精度に大きく影響を与え、得られた定量分析値の信頼性は厳密な意味で確保できていないと言える。

③以外に関しては、方法論および実際の操作の妥当性検証の実施が要求される。しかしながら、実験誤差を極力小さくし、その研究方法や実験系自体の再現性を確保しているのが現状である。

一般的に分析機器の検出器の応答能は一定ではなく、経時的に変動しているため、試料測定毎の検量線の作成が要求される。しかし、検量線作成は多大な時間と労力を要し、結果として迅速且つ精確な分析を妨げることとなる。このことから、昨年度は、GC/MS における機器の不確かさを小さくするため、機器の検出感度のばらつきを補正し、一度設定した検量線を用いて、以降の分析を可能とする方法について、その有効性を検討した。

今年度は PAH について、精確な定量分析が可能な普遍的な検量線を作成し、迅速且つ網羅的な定量的スクリーニングが可能で、国際単位系 (SI) へのトレーサビリティを確保し、分析の一連の不確かさを明示することが可能な自動定量分析法の方法論の開発を試みた。すなわち、PAH の市販試薬製品について、qNMR を用いた計量学的に信頼性の高い純度値を求めた。また、昨年度構築した複数の内標準物質 (Internal Standard: IS) およびチューニングデータを用いた新規補正法 Multi Internal standards Calibration Objective (MICO) が GC/MS データベース構築に応用可能と考えられたため、定量分析値の信頼性の更なる向上を目指し、測定誤差を消去し、且つ、迅速、精確、網羅的な多次元データベースを用いた定量分析法 (Multi Dimensional Property Database - MICO - quantitative GC/MS (MDPD-MICO-qGC/MS)) を開発した。

B. 研究方法

1. 試薬および試液

PAH は、qNMR による純度測定には市販標準品または試薬 9 製品を、MDPD には PAH 18 種混合標準液 (AccuStandard, Cat. No. M-610-QC-FL) を用いた。純度測定に供した対象化合物名および化学式、分子量、メーカー、製品番号、Lot 番号を Table 1 に示す。

NMR の基準物質には、認証標準物質 (Traceable Reference Material: TRM) である 1,4-Bis(trimethylsilyl)benzene-d_4 (BTMSB, 和光純薬工業株式会社)を、BTMSB の校正には CRM である Diethyl phthalate (DEP, NMJ CRM 4022-b, 純度 99.98 ± 0.09 w/w%, 独立行政法人 産業技術総合研究所) を使用した。重アセトン (acetone-d_6) は、Isotec 製 (99.9 atom %D) を用いた。

GC/MS の IS は BTMSB、DEP および Bisphenol-A (NMJ CRM 4030-a, 純度 99.92 ± 0.06 w/w%, 独立行政法人 産業技術総合研究所) の 3 種を選定した。Acetone は和光純薬工業株式会社 高速液体クロマトグラフ用を、精製水はミリ–Q (超純水) を用いた。

2. 装置および器具

核磁気共鳴装置 (NMR) はオートサンプラー付き JNM-ECA (600 MHz, 日本電子株式会社 (現：株式会社 JEOL RESONANCE))、qNMR および qNMR 多変量解析のケミカルシフト値は、BTMSB を基準シグナル (0 ppm) とし、δ 値を ppm 単位で表した。

GC/MS は Shimadzu GCMS-QP2010 Plus (島津製作所株式会社) を用いた。

なお、ウルトラミクロ天秤は XP2U (メトラートレド株式会社) を用い、試料の秤量値は最小目盛 0.0001 mg まで読み取った値を用いた。

標準液および試料溶液の調製には、化学用体積計 (5-20 mL メスフラスコ、10-500 μL マイクロシリンジ) または電動オートピペッター(マルチピペット Xstream (エッペンドルフ株式会社)、10 mL (不確かさ±0.4%)、1-5 mL (不確かさ±

0.5%))を用いた。

3. qNMRによるPAHの純度決定

3-1. qNMR 標準液の調製および濃度校正

qNMR 標準液の調製および標準液中の BTMSB の濃度校正は既報に準じた。すなわち、BTMSB 約 10 mg を精密に量り取り、Aacetone-d_6 50 mL に定容したものを qNMR 用標準液とした。qNMR 用標準液中の BTMSB の濃度を下記に従い、DEP により校正して求めた。CRM の一つである DEP 約 10 mg を精密に量り取り、qNMR 用標準液 1.0 mL に溶解した。この溶液 0.6 mL を NMR 試験管 (5 mm φ × 200 mm, 和光純薬工業株式会社) に封入したものを BTMSB 濃度校正用試料溶液とした。この溶液を qNMR に付し、DEP の CH_2 × 2 および BTMSB の CH_3 × 6 に由来するシグナル面積、分子量、濃度等を式(1) に代入し、qNMR 用標準液中の BTMSB の濃度を校正した。

$$W_{BTMSB} = \frac{M_{BTMSB} \times I_{BTMSB}}{H_{BTMSB}} / \frac{M_{DEP} \times I_{DEP}}{H_{DEP} \times W_{DEP}} \times \frac{P_{DEP}}{100} \quad \text{--- (1)}$$

ただし、W_{BTMSB}, W_{DEP} = BTMSB および DEP の濃度 (mg/mL)、M_{BTMSB}, M_{DEP} = BTMSB および DEP の分子量 (226.49832 および 222.23370)、H_{BTMSB}, H_{DEP} = BTMSB の CH_3 × 6 および DEP の CH_2 × 2 のプロトン数、I_{BTMSB}, I_{DEP} = BTMSB の CH_3 × 6 および DEP の CH_2 × 2 のシグナル面積、P_{DEP} = DEP の純度 (99.98 w/w%)。

3-2. PAH の純度決定

PAH 標準品を約 10 mg 精密に量り取り、予め調製した qNMR 用標準液 1.0 mL に溶解した。これらの溶液 0.6 mL を NMR 試験管に封入したものを試料溶液とした。この試料溶液を qNMR に付し、BTMSB のシグナル強度面積、PAH に由来するそれぞれの特定シグナルの相対面積、分子量、濃度等を式(2) に代入し、PAH の純度を算出した。この一連の操作を 3 試行し、各シグナルより算出された純度値の平均 (n=3 Ave.) を最終的に PAH の純度値として表した。

$$P_{PAH} = \frac{I_{PAH} / H_{PAH}}{I_{BTMSB} / H_{BTMSB}} \times \frac{M_{PAH} / W_{PAH}}{M_{BTMSB} / W_{BTMSB}} \times 100 \quad \text{--- (2)}$$

ただし、W_{BTMSB}, W_{PAH} = BTMSB および PAH の濃度(mg/mL)、M_{BTMSB}, M_{PAH} = BTMSB および PAH の分子量 (226.49832 および Table 1)、I_{BTMSB}, I_{PAH} = BTMSB および PAH の特定基のシグナル強度面積、H_{BTMSB}, H_{PAH} = BTMSB および PAH の特定基のプロトン数、P_{PAH} = PAH の純度 (%)。

3-3. qNMR 測定条件および解析処理

qNMR 測定条件の基本情報は Table 1-2 に示した。qNMR データ解析には、得られた Free Induction Decay (FID) 信号データを定量解析ソフトウェア(日本電子株式会社 (現：株式会社 JEOL RESONANCE), Alice2 for qNMR) に導入して自動処理した。すなわち、このソフトウェア上で、qNMR データをフーリエ変換および自動位相調整を行い、BTMSB および特定シグナルの積分範囲設定等を設定後、予め入力した BTMSB および PAH の濃度、分子量、特定基のプロトン数等の化合物情報から自動解析処理を行い、定量値 (純度%) を式(2) に従い算出した。

4. GC/MS による PAH の分析条件

GC/MS の測定条件は以下に記述する。

GC/MS 条件: カラム, SGE forte GC Capillary Column BPX-5 (0.25 mm i.d.×30 m, 0.25 μm); 注入方法, スプリットレス; 注入量, 1 μL; インサート, 片側テーパーウールあり; キャリヤーガス, He; カラム温度, 45℃ - (30℃/min) - 190℃ - (2℃/min) - 200℃ - (15℃/min-260℃) – (5℃/min)

- 315℃ (10min); イオン化法, EI; イオン化電圧, 70 eV; インターフェイス温度, 280℃; イオン源温度, 300℃; 測定モード, スキャンモード; スキャン範囲 (*m/z*), 50-550。測定前に EPA625 の DFTPP チューニングを行った (1 回/日)。その後、検量線を作成し、検量線を作成した日とは別の日に測定試料を測定した。

C. 結果及び考察

1. qNMR による PAH の純度決定

qNMR を用いて、PAH の市販試薬製品の純度値を測定した。qNMR スペクトル上に観察された基準物質 BTMSB および PAH に由来する各シグナル面積、水素数、濃度等を関係式(2) に代入し、それぞれの純度値を算出した。定量に用いた各シグナルおよびそれぞれのシグナルより算出された純度値の平均 (シグナル間 Ave.) を PAH の純度値として表した (Table 3)。その結果、Anthracene 98.3% (シグナル間 RSD 0.2%)、Fluoranthene 99.1% (0.4%)、Benz[*a*]anthracene 95.2% (0.6%)、Benzo[*b*]fluoranthene 99.1% (0.6%)、Benzo[*k*]fluoranthene 98.3% (0.5%)、Benzo[*a*]pyrene 90.2% (0.9%)、Indeno[1,2,3-*cd*]pyrene 90.1% (1.0%)、Dibenz[*a,h*]anthracene 95.7% (0.2%)、Benzo[*g,h,i*]perylene 81.0% (0.2%) の純度値を与えた。製品によりその純度値は大きく異なり、81.0 ± 0.2 ~ 99.1 ± 0.6 % (Ave. ± RSD) の幅があることがわかった。また、試薬製品に記載されていたクロマトグラフィーを用いた面積百分率による純度値と比較した (Table 4)。qNMR により得られた純度値 (n = 3 Ave.) が 95.3%の Benz[*a*]anthracene、76.1%の Benzo[*g,h,i*]perylene については、製品に純度値の記載がなかったが、仮にこれらの純度を 100%として定量用標準品の代用とし、検量線を作成し定量分析を行ったとすると、得られる定量値は 4.7 および 23.9%の誤差を生じることになることが示唆された。

2. MDPD における純度情報の役割

クロマトグラフィーは、相対ピーク面積比から定量する相対分析法であることから、分析値の信頼性確保や精度管理には、分析対象の標準物質の純度が精確に値付けられていることが不可欠である。そのため、濃度が値付けされた標準物質の使用が必須となりつつあるが、すべての化合物に対して、値付けされた標準物質を供給・入手することは現状では困難である。

標準物質として代用される市販試薬や市販標準品を定量用標準品は、科学的な根拠に基づく純度の証明がほとんどの場合なされていないという問題がある。そのため、これらを用いて定量値を求めたとしても、現状では、SI へのトレーサビリティの確保が不可能となっている。

我々はこれまでに、定量値の信頼性を飛躍的に向上させるため、標準物質に対しては、NMR を用いた SI にトレーサブルな定量分析法 qNMR を開発した。この qNMR および qGC/MS の情報を組み合わせ、測定対象化合物に関する多次元情報 (純度、NMR スペクトル、保持時間、検量線情報、MS スペクトル等) による総合的なデジタルデータベース MDPD を構築することで、これを参照することによって、測定対象の SI トレーサビリティが確保された精度の高い迅速定量分析法の確立を可能とすると考えられた (Fig. 1)。

そこで、本測定で得られた PAH の純度および NMR スペクトル等の化合物情報についても、MDPD に追加し、データの拡充を進めた。

3. MICO-qGC/MS を用いた MDPD の構築

昨年度は、従来のIS一つで補正する内標準法を見直し、複数のISやチューニング結果を用いて機器のドリフトなどの感度変化をキャンセルアウトする定量値の新規補正方法 MICOを考案した。MICOは、装置内変動および装置間変動を補正し、さらに不確かさの小さい定量値

を算出できることから、今年度は、PAH 18種について、多次元データベースにMICOを適用した定量分析法 MDPD-MICO-qGC/MS を構築した。

検量線を作成するための標準試料は、PAHを18種混合した標準液と内標準物質を3種混合した標準液を作製し、それぞれマイクロシリンジで精密にはかり、アセトンで定容した。PAHの検量線は、各内標準物質との相対比により作成した。すなわち、1被検物質あたり、3本の検量線を作成してデータベースに登録した。

データベースの検量線を用いて定量値を算出した。Table 5から明らかなように、想定濃度と定量値 (日の異なる5回の試行の平均) がよく一致した。また、日の異なる5回の試行の相対標準偏差 (RSD) も小さく、定量値が想定濃度の20%以内と精確に定量できた測定は18種を5回試行した計90測定のうち93.3%であった。この結果は、一度標準物質を用いて検量線を作成し、これをデジタルデータ化することにより、MDPD-MICO-qGC/MSが高い分析精度を実現できることを示している。化合物の多次元情報をデジタル登録する際には時間がかかるものの、一旦登録した測定対象化合物については、再度検量線作成を必要とせず、すなわち、測定対象と同一の標準物質を用いることなく、迅速且つ網羅的な定量分析を可能とする方法となった。

D. まとめ

PAH については、純度、NMR スペクトル、保持時間、検量線情報、MS スペクトル等一連の分析操作で重要な多次元情報を得て、デジタルデータベース化することにより、標準物質を用いることなく、より精確な定量分析が可能な新規分析法を構築することができた。

本研究により、これまでに我々が開発したqNMR およびqGC/MS の技術を応用することによって、環境中の化学物質の自動モニタリングは、科学的な根拠に基づいた、且つ、計量学的に定量値の信頼性を確保した定量分析値を算出可能と考えられる。

今後も環境分析において、微量で精確な分析が求められる化合物に対し、MDPD-qNMR-MICO-GC/MS システムの整備を行っていく予定である。

E. 研究発表

1. 論文発表

なし

2. 学会発表

1) 田原麻衣子, 杉本直樹, 小林憲弘, 久保田領志, 穐山浩, 五十嵐良明: GC/MS データベースを用いた定量分析への新規キャリブレーションシステムの適用, 第 49 回全国衛生化学技術協議会年会, 2012.11.21-22, 香川.

Table 1 Information of commercial PAH reagent products

PAH	Formula	Molecular mass	Manufacturer*	Code No.	Lot No.
Anthracene	$C_{14}H_{10}$	178.22920	Wako	015-04211	STF2839
Fluoranthene	$C_{16}H_{10}$	202.25060	TCI	F0016	YZJ7E
Benz[*a*]anthracene	$C_{18}H_{12}$	228.28788	Kanto	04822-60	106N2026
Benzo[*b*]fluoranthene	$C_{20}H_{12}$	252.30928	Wako	028-13651	ALQ8569
Benzo[*k*]fluoranthene	$C_{20}H_{12}$	252.30928	ACROS	279732500	A0199983
Benzo[*a*]pyrene	$C_{20}H_{12}$	252.30928	Sigma-Aldrich	B1760	090M1400V
Indeno[1,2,3-*cd*]pyrene	$C_{22}H_{12}$	276.33068	Wako	091-04451	EPH5305
Dibenz[*a,h*]anthracene	$C_{22}H_{14}$	278.34656	Wako	041-26791	EPQ4053
Benzo[*g,h,i*]perylene	$C_{22}H_{12}$	276.33068	TCI	B2983	EGTSJ

Wako: Wako Pure Chemical Industries, Ltd., TCI: Tokyo Chemical Industry Co., Ltd.,
Kanto: Kanto Chemical Co., Inc., ACROS: ACROS ORGANICS,
Sigma-Aldrich: Sigma-Aldrich Co, LLC

Table 2 Instruments and acquisition parameters

Spectrometer	JNM-ECA600 (JEOL)
Probe	5 mm broadband autotune probe
^{13}C decoupling	Multi pulse decoupling with Phase and Frequency switching (MPF-8)
Spectral width	-5 ~ 15 ppm
Data points	64000
Auto filter	on (8 times)
Flip angle	90°
Pulse delay	60 s (> 5*T_1)
Scan times	8
Sample spin	no spin
Probe temperature	22-25℃
Sample solvent	Acetone-d_6
qNMR reference material	1,4-BTMSB-d_4
Window function	—

Table 3 Calculated purities of samples from listed proton signals

PAH		Signal 1	2	3	4	5	6	7	8	9	10	Average (%, RSD %)
Anthracene	a)	4H, m	4H, m	2H, s								
	b)	7.25	7.82	8.29								
	c)	98.6	98.3	98.1								98.3 (0.2)
Fluoranthene		2H, m	2H, m	2H, d	2H, m	2H, d						
		7.17	7.45	7.68	7.79	7.85						
		98.5	99.0	99.7	99.4	99.0						99.1 (0.4)
Benz[*a*]anthracene		2H, m	1H, t	2H, m	1H, d	1H, d	1H, m	1H, m	1H, s	1H, d	1H, s	
		7.35	7.41	7.48	7.65	7.69	7.88	7.99	8.26	8.73	9.16	
		96.2	94.2	94.5	96.1	95.2	95.0	94.9	95.3	95.6	95.5	95.2 (0.6)
Benzo[*b*]fluoranthene		2H, m	1H, t	1H, t	1H, t	1H, m	3H, m	1H, s	1H, d	1H, d		
		7.22	7.45	7.50	7.60	7.81	7.92	8.23	8.39	8.57		
		99.3	98.6	98.0	99.4	99.1	99.5	100.0	99.3	98.9		99.1 (0.6)
Benzo[*k*]fluoranthene		2H, m	2H, t	2H, d	2H, m	2H, d	2H, s					
		7.29	7.50	7.70	7.77	7.94	8.26					
		99.0	98.3	97.6	98.6	98.2	98.0					98.3 (0.5)
Benzo[*a*]pyrene		1H, t	1H, t	2H, d+t	1H, d	1H, d	1H, d	1H, d	1H, d	1H, s	2H, d+d	
		7.59	7.64	7.79	7.85	7.95	8.09	8.14	8.19	8.42	8.96	
		89.5	90.6	91.0	90.7	90.7	89.5	89.0	89.6	89.9	91.6	90.2 (0.9)
Indeno[1,2,3-*cd*]pyrene		2H, t+t	2H, t+d	1H, d	2H, d+d	2H, d+d	2H, d+d	1H, s				
		7.25	7.90	7.95	8.01	8.12	8.30	8.57				
		91.1	88.7	89.9	89.8	90.9	89.2	90.8				90.1 (1.0)
Dibenz[*a,h*]anthracene		2H, t	2H, t	2H, d	2H, d	2H, d	2H, d	2H, s				
		7.43	7.51	7.62	7.76	7.85	8.78	9.17				
		95.6	95.8	95.9	96.0	95.9	95.5	95.4				95.7 (0.2)
Benzo[*g,h,i*]perylene		2H, t	2H, d	2H, d	2H, d	2H, d						
		7.86	7.97	8.02	8.25	8.97						
		81.3	81.1	80.8	80.9	81.0						81.0 (0.2)

a) Upper column shows a number of proton with the spin-spin coupling (s: singlet, d: doublet, t: triplet, m: multiplet).

b) Middle column shows the signal region (ppm).

c) Lower column shows the purity of each signal (%).

Table 4 Summary of commercial reagent products purities calculated by qNMR and labeled percentage of peak area on chromatogram by manufacturer

PAH	Purity (%)			
	qNMR (n = 3 Ave., RSD %)		Manufacturer *	
Anthracene	98.6	0.54	> 99.5	GC
Fluoranthene	99.2	0.10	—	—
Benz[*a*]anthracene	95.3	0.21	—	—
Benzo[*b*]fluoranthene	99.3	0.17	99.9	HPLC/UV
Benzo[*k*]fluoranthene	98.2	0.27	> 99	—
Benzo[*a*]pyrene	90.2	0.04	> 96	HPLC
Indeno[1,2,3-*cd*]pyrene	91.0	0.95	96.2	HPLC/UV
Dibenz[*a,h*]anthracene	96.0	0.31	97.1	HPLC/UV
Benzo[*g,h,i*]perylene	76.1	6.62	—	—

* The purity means the area percentage of main peak on chromatogram.

Table 5 Calculated quantitative value by MDPD-MICO-qGC/MS

PAH	Quantitative ion (*m/z*)	Concentration (mg/L)	Quantitative value (mg/L)	RSD (%)
Naphthalene	128	1	0.95	12.5
1-Methylnaphthalene	142	1	0.95	11.9
2-Methylnaphthalene	142	1	0.96	12.7
Acenaphthylene	152	1	0.95	11.8
Acenaphthene	153	1	0.95	11.0
Fluorene	166	1	0.96	9.6
Phenanthrene	178	1	0.96	9.0
Anthracene	178	1	0.96	10.3
Fluoranthene	202	0.1	0.098	6.2
Pyrene	202	0.1	0.098	6.2
Benz[*a*]anthracene	228	0.1	0.098	6.8
Chrysene	228	0.1	0.099	6.7
Benzo[*b*]fluoranthene	252	0.1	0.101	12.7
Benzo[*k*]fluoranthene	252	0.05	0.050	14.1
Benzo[*a*]pyrene	252	0.1	0.098	13.7
Indeno[1,2,3-*cd*]pyrene	276	0.1	0.104	23.6
Dibenz[*a,h*]anthracene	278	0.1	0.098	22.1
Benzo[*g,h,i*]perylene	276	0.1	0.104	23.2

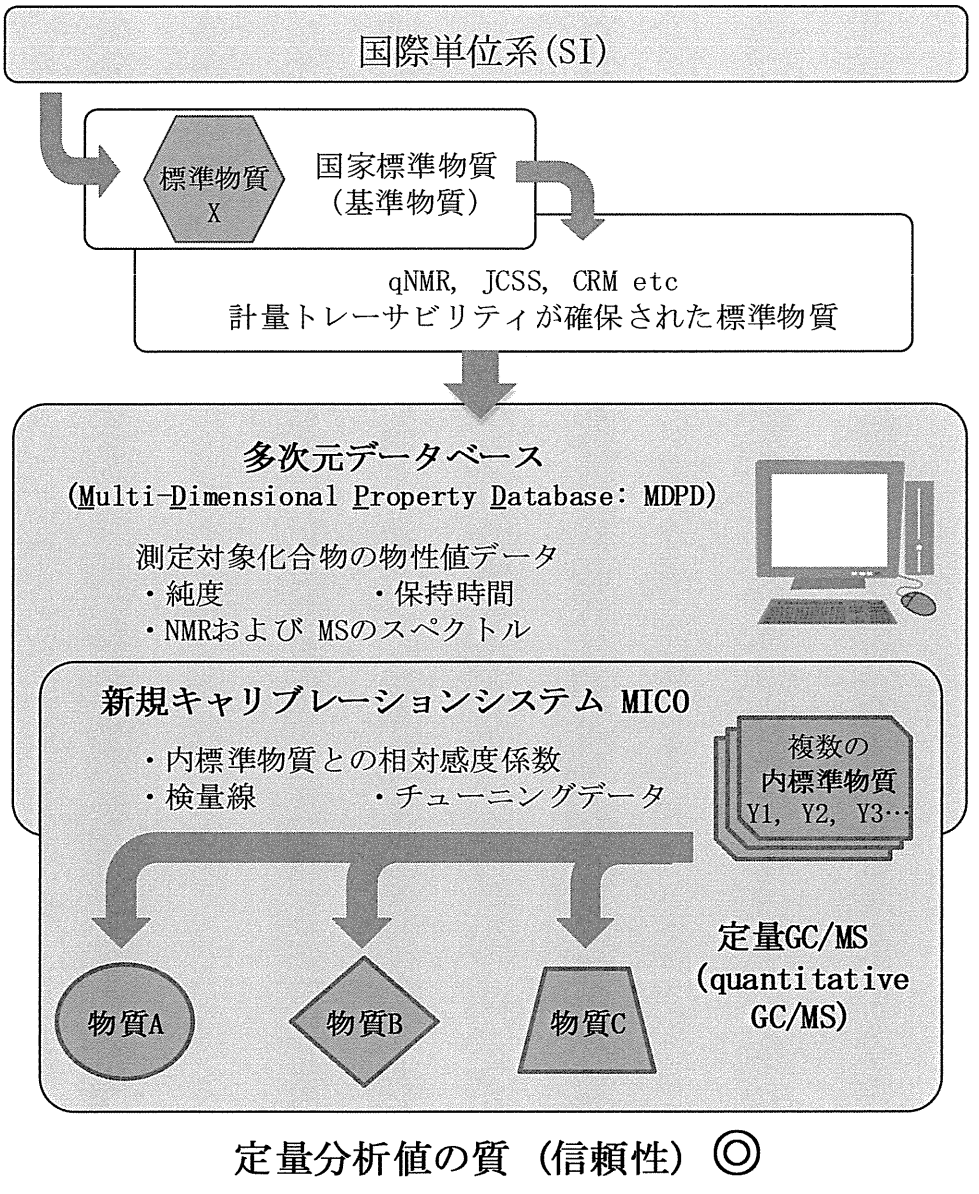

Fig. 1 Strategy of building up SI traceability system for quantitative value using MDPD

Ⅲ．研究成果の刊行に関する一覧表

別紙４

研究成果の刊行に関する一覧表レイアウト

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Tnag, N., Yoda, Y., Otani, N., Kameda, T., Toriba, A., Hayakawa, K., Shima, M	Personal and atmospheric concentrations of ozone in southeastern Hyogo prefecture, Japan.	Chemical & Pharmaceutical Bulletin	**60** (8)	962-966	2012
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Kakikawa, M., Yamamoto, T., Chowdhury, V. S., Satoh, Y., Kitamura, K., Sekiguchi, T., Funahashi, H., Omori, K., Endo, M., Yano, S., Yamada, S., Hayakawa, K., Chiba, A., Srivastav, A. K., Ijiri, K., Seki, A., Hattori, A., Suzuki, N.	Determination of calcium sensing receptor in the scales of goldfish and induction of its mRNA expression by acceleration loading.	Biological Sciences in Space	**26**	26-31	2012

Omori, K., Wada, S., Maruyama, Y., Hattori, A., Kitamura, K., Sato, Y., Nara, M., Funahashi, H., Yachiguchi, K., Hayakawa, K., Endo, M., Kusakari, R., Yano, S., Srivastav, A.K., Kusui, T., Ejiri, S., Chen, W., Tabuchi, Y., Furusawa, Y., Kondo, T., Sasayama, Y. Nishiuchi, T., Nakano, M., Sakamoto, T., Suzuki, N.	Prostaglandin E2 increases both osteoblastic and osteoclastic activities in the scales of goldfish and participates in the calcium metabolism in goldfish	Zoological Science	**29**	499-504	2012
鈴木信雄，舟橋久幸，耿 啓達，柿川真紀子，山田外史，廣田憲之，北村敬一郎，清水宣明，早川和一，三島弘幸，岩坂正和，上野照剛，大森克徳，矢野幸子，池亀美華，田渕圭章，和田重人，近藤隆，服部淳彦	魚類のウロコを用いた評価系の開発と骨代謝研究への応用	まぐね	**7**	174-178	2012
Chizhova, T. Hayakawa, K., Tischenko, P. Nakase, H., Koudryashova,Yu.	Distribution of PAHs in the northwestern part of the Japan Sea.	Deep-Sea Research part II	**86-87**	19–24	2013
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Yano, S., Kitamura, K., Satoh, Y., Nakano, M., Hattori, A., Sekiguchi, T., Ikegame, M., Nakashima, H., Omori, K., Hayakawa, K., Chiba, A., Sasayama, Y., Ejiri, S., Mikuni-Takagaki, Y., Mishima, H., Funahashi, H., Sakamoto, T., Suzuki, N.	Static and dynamic hypergravity responses of osteoblasts and osteoclasts in medaka scales.	Zoological Science	**30** (3)	217-223	2013
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Yachiguchi, K., Matsumoto, N., Haga, Y., Suzuki, M., Matsumura, C., Tsurukawa, M., Okuno, T., Nakano, T., Kawabe, K., Kitamura, K., Toriba, A., Hayakawa, K., Chowdhury, V.S., Endo, M., Chiba, A., Sekiguchi, T., Nakano, M., Tabuchi, Y., Kondo, T., Wada, S., Mishima, H., Hattori, A., Suzuki, N.	Polychlorinated biphenyl (118) activates osteoclasts and induces bone resorption in goldfish.	Environmental Science and Pollution Research		in press	2013

Ⅳ. 研究成果の刊行物・別刷

Personal and Atmospheric Concentrations of Ozone in Southeastern Hyogo Prefecture, Japan

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Twenty-one data sets composed of readings collected by atmospheric ozone monitors worn by individuals on their clothing and installed outside their home or office were collected using Ogawa passive ozone samplers in southeastern Hyogo prefecture, Japan from September 12 to 13, 2011. The concentrations of personal and outdoor ozone ranged from not detectable to 23.2 ppb and from 4.7 to 38.3 ppb, respectively. The mean concentration of personal exposure to ozone was 3.7 ppb and was significantly lower than that of outdoor ozone (18.5 ppb). This suggests that the concentrations of outdoor ozone affect personal ozone exposure. However, in this study, we found no correlation between the concentrations of personal ozone and the total time spent outdoors or the time of day the individual was outside. In contrast, the mean concentrations of outdoor ozone were similar to those of ozone measured at the 12 nearest Ambient Monitoring Stations (AMSs). However, when the AMS was situated near a main road, the regional ozone levels were underestimated.

Key words ozone; air pollution; personal exposure; nitrogen dioxide; Ogawa sampler

Ozone is a strong oxidant and is mainly formed by photochemical reactions from nitrogen oxides and/or reactive volatile organic compounds in the atmosphere. Acute and chronic effects of ozone exposure on human respiratory system have been investigated by many epidemiological studies.[1–3] Some researchers have also reported that ozone had negative effects on cardiovascular and respiratory mortality.[4–6] A strong relationship has been noted between measured outdoor ozone levels and ambient ozone levels measured by the nearest Ambient Monitoring Station (AMS).[7] Ozone concentrations were usually the highest in outdoor and lowest in indoor environment.[8,9] Therefore, in the past, these AMS data were sometimes used to evaluate the personal health risk of ozone.[5,6] However, the specific location of the AMS site and wind direction around the AMS affect its value for evaluating ozone levels at specific localities. The passive ozone sampler, named Harvard or Ogawa passive sampler, developed by Koutrakis *et al.*[10] is a useful tool to accurately measure ozone concentrations in a much smaller area.[8,9,11,12] The passive ozone sampler is able to provide a precise measure of the ozone to which a single person may be exposed, which we call the "personal ozone" in this paper. These samplers can measure personal ozone exposure with a precision of ±4 ppb and relative error of ±10%.[13]

In this study, passive ozone samplers were used to evaluate the ozone exposure levels of selected personnel from Hyogo College of Medicine and of their outdoors. This is the first study to determine the ozone exposure levels for people living in Japan. This study also investigates the relationship between personal outdoor ozone exposure levels and the AMS ozone data recorded by the nearest outdoor sampler.

Experimental

Sampling and Pretreatment Procedures Twenty-one employees of Hyogo College of Medicine were selected as study participants. This study was approved by the ethics committee of Hyogo College of Medicine, and informed consent was obtained from each participant before the study. All participants live in five cities of southeastern Hyogo prefecture and their houses are located there (Fig. 1). Each person and house was monitored by a set of personal/outdoor passive ozone samplers (Ogawa sampler manufactured by Ogawa and Co., Ltd., Kobe, Japan). The personal sampler was pinned to the top front side of participant's clothes; the outdoor sampler was placed in a well-ventilated area outside the participant's house. Sampling was performed simultaneously starting at 07:00 September 12, 2011. A total of forty-two 24-h samples were collected and stored in a refrigerator (4°C) until they were analyzed. The study participants were also instructed to record their daily activities from 7:00 to 12:00, from 12:00 to 18:00, and from 18:00 to 24:00, so that the amount of time spent outdoors could be estimated (Fig. 2).

The passive ozone sampler consists of two glass fiber filters coated with sodium nitrite and potassium carbonate.[14] The mechanism is based on the oxidation reaction of nitrite by ozone to produce nitrate. In this study, the mean concentrations of ozone during the sampling period are estimated by the amount of produced nitrate and collection rate (21.8 mL/min).[14] The filters were treated according to the Ozone Passive Sampler Protocol published by Ogawa & Co., Ltd.[14] Two glass fiber filters were placed in a vial and 5 mL ultrapure water (Milli-Q) was poured in the vial. After shaking gently, the vial was left to stand for approximately 30 min. About 1 mL of supernatant solution was filtered (Cosmonice Filter (W), pore size 0.45 μm, φ13 mm, Nacalai Tesque, Kyoto, Japan) and 100 μL of filtrate was injected into the HPLC system described below.

HPLC System and Chemicals Nitrate was analyzed by using an HPLC system with an UV detector. The HPLC system consisted of a mobile phase pump (SI-1/2001, Shiseido, Tokyo, Japan), an UV-visible detector (SI-1/2002, Shiseido, Tokyo, Japan), a chromatopac integrator (C-R7A, Shimadzu, Kyoto, Japan), a degasser (SI-1/2009, Shiseido, Tokyo, Japan), a column oven with a manual injector (SI-1/2004,

The authors declare no conflict of interest.

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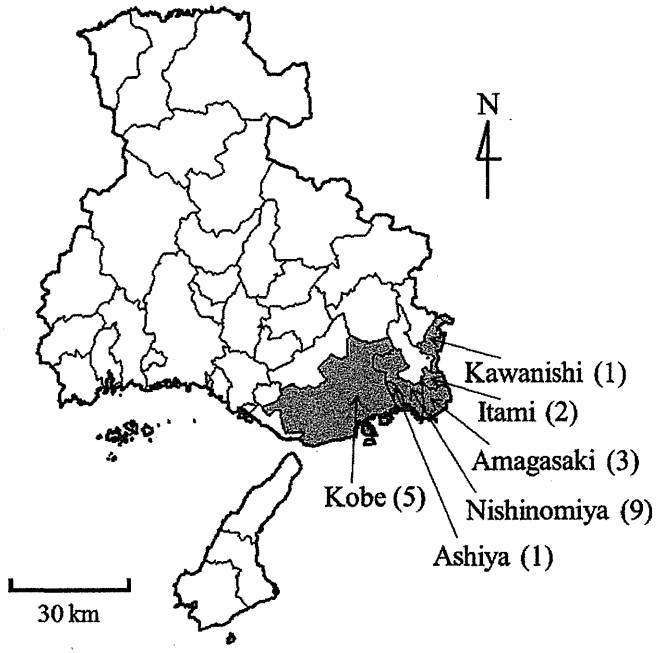


Fig. 1. Cities in Southeastern Hyogo Prefecture in Which Samples Were Collected

Numbers in parentheses indicate the number of samples collected in each city.

Shiseido, Tokyo, Japan), a guard column (Shim-pack IC-GA2, 4.6 i.d.×10 mm, Shimadzu, Kyoto, Japan), and a non-suppressor type analytical column (Shim-pack IC-A3, 4.6 i.d.×150 mm, Shimadzu, Kyoto, Japan). The mobile phase was 0.4 mM disodium phthalate (Tokyo Kasei, Tokyo, Japan) and the flow rate was 1.0 mL/min. Both the guard column and the analytical column were maintained at 40°C in the column oven. Standard nitrate (sodium nitrate) was purchased from Kishida Chemical Co., Ltd. (Osaka, Japan).

Quality Control Indirect photometric detection ion chromatography was used for nitrate analysis. A standard solution of nitrate (100 μL) was injected into the analysis system to verify the method. The calibration curve showed good linearity (r=0.9933) from 0.1 to 1.0 μg/mL. The detection limit (S/N=3) was 0.1 μg/mL and the relative standard deviation (n=3) was 4%.

In order to assess the accuracy of the passive samplers, data from collocated passive samplers were compared to those obtained from a UV Ozone Monitor (EG-700, EBARAJITSU-GYO Co., Ltd., Kanagawa, Japan) at the roof of No. 9 building of Hyogo College of Medicine. The mean (±S.D.) ozone concentrations of five 24-h data sets obtained from passive samplers and Ozone Monitor were 23.5 (±2.96) ppb and 23.9 (±2.14) ppb, respectively. In addition, the Ozone Monitor-passive sampler/Ozone Monitor ratio of each data set was less than 10% (in the range from 0.9 to 8.4%). Approximately 10% of the samplers from the same batch were used as field blanks. The mean (±S.D.) concentrations of four field blanks were 11 (±1.1) ppb. The values from the field blanks were subtracted from the ozone measurement for calculation of the concentrations of nitrate collected by the passive samplers.

Data Analysis For statistical treatment, correlation analysis and multiple regression analysis were performed by using a statistical analysis program (PASW Statistic 18, IBM, U.S.A.). For the multiple regression analysis, the following form of the model was used:

$$C_o = \beta_1 C_a + \beta_2 P + \beta_3 D + \varepsilon \quad (1)$$

where C_o and C_a are the outdoor and the AMS ozone concentrations, respectively. P is the relative position of the outdoor sampler with respect to the nearest AMS (based on the wind direction including leeward, windward, and parallelism); D is the distance between the outdoor sampler and its nearest AMS and ε is the error term. The distance between the outdoor samplers and the nearest AMS was between 0.5 and 4.4 km.

Results and Discussion

Outdoor Ozone A total of 21 outdoor ozone samples were collected for this study. The mean and median ozone concentrations in these sample were 18.5 and 17.0 ppb, respectively, the same as the concentration levels recorded from the 12 AMS sites nearest to the outdoor samplers (mean, 16.2 ppb; median, 15.5 ppb, Fig. 3).[15] However, the concentrations of outdoor ozone measured at study participants' houses ranged from 4.7 to 38.3 ppb, while those of ozone measured by the AMS ranged from 9.5 to 20.2 ppb. The difference between the two concentration ranges was considerably large (Fig. 3). No clear relationship was observed between the outdoor and AMS ozone concentrations (Fig. 4, solid line; n=21, r=0.161, p=0.481). However, because ozone reacts with nitric oxide in the atmosphere, local variations in nitric oxide concentrations may affect at atmospheric ozone levels.[16] When nine data points for the outdoor samplers and the AMS's located within 100 m of main road were deleted, a significantly positive correlation between the outdoor ozone concentrations and the AMS ozone concentrations exists (Fig. 4, dotted line; n=12, r=0.711, p=0.001). Therefore, these results suggest that the ozone concentrations recorded by the AMS, which keeps is placed away from the main road, could represent the mean levels of outdoor ozone concentrations at the home around the AMS.

In order to further evaluate the effects of wind direction and distance on the ozone concentrations determined from outdoor samplers with respect to its nearest AMS, a multiple regression analysis was performed. As shown in Eq. 2, no relationship was observed between the outdoor ozone concentrations and the relative position of the AMS (p=0.89) or the distance (p=0.11).

$$C_o = 42.6 - 1.02 C_a - 0.46 P - 3.79 D \quad (2)$$

Similarly, if the same nine values for the outdoor samplers and the AMS mentioned above were ignored (samples collected within 100 m of a main road), the analysis result was

$$C_o = 0.26 + 1.08 C_a - 0.80 P - 0.58 D \quad (3)$$

In this model, only the coefficients of outdoor ozone concentrations and the AMS ozone concentrations were significant (p=0.05). The relative position and the distance are less important for the outdoor ozone concentrations measured by the Ogawa samplers.

Personal Ozone This is the first study to report the personal exposure levels of ozone in Japan. A total of 21 personal ozone samples were obtained for this study. The mean and the median concentrations of personal ozone samples were 3.7 and 2.7 ppb, respectively, and these concentrations were significantly lower than outdoor ozone (Fig. 3). The range of

Questionnaire on Ozone Exposure Investigation

1. ID _____

2. Address _____

3. Start Time

( )-1 Sep. 12 2011 _____ H. _____ M. (personal)

( )-2 Sep. 12 2011 _____ H. _____ M. (outdoor)

4. Stop Time

( )-1 Sep. 13 2011 _____ H. _____ M. (personal)

( )-2 Sep. 13 2012 _____ H. _____ M. (outdoor)

5. Time in outdoor

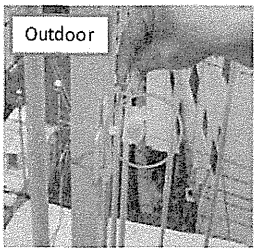
06:00 - 12:00 _____ H. _____ M.

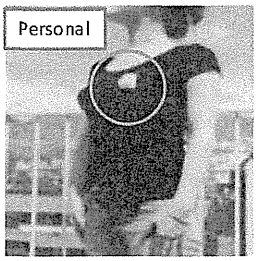
12:00 - 18:00 _____ H. _____ M.

18:00 - 24:00 _____ H. _____ M.

6. Sampling Method

* Open the container and remove the sampling badge from the resealable bag.
* Check that the ID label on the bottle; (ID)-1 use for personal ozone and (ID)-2 use for outdoor ozone.
* Place sampling badge as same as the following Figures.





* After sampling, replace the re-sealable bag in the brown storage bottle and tighten cap securely.
* Record daily activities during sampling period.

Noto

Thank you very much for your collaboration!

Department of Public Health,
Hyogo College of Medicine

Fig. 2. Questionnaire on Ozone Exposure Investigation

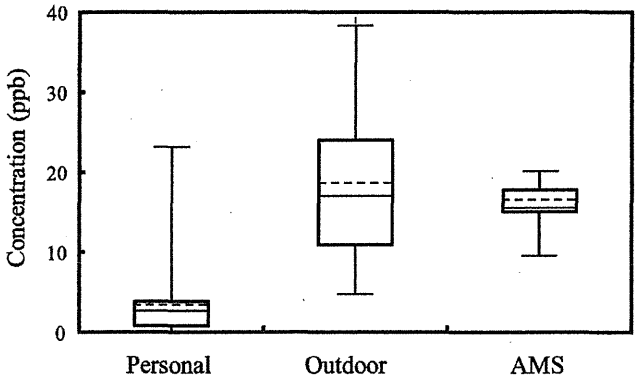


Fig. 3. Ozone Concentrations Found in Personal and Outdoor Samples and Ozone Levels Recorded by the Ambient Monitoring Stations (AMS)

Error bars indicate the range from minimum to maximum concentrations of each sample set. The upper and lower edges of each box represent the 75th and 25th percentile concentration ratios, respectively. Within each box, mean and median concentrations are indicated by the dashed and solid lines, respectively.

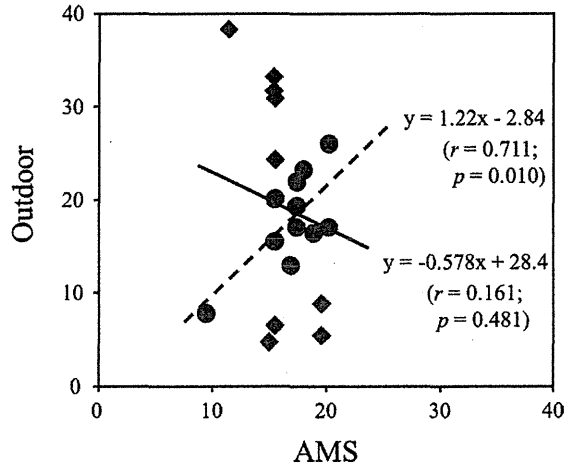


Fig. 4. Correlation between Outdoor Ozone Concentrations and the AMS Ozone Concentrations

Solid line: all data (n=21), Dotted line: nine data points for the outdoor samplers and the AMS's located within 100m of main road were deleted (n=12).

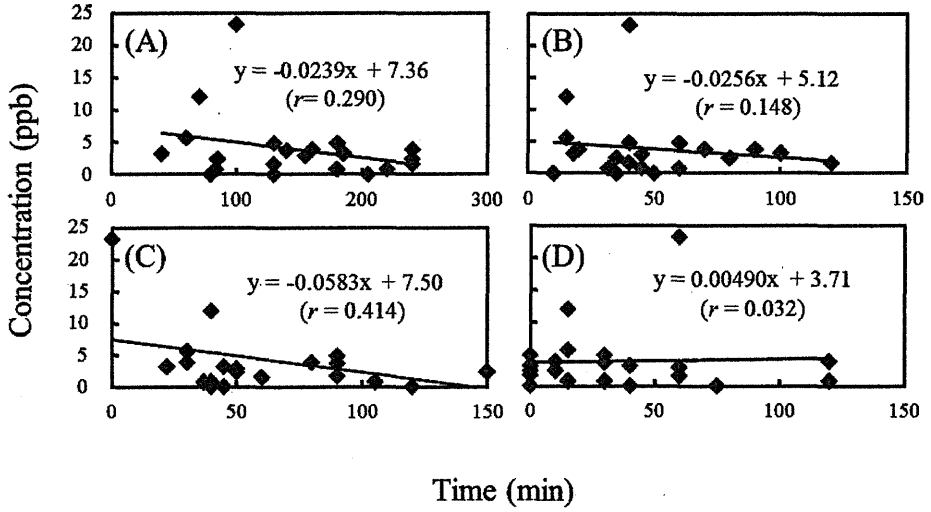


Fig. 5. Distribution of Data Points Showing the Lack of Correlation between Personal Ozone Concentrations and the Total Amount of Time Spent Outdoors

(A) 07:00–24:00; (B) 07:00–12:00; (C) 12:00–18:00; (D) 18:00–24:00.

concentrations for personal ozone was from not detectible to 23.2 ppb. Compared with other studies using the same sampling methodology, the mean concentrations of personal ozone found in this study were similar to levels as in Nashville (3.5 ppb)[9] and lower than those in Mexico City (7.8 ppb).[16]

Atmospheric ozone is formed during daylight from complex mixtures of nitrogen oxides and reactive volatile organic compounds by photochemical reactions.[17] At night, however, ozone reacts with nitrogen monoxide and disappears slowly.[18] Therefore, ozone concentrations are usually the highest in the afternoon and low in the morning. Lee *et al.* reported that the concentrations of personal ozone were affected not only by the total amount of time spent outdoors but also the time of day the personal was outside.[9] In this study, we plotted the concentrations of personal ozone and personal daily activities, as shown in Figs. 5A through D. We, however, found no correlation between the concentrations of personal ozone and the total time spent outdoors or the time of day the individual was outside. Furthermore, we also found no correlation between the concentrations of personal ozone and the concentrations of outdoor ozone at the same locality (r=−0.208, p=0.352). We cannot explain these results, perhaps because our questionnaire did not include questions about the office or house construction, equipment, and outdoor environment that they spent during sampling period. Items such as a window fan,[9] a laser printer,[19] or an air cleaner[20] might increase ozone levels, and the close proximity of a main road might be decrease the ozone level.[9] An improved questionnaire will be used in our next study.

In conclusion, the concentrations of personal ozone and outdoor ozone in southeastern Hyogo were investigated by using the Ogawa sampler. The concentrations of personal ozone were significantly lower than that of outdoor ozone. This suggests that the concentrations of outdoor ozone are important for personal ozone exposure. However, our data found no correlation between the concentrations of personal ozone and the total time spent outdoors or the time of day the individual was outside. In contrast, according to our data, the regional ozone levels may be underestimating by installation feature of the AMS, such as near the main roads.

Acknowledgments This work was supported in part by the Grants-in-Aid for Scientific Research (Nos. 23249034, 21256001, and 21590132) from the Ministry of Education, Culture, Sports, Science and Technology of Japan. The authors thank the staff of the Center of Hyogo Unit of the Japan Environment and Children's Study for participating in this study.

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