	3.1	10.2	8.1	2.6	8.0	14.6	11.0	9.1	12.1	7.8	7.8
10	Fish and seafoods	Salmon, tuna, shrimp, dried fish	87.5	28	ND	0.2	0.5	2.8	2.0	2.3	4.1	0.5	0.9	4.1	1.1	1.9	1.9
11	Meat and eggs	Beef, chicken, egg, sausage	113.7	20	ND	3.5	6.3	5.0	2.3	2.7	5.0	5.1	3.3	5.1	4.6	4.3	4.3
12	Milk and milk products	Milk, cheese	147.5	12	3.0	3.2	4.3	3.3	3.3	2.5	3.2	5.3	3.8	2.4	5.5	5.2	3.8
13	Spices	Soy sauce, salt, pepper	90.3	19	9.8	33.9	17.7	7.3	6.3	15.8	14.1	9.6	15.0	7.5	34.6	15.6	15.6

Table 4. Estimated daily intake of nitrates

Food groups No.	Food group	Codes for each laboratory where the samples were prepared										
		A	B	C	D	E	F	G	H	I	J	K
Estimated daily intake of nitrate (mg/man/day)												
1	Rice	1.2	0.4	1.4	3.2	1.0	1.9	4.4	1.1	1.1	1.9	0.7
2	Cereals, potatoes and nuts	1.7	1.1	0.7	3.3	2.6	7.8	18.7	12.4	3.4	8.3	16.9
3	Sugar and confectionery	1.4	1.5	1.3	2.1	1.3	1.0	0.5	1.5	1.1	0.2	1.2
4	Fat and oils	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	Pulses and its processed products	0.0	0.1	0.1	0.3	0.3	0.2	0.5	0.1	0.2	0.4	0.2
6	Fruits	1.0	2.1	3.7	2.9	1.4	2.2	1.5	4.5	4.8	3.1	3.0
7	Colored vegetables	65.7	54.5	71.4	76.5	65.8	44.6	77.5	55.2	28.6	55.5	79.5
8	Vegetables, seaweeds and mushrooms	112.2	116.2	56.0	143.9	112.1	127.1	84.4	165.1	161.3	142.8	84.9
9	Preference beverages	0.4	3.4	1.8	6.3	4.9	1.5	4.3	7.9	6.0	4.8	6.2
10	Fish and seafoods	0.0	0.0	0.1	0.2	0.3	0.3	0.4	0.1	0.1	0.2	0.1
11	Meat and eggs	0.0	0.5	0.9	0.5	0.3	0.3	0.5	0.5	0.3	0.5	0.6
12	Milk and milk products	0.4	0.5	0.6	0.5	0.4	0.5	0.7	0.5	0.3	0.7	0.8
13	Spices	0.9	2.9	1.8	0.7	0.7	1.3	1.3	1.2	1.4	0.7	3.0
Sum of the estimated nitrate daily intake		184.9	183.2	139.8	240.5	191.1	188.6	194.8	250.1	208.6	219.0	197.1

結 果

1. 分析方法の妥当性評価

分析に先立ち、分析方法の妥当性の検証を行った。検証は、平成19年11月に通知された食品中に残留する農薬等に関する試験法の妥当性評価ガイドライン（食安発第1115001号）に準じた方法で実施した。

添加回収試験の対象食品群として、比較的硝酸塩濃度の低い5群（豆類）と12群（乳類）を選択した。添加濃度を1.25 µg/g、5 µg/gの2レベルとし、蒸留水を用いた操作ブランク1検体、5群および12群の無添加試料を各1検体、高レベル添加試料ならびに低レベル添加試料それぞれ2検体を併行して1日に分析し、これを5日間繰り返した。試料量は共通して5gとした。分析結果をTable 1に、回収率、一元配置の分散分析により得られた分散に基づき算出した併行精度、室内精度をTable 2に示した。いずれの食品群においても、回収率は80%以上、室内精度は相対標準偏差(RSD)として5%以下であり、本試験法実施の妥当性が確認された。

2. 硝酸塩濃度および摂取量

Table 3に11セットのTD試料中の硝酸塩濃度を食品群ごとに示すとともに、各食品群の一日摂取重量と試料調製に使用した食品の数を示した。11か所の結果を平均すると、有色野菜の群（7群）の濃度が約700 µg/gで最も高く、その他の野菜の群（8群）がそれに続き、この2つの食品群中の硝酸塩濃度は他の群の20倍以上であった。7群と8群の濃度の大小関係は試料セットにより異なり、全11試料セット中4つのセットでは8群の濃度が7群よりも高い結果となった。

雑穀（2群）、菓子（3群）、果実（6群）の群の硝酸塩濃度は平均20 µg/g程度であった。これ以外の米（1群）、油脂（4群）、豆類（5群）、魚介（10群）、肉・卵（11群）、乳製品（12群）の試料中の硝酸塩濃度は低く、10 µg/gを超える試料は見られなかった。いずれの食品

群でも、硝酸塩濃度は大きな変動幅を示し、試料調製に用いた個別食品中の硝酸塩濃度が広い範囲に分布していることが考えられる。特に雑穀（2群）は濃度の範囲が大きく2.3~68.9 µg/gで、最大値と最小値の比は約30であった。

Table 4には各試料検体中の硝酸塩濃度とその重量から計算した、一日当たりの硝酸塩摂取量を示す。食品群ごとの濃度から予測されたように、有色野菜の群（7群）およびその他の野菜の群（8群）からの摂取が全体の80%以上を占め、他の食品群からの摂取は全体としてわずかであった。硝酸塩濃度の平均値は有色野菜の群（7群）のほうが高かったが、摂取量はその他の野菜の群（8群）のほうが高く、この関係が逆転しているのは1機関のみであった。

推定された硝酸塩総摂取量は140~250 mg/man/dayの範囲にあり、平均は200 mg/man/dayであった。

考 察

TD試料11セットの分析値に基づき推定した硝酸塩の摂取量は平均200 mg/man/dayであり、体重当たりとすると4 mg/kg bw/dayであった。この値はJECFAが定めた硝酸塩の1日許容摂取量(ADI) 3.7 mg/kg bw/dayを8%程度超えている。使用した11試料において、JECFAのADIを超える摂取量推定値(185 mg/man/day)を与えたものは8試料であった。

昭和51年~60年に実施された「日本人の食品添加物1日摂取量実態調査研究」⁶⁾では、加工食品由来の硝酸塩摂取量は35.5 mg/man/day (0.71 mg/kg bw/day)であり、果実・野菜・海草を原材料とする加工食品からの摂取が主であり、全体の70%を占めると報告されている。この傾向は、今回の調査結果と同じであった。同時に報告された生鮮食品からの摂取量は224.1 mg/man/day (4.5 mg/kg bw/day)で、加工食品に由来する摂取量の6倍

であり、両者を合わせた摂取量は 260 mg/man/day (5.2 mg/kg bw/day) であり、本研究で推定された摂取量よりも高い結果であった。同様に、平成 10~11 年の「食品添加物一日摂取量総点検調査」⁷⁾ では 190 mg/man/day (3.8 mg/kg bw/day)、平成 15 年の TD 試料 3 種による調査では 184~227 mg/man/day (3.7~4.5 mg/kg bw/day) であった。今回の調査結果の平均である 200 mg/man/day の推定摂取量は、平成 10~11 年および 15 年の調査とほぼ同程度である。これらの結果から、日本人の硝酸塩摂取量は、JECFA の設定した ADI をやや超えた水準で変わらず推移していることが示された。いずれの摂取量調査においても、硝酸塩の大部分が果実・野菜・海草の群から摂取されていた。

辻らの報告⁵⁾ によれば、ハウレンソウ (3,560 $\mu\text{g/g}$)、ターサイ (5,670 $\mu\text{g/g}$)、チンゲンサイ (3,150 $\mu\text{g/g}$)、しゅんぎく (4,410 $\mu\text{g/g}$)、サラダ菜 (5,360 $\mu\text{g/g}$)、サニーレタス (1,230 $\mu\text{g/g}$)、白菜 (1,040 $\mu\text{g/g}$)、大根 (1,060 $\mu\text{g/g}$) に高濃度の硝酸塩が含まれている。前の 4 種類は 7 群に、残りは 8 群に含まれる野菜である。今回の調査結果では、7 群の硝酸塩濃度はおよそ 700 $\mu\text{g/g}$ 、8 群は 600 $\mu\text{g/g}$ で個々の野菜の分析により得られた濃度に比べ低濃度であった。

今回使用した国民健康・栄養調査結果では、ハウレンソウの 1 日当たりの摂取量は全国平均で 19.8 g であった。辻らが報告⁵⁾ したハウレンソウ中の硝酸塩濃度 3,560 $\mu\text{g/g}$ から、ハウレンソウに由来する硝酸塩摂取量を計算すると 70 mg/man/day (1.4 mg/kg bw/day) であり、ADI の 38% に相当する。同様に白菜および大根からの摂取量を求めると、それぞれ 0.4 mg/kg bw/day および 0.8 mg/kg bw/day となり、これら 3 種の野菜で ADI のほぼ 70% にあたる 2.6 mg/kg bw/day を摂取していると推定される。このほかに、葉菜漬物、沢庵も 18 g 程度摂取されている。漬物類にも原料である白菜、大根と同程度の硝酸塩が含まれているため、これらを含めた 8 群から

の摂取量が多くなったと考えられる。

結 論

11 か所で調製したマーケットバスケット方式によるトータルダイエツト試料を分析し、硝酸塩の摂取量を推定した。分析方法の妥当性を回収率および室内精度により確認した。推定された摂取量は 4.0 mg/kg bw/day であり、JECFA の定めた ADI 3.7 mg/kg bw/day を 8% 超過していた。硝酸塩の主要な摂取源は、7 群 (有色野菜) および 8 群 (その他の野菜、漬物、海草) であり、これらの群からの摂取量が総摂取量の 80% 以上を占めていた。

文 献

- 1) Mensinga, T. T., Speoijers, G. J., Meulenbelt, J. Health implication of exposure to environmental nitrogenous compound. *Toxicol. Rev.* 22, 41-51 (2003).
- 2) Maric, P., Ali, S. S., Heron, L. G., Rosenfeld, D., Greenwood, M. Methaemoglobinaemia following ingestion of a commonly available food additive. *Med. J. Aust.* 188, 156-158 (2008).
- 3) Lijinsky, W. *N*-Nitroso compounds in the diet. *Mutat. Res.* 443, 129-138 (1999).
- 4) Sugimura, T. Nutrition and dietary carcinogens. *Carcinogenesis* 21, 387-395 (2000).
- 5) Tsuji, S., Kohsaka, M., Morita, Y., Shibata, T., Kaneta, N., Wakabayashi, K., Uchibori-Hase, S., Ide, S., Fujiwara, K., Suzuki, H., Ito, Y. Naturally occurring of nitrite and nitrate existing in various raw and processed foods. *Shokuhin Eiseigaku Zasshi (J. Food Hyg. Soc. Japan)* 34, 294-302 (1993).
- 6) 伊藤善志男編, 「日本人の食品添加物 1 日摂取量実態調査研究—食品中の食品添加物残存量測定による調査研究のまとめ—」, 厚生省生活衛生局食品化学課 (1987).
- 7) 食品添加物研究会編, 「あなたが食べている食品添加物, 食品添加物一日摂取量の実態と傾向 総合版」(2001).

Accumulation and Localization of Cesium in Edible Mushroom (*Pleurotus ostreatus*) Mycelia

HIDEO SUGIYAMA,^{*†} MITSUKO N. TAKAHASHI,[†] HIROSHI TERADA,[†]
 CHIKAKO KUWAHARA,[‡] CHIKA MAEDA,[§] YOJIRO ANZAI,[§] AND FUMIO KATO[§]

Department of Environmental Health, National Institute of Public Health, 2-3-6 minami Wako-shi, Saitama 351-0197, Japan, Chemistry Division, Kanagawa Prefectural Institute of Public Health, Chigasaki-shi, Kanagawa 253-0087, Japan, and Department of Microbiology, School of Pharmaceutical Sciences, Toho University, Funabashi-shi, Chiba 274-8510, Japan

The characteristics of Cs accumulation and localization in edible mushrooms were examined using the mycelia of *Pleurotus ostreatus*-Y1. Scanning electron microscope images revealed the existence of white spots, and energy dispersive X-ray microanalyzer analysis indicated the presence of larger amounts of Cs and P in these spots in mycelia cultured on medium containing 25 mM CsCl. The ¹³⁷Cs activities in the mycelia were approximately 4–6 times higher than those in water used for ¹³⁷Cs elution. Higher Cs concentrations in the sediment fraction including vacuolar pellets were obtained compared to the upper fractions. It was observed that yellowish spots caused by the fluorescence of 4',6-diamidino-2-phenylindole (DAPI)-stained polyphosphate were localized in the mycelia. The higher fluorescence intensity of the yellowish-grained spots was measured in comparison with other regions in the mycelium. These results suggested that Cs in the mycelia was trapped by polyphosphate in vacuoles or other organelles.

KEYWORDS: Accumulation; localization; cesium; potassium; mushroom; mycelium; *Pleurotus ostreatus*

INTRODUCTION

After the Chernobyl accident in 1986, it was reported that wild mushrooms in European countries contained high levels of ¹³⁷Cs (1–4). In addition, wild mushrooms with higher ¹³⁷Cs contents (less than detectable-1070 Bq kg⁻¹ fresh wt., <0.4–1260 Bq kg⁻¹ fresh wt., and not detectable-570 Bq kg⁻¹ fresh wt.) were found in Japan (5–9). Recent reports showed that the levels of ¹³⁷Cs in wild mushrooms were still high in Europe (10, 11). For example, the highest activity concentrations of ¹³⁷Cs were 2263 Bq kg⁻¹ (dry wt.) in *Xerocomus badius* from the Czech Republic, 966 Bq kg⁻¹ (dry wt.) in *Suillus luteus* from Slovakia, and 401 Bq kg⁻¹ (dry wt.) in a mushroom from Turkey, and it is internationally recognized that mushrooms are capable of accumulating ¹³⁷Cs from the environment. In Japan, radioactivity monitoring (permissible limit of 370 Bq kg⁻¹ for ¹³⁷Cs + ¹³⁴Cs) of mushrooms imported from European countries has been undertaken by the Ministry of Health, Labor and Welfare since the Chernobyl accident.

However, an explanation of why there is a high concentration and accumulation of ¹³⁷Cs in mushrooms has not been established. Therefore, investigations have been carried out on soil-mushroom or mushroom mycelia systems (12–15). The present

studies have focused on the cultivation of the edible, saprophytic mushroom *Pleurotus ostreatus* to investigate its accumulation of ¹³⁷Cs and stable Cs. *P. ostreatus* is a very popular, edible mushroom in Japan, and its cultivation is relatively easy. The ease of cultivation of *P. ostreatus* enables the establishment of experimental conditions for ¹³⁷Cs or stable Cs tracing and allow its use as an effective tool to study the translocation characteristics and mechanisms of accumulation of Cs. Previously, the following findings using the *P. ostreatus*-Y1 strain were obtained; high ¹³⁷Cs contents were detected not only in mycorrhizal mushrooms in the field but also in the fruiting body of saprophytic mushrooms depending on the concentration of Cs in the culture medium (9, 16); Cs⁺ and analogous alkali metals such as K⁺ and Rb⁺ were competitively taken-up by mushroom mycelia (17); and NMR measurement of the mushroom fruiting body and mycelia revealed the existence of Cs in ionized as well as other forms (9, 18).

In the present study, further experiments were conducted using the *P. ostreatus*-Y1 strain involving observations using a scanning electron microscope (SEM)—energy dispersive X-ray microanalyzer (EDX) and elemental analysis, elution of ¹³⁷Cs from mycelia, fractionation of Cs contents in mycelium cells by density-gradient centrifugation, and fluorescence microscopy of polyphosphate in mycelia using DAPI staining to investigate the characteristics of Cs accumulation and localization in mushroom mycelia.

* Corresponding author. Phone: +81 48 458 6263. Fax: +81 48 458 6270. E-mail: sugiyama@niph.go.jp.

[†] National Institute of Public Health.

[‡] Kanagawa Prefectural Institute of Public Health.

[§] Toho University.

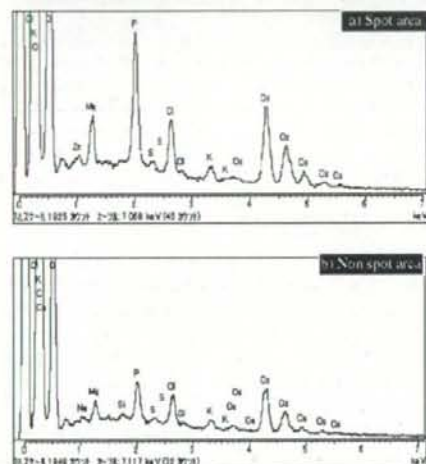
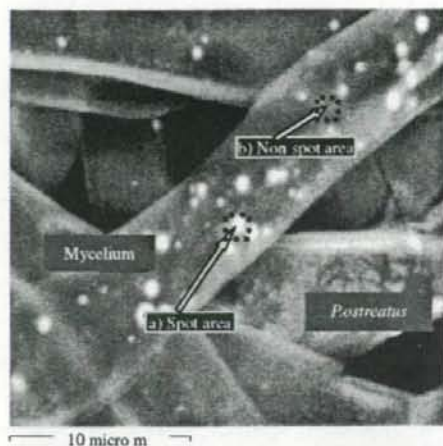


Figure 1. Image and spectra of *P. ostreatus*-Y1 mycelia using a scanning electron microscope (SEM) and energy dispersive X-ray microanalyzer (EDX). The rectangles with dotted lines indicate the scanning areas with a spot (a) and without a spot (b) for the spectra of the spot area and nonspot area.

MATERIALS AND METHODS

This study used the *P. ostreatus*-Y1 strain, a popular, edible, saprophytic mushroom in Japan.

SEM-EDX Observation of *P. ostreatus* Mycelia. *P. ostreatus* mycelia were cultivated at 27 °C for seven days on the upper side of a membrane filter (Millipore, pore size: 0.45 μ m) placed on yeast extract–malt extract agar medium (0.4% yeast extract (Difco), 1% malt extract (Difco), and 0.4% glucose (Wako), pH 7.2) containing 25 mM CsCl (Wako) in a petri plate. Parts of mycelia on the filter were smeared onto double-sided carbon tape (Shinto Paint, 8W mm for Al specimen mounting; Hisco, 15 ϕ \times 6H mm for SEM without vapor deposition). Observation of images and element identification in *P. ostreatus* mycelia samples were performed by SEM-EDX (Hitachi S-3000N) in low vacuum mode (50 Pa) at 15 kV. Identification and semiquantitative analysis of elements were carried out using the spectra and peak heights of the spectra using EDX-Analysis Manager (Horiba).

Uptake of ^{137}Cs into *P. ostreatus* Mycelia and Elution. *P. ostreatus* mycelia were incubated by shaking in liquid medium (0.4% yeast extract, 1% malt extract, and 0.4% glucose, pH 7.2) containing 4.4 kBq ^{137}Cs (The Japan Radioisotope Association, $^{137}\text{CsCl}$ standard solution 56-CS005, ^{137}Cs 370 kBq kg $^{-1}$) and 5 mM CsCl using 6 baffles

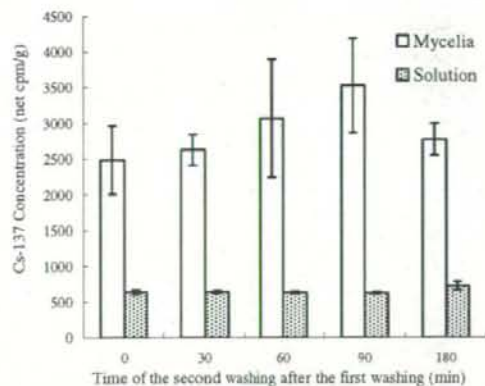


Figure 2. Concentrations of ^{137}Cs in *P. ostreatus*-Y1 mycelia and solutions after washing with distilled water. *P. ostreatus*-Y1 mycelia were cultured in YM liquid medium containing 4.4 kBq of $^{137}\text{CsCl}$ and 5 mM CsCl. The ^{137}Cs concentrations of the samples were determined by comparing with a $^{137}\text{CsCl}$ standard solution with an auto gamma-ray counter. Error bars indicate the mean value \pm standard deviation of three replicates.

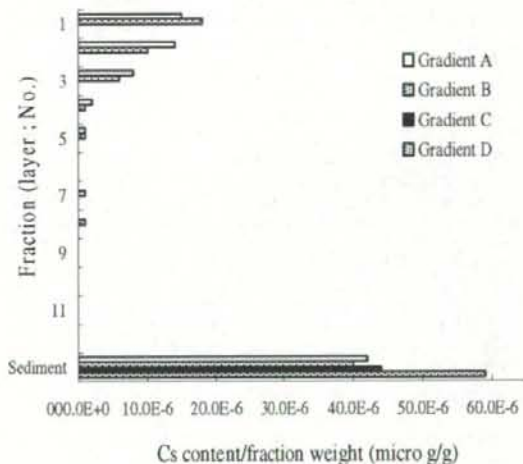


Figure 3. Distribution of Cs in each fraction of *P. ostreatus*-Y1 mycelia cultured on YM agar with 15 mM CsCl following density-gradient centrifugation.

flasks at 27 °C for seven days. After incubation, the culture fluids were centrifuged at 500 rpm for 10 min (15 °C). Each pelleted mycelia sample was combined in a centrifuge tube, and the mycelia were suspended in 35 mL of distilled water. Approximately 5 g of mycelia was shaken in distilled water (35 mL) for the first wash using a test tube mixer (Vortex) for 1 min. For ^{137}Cs elution experiments, a total of 15 2-mL aliquots of the mycelia suspension were collected. At time points of 0, 30, 60, 90, and 180 min after the start of ^{137}Cs elution, the samples were centrifuged (3000 rpm, 10 min) in triplicate for each time point to isolate mycelia from the mycelia-washing solution. Approximately 0.3–0.4 g of fresh mycelia and 2 mL of wash solution were obtained per sample. ^{137}Cs activities in the mycelia (sediment before and after washing), solutions for elution (supernatant before and after washing), and $^{137}\text{CsCl}$ standard solution in 0.5 mL tubes were measured with an auto well γ -ray counter (Aloka; Auto Well Gamma System ARC-380CL) for 10 min. The ^{137}Cs contents (cpm g $^{-1}$) of the samples were determined on the basis of $^{137}\text{CsCl}$ standard solution activities and the weight of samples. Loss of ^{137}Cs from the mycelia during the first wash was about 47%.

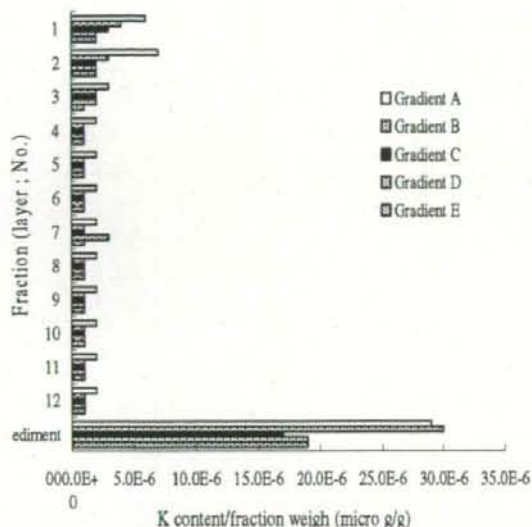


Figure 4. Distribution of K in each fraction of *P. ostreatus*-Y1 mycelia cultured on YM agar following density-gradient centrifugation. The basic K content in YM medium was 8.4 mM.

Measurement of Cs and K Contents in *P. ostreatus* Mycelia Fraction by Cell Fractionation. *P. ostreatus* mycelia incubated on YM agar medium (0.4% yeast extract, 1% malt extract, 0.4% glucose, and 1.8% agar, pH 7.2) containing 15 mM CsCl at 27 °C for 20 days were harvested and washed with distilled water. The mycelia were disrupted using a Bead-Beater (Biospec Products, Bartlesville, OK, USA). The chamber was filled with 1 M sorbitol (Sigma), and mycelia were homogenized with glass beads (diameter: 0.5 mm) in a vessel filled with crushed ice for 1 min. The homogenized mycelia were fractionated according to the revised method for isolating vacuolar and mitochondrial pellets (19, 20). The homogenized mycelia were centrifuged at 600g for 5 min (15 °C), and the supernatant was additionally centrifuged at 15,000g for 20 min (15 °C) to obtain the sediment. The sediment was suspended in 6 mL of 1 M sorbitol. Eighteen milliliters of 1.8 M sucrose (Sigma) + 1 M sorbitol, 12 mL of 1.8 M sucrose and 6 mL of mycelia suspension in 1 M sorbitol were added to a centrifuge tube in the order of lower to upper layers, and then centrifuged at 43,000g for 120 min (4 °C). In addition to the above-mentioned mycelia suspension, control samples were also prepared according to the procedures described above, except that the top layer was changed to 1 M sorbitol solution containing 5 mM CsCl or 1 M sorbitol containing 5 mM KCl (Wako). A total of 12 layers (3-mL aliquots of both mycelia suspension and controls) were pipetted into clean tubes to fractionate the liquid and sediment layers. These fractions were digested individually with approximately 20 mL of 14 M HNO₃ (Wako) and 2 mL of 30% H₂O₂ (Wako) at 160 °C for 4–12 h on a hot plate. The digested samples were diluted with up to 10 mL of 0.5 N HCl (Wako and MilliQ Element), and then the Cs and K contents were analyzed separately by flame atomic absorption spectrometry (Hitachi A-2000) using hollow cathode lamps (Hamamatsu Photonics, Cs; 852.1 nm absorption lines and Hitachi, K; 766.5 nm absorption lines). Similarly, flame atomic absorption spectrometry of the control CsCl and KCl fractions was performed without the wet digestion. The K content in the YM medium as a blank value was 8.4 mM.

Detection of Polyphosphate by Staining of *P. ostreatus* Mycelia. *P. ostreatus* was inoculated onto a YM agar plate (0.4% yeast extract, 1% malt extract, 0.4% glucose, and 1.8% agar, pH 7.2) containing 15 mM CsCl and incubated at 27 °C for seven days. The control sample (without Cs) was also incubated under the same conditions. Cultivated mycelia were carefully scraped from the plate and stained with 50 μg mL⁻¹ of DAPI (ABD Bioquest) for 10 min. Stained mycelia were

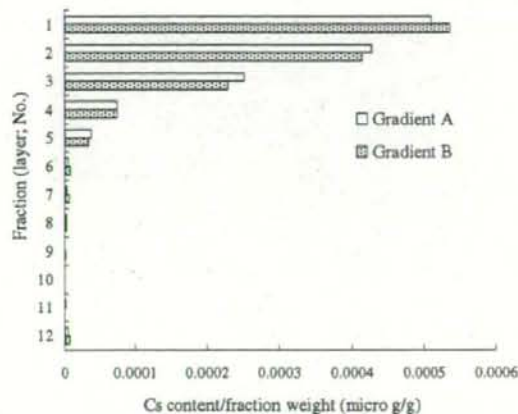


Figure 5. Distribution of Cs in each fraction of the control solution containing 5 mM CsCl following density-gradient centrifugation.

washed with distilled water and observed by fluorescence microscopy using a confocal laser scanning microscope (Carl Zeiss LSM 510 META). By DAPI staining, the fluorescence of polyphosphate (polyphosphate-DAPI) and DNA-DAPI can be observed around 526 nm (yellowish fluorescence) and 456 nm (bluish fluorescence), respectively (21). The presence of fluorescence corresponding to polyphosphate in mycelia and its morphology were observed.

RESULTS AND DISCUSSION

SEM Observation and Qualitative Elemental Analysis of *P. ostreatus* Cultivated Mycelia. SEM equipped with EDX, which can detect the presence of elements in the microstructure of plant tissue, is one of the most effective techniques in elemental analysis. As shown in Figure 1a, a nonuniform distribution of circular white spots was observed in *P. ostreatus* mycelia cultivated in the presence of 25 mM CsCl. In addition, the spectra obtained from elemental analysis revealed that the Cs, P, and Mg concentrations of these spots were clearly higher compared to that of other regions without spots (Figure 1b). By SEM-EDX analysis, it was shown previously that the Cs content of the mycelial root (old regions of the hyphae) of *P. ostreatus* is approximately 5-times higher than that of the mycelial tip (growing regions of the hyphae) (22). In addition, in the previous study on Cs accumulation by soil microorganism (23) concerning the transfer of Cs from soil to mushroom (24), the presence of white spots at regular intervals was also confirmed using the mycelia of a soil filamentous bacterium (*Streptomyces lividans*) by SEM, and semiquantitative elemental analysis by EDX revealed that the Cs and P contents in these spots were as high as in *P. ostreatus* mycelia used in the present study. K, a chemical analogue of Cs, and P, an essential element transported by arbuscular mycorrhizal fungi (AMF), have been suggested to influence the uptake, translocation, and transfer of radiocesium by AMF (25). It was demonstrated that the transport of ¹³⁴Cs was affected by P independently of the concentration of K, and increasing the concentration of P in the hyphal compartment resulted in higher uptake and accumulation of ¹³⁴Cs in roots and shoots of *M. truncatula* plants (26). From these results, it was considered that Cs trapped in *P. ostreatus* mycelia was accumulated in specific regions with higher levels of P and concentrated. As specific regions where higher levels of P are present in mycelia, vacuoles accumulating polyphosphate have been suggested (27). The white spots where

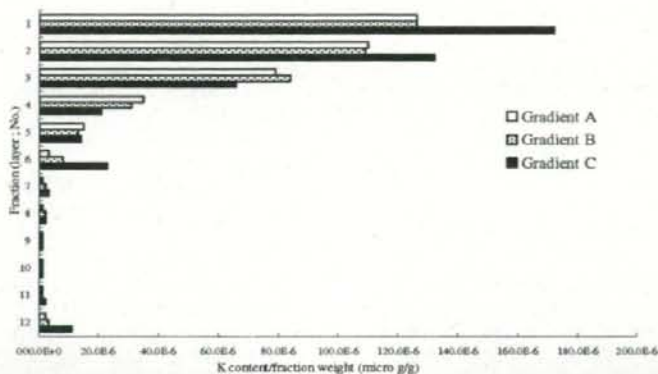


Figure 6. Distribution of K in each fraction control solution containing 5 mM KCl following density-gradient centrifugation.

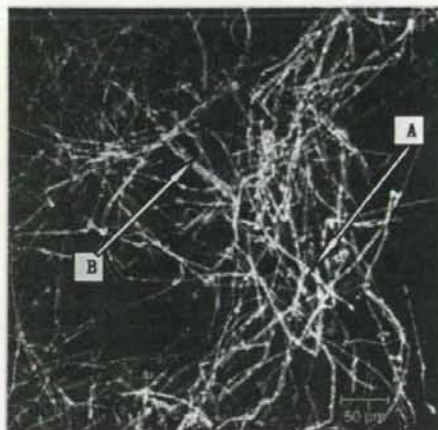


Figure 7. Image of 4',6-diamidino-2-phenylindole (DAPI)-stained *P. ostreatus*-Y1 mycelia cultured on a YM agar plate containing 15 mM CsCl with laser-scanning confocal microscopy. A, polyphosphate-DAPI (yellowish granular fluorescence) around 526 nm; B, DNA-DAPI (bluish granular fluorescence) around 456 nm.

the Cs and P contents were higher than other regions, observed in the mycelia cultivated with CsCl by SEM could be a result of Cs⁺ trapped by polyanionic polyphosphate.

Uptake of ¹³⁷Cs into *P. ostreatus* Cultivated Mycelia and Elution Fractions. The ¹³⁷Cs contents in mycelia and the elution fractions after washing were investigated. The rate of uptake of Cs into *P. ostreatus* mycelia cultured for seven days and elution by washing was investigated using ¹³⁷Cs. Figure 2 shows the ¹³⁷Cs concentrations in water used for ¹³⁷Cs elution and mycelia at each time point in the second wash that was retained after the first wash. Mycelia of *P. ostreatus* cultivated in YM liquid medium containing ¹³⁷Cs with shaking were harvested by centrifugation and washed with distilled water. In Figure 2, error bars indicate the mean value ± the standard deviation of three replicates. The ¹³⁷Cs concentrations in each 2 mL aliquot of water at 0, 30, 60, 90, and 180 min after the start of elution were almost constant. These results suggested that the elution of ¹³⁷Cs from mycelia into distilled water occurred rapidly after the start of elution and that the increase or decrease in the amount of ¹³⁷Cs eluted with time was small. The ¹³⁷Cs activities in the water used for the ¹³⁷Cs elution experiment were approximately 4–6 times higher than those in the mycelia after washing. The previous ¹³³Cs NMR studies showed that the

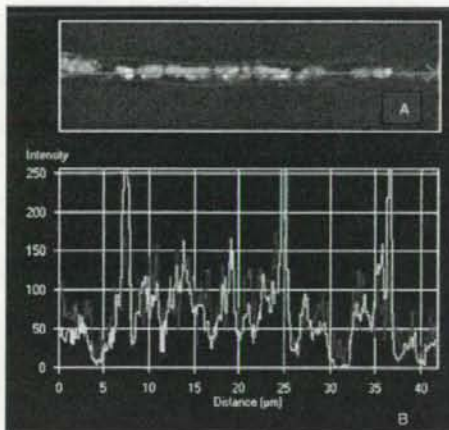


Figure 8. Image and fluorescence intensity caused by polyphosphate-4',6-diamidino-2-phenylindole (DAPI)-stained *P. ostreatus*-Y1 mycelia cultured on a YM agar plate containing 15 mM CsCl by laser-scanning confocal microscopy (526 nm). (A) Image of fluorescence from polyphosphate-DAPI in the mycelium. (B) Fluorescence intensity of the mycelium. The scale of the horizontal axis of the graph (B) is the same scale as the fluorescence microscopy image of *P. ostreatus* mycelia (A).

spectra exhibited two resonance signals arising from the mycelia and the fruiting bodies, whereas one signal was detected from CsCl standard solution (0 ppm) and the other lower magnetic field. This suggested that Cs existed in a different state in the samples (9, 18). Taking these results into consideration, part of the ¹³⁷Cs was concluded to be in its ionized form in the mycelia and could be eluted by washing, but the other Cs form was trapped by polyphosphate and remained in the mycelia even after washing.

Presence of Cs and K in *P. ostreatus* Mycelia Fraction as Assessed by Cell Fractionation. After density gradient centrifugation of the mycelial homogenate of *P. ostreatus*, 13 fractions were obtained. Yellowish suspensions, fractions 3 and 13 (sediment), were regarded as containing mitochondrial and vacuolar material, respectively (19, 20). However, confirmation of the composition by electron microscopy was not performed. The results of atomic absorption spectrometry of Cs and K in each fraction are shown in Figure 3 (for Cs obtained from four experiments) and Figure 4 (for K obtained from five experiments), respectively. In both graphs, the contents of Cs or K derived from *P. ostreatus* mycelia are expressed as concentra-