表 1　調査対象としたカーペットの製品情報

Sample No.	パイル		原産	特殊加工							
	素材	長さ (mm)		撥水	抗菌	防/消臭	防ダニ	防汚	防炎	AB*	防音
01	ウール 100%	3-5	日本	-	○	-	○	-	○	-	○
02		-	日本	-	-	-	○	-	○	○	-
03		2.5-7	日本	-	○	-	○	-	○	-	-
04		約 25	日本	-	-	-	-	○	-	-	-
05	ナイロン 100%	約 21	日本	-	○	○	○	-	○	○	○
06		14-5-3	日本	-	○	-	○	-	-	-	-
07		約 6-22	日本	-	-	-	○	-	○	○	-
08		約 3-6.5	日本	○	-	-	-	-	-	-	-
09	ポリエステル 100%	約 6	日本	-	-	○	○	-	-	-	-
10		-	中国	-	-	-	-	-	-	-	-
11		約 15-33	タイ	-	-	○	○	-	-	-	-
12		5-16	日本	-	○	-	○	-	-	-	-
13	アクリル 100%	約 50	日本	-	○	○	○	-	-	-	-
14		約 9-10	中国	-	-	-	-	-	-	-	-
15		-	中国	-	-	-	-	-	-	-	-
16		約 3-12	日本	-	○	○	-	○	-	-	-
17	ポリプロピレン 100%	-	日本	-	-	-	○	-	-	○	-
18		約 20・50	中国	-	-	-	-	-	-	-	-
19		約 3.5-17	日本	-	-	-	○	-	-	-	-
20		約 2.5-12.5	日本	-	-	-	-	-	-	-	-

* AB：アレルバスター機能（低ホルムアルデヒド等加工）

表 2　カーペットからの TVOC 放散速度と室内空気中濃度増分予測値

Sample ID	TVOC 放散速度 ($\mu g/m^2/h$)	室内空気中増分予測値 ($\mu g/m^3$)
01	2681	2145
02	7269	5815
03	1373	1099
04	150	120
05	1907	1525
06	3123	2498
07	915	732
08	707	566
09	2507	2005
10	40	32
11	4163	3330
12	212	170
13	457	366
14	280	224
15	77	62
16	911	728
17	2248	1799
18	926	741
19	302	241
20	3016	2413

表 3　カーペットから放散される主な VOCs とその放散速度

Sample No. (TVOC) (Tolune μg/m^2/h)	Major Components	Emission rate (Toluene μg/m^2/h)
01	1-Hexanol	387
(2,681)	3-Ethyl-2-methyl-1-heptene	273
	2-Ethyl-1-hexanol	266
	8-methyl-1-Decene	198
	1-Octanol	167
02	1-Hexanol	5,203
(7,269)	1-Octanol	1,177
	2-Ethyl-1-hexanol	292
	1-Decanol	207
	Diethyltoluamide	151
03	1-Butanol	1,153
(1,373)	β-Pinene	54
	2-Ethyl-1-hexanol	51
	Toluene	45
	α-Pinene	38
04	Toluene	51
(150)	2-Ethyl-1-hexanol	43
05	Oxime-2-Butanone	815
(1,907)	2-Ethyl-1-hexanol	228
	2-propoxy-Ethanol	101
	Toluene	90
	8-methyl-1-Decene	70
06	Oxime-2-Butanone	1,725
(3,123)	2-Ethyl-1-hexanol	618
	Toluene	43
	Pentadecane	43
	Tetradecane	35
07	2-Ethyl-1-hexanol	327
(915)	1-Hexanol	154
	Propylene glycol	143
	1-Methoxy-2-propanol	59

	3-Hydroxy-2,4,4-trimethylpentyl 2-methyl-propanoate	58
08	D-Limonene	173
(707)	3-Methoxy-3-methylbutanol	152
	1-Hexanol	124
	α-Pinene	81
	Toluene	64
09	2-Ethyl-1-hexanol	1,437
(2,507)	1-Hexanol	391
	Diethyltoluamide	240
	1-Octanol	147
	Toluene	61
10 (40)	Toluene	40
11	D-Limonene	357
(4,163)	3-Ethyl-2-methyl-1-heptene	354
	(E)-3-Methyl-5-undecene	352
	1,2,3,4,5-Pentmethyl-cyclopentane	265
	(E)-2,2-Dimethyl-3-decene	182
12	2-Ethyl-1-hexanol	89
(212)	D-Limonene	57
	Toluene	50
13	2-Ethyl-1-hexanol	280
(457)	Toluene	89
	Decane	38
14	3-Cyclohexen-1-yl-benzene	81
(280)	Toluene	50
	2-Ethyl-1-hexanol	43
	Hexamethyl-cyclotrisiloxane	23
15 (77)	4-(1-Trichlorosilyl-3,3-dimethylbutyl)cyclopentene	77
16	Octamethyl-cyclotetrasiloxane	307
(911)	2-Ethyl-1-hexanol	242
	Hexamethyl-cyclotrisiloxane	207
	Decamethyl-cyclopentasiloxane	95
	Toluene	28
17	2-Ethyl-1-hexanol	862
(2,248)	1-Hexanol	447
	Diethyltoluamide	248

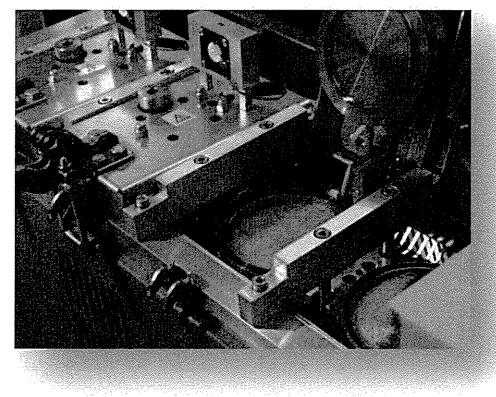
	1-Octanol	140
	Toluene	83
18	4,6-Dimethyl-dodecane	269
(926)	5-(2-methylpropyl)-Nonane	135
	Toluene	122
	p-Xylene	95
	5-(2-methylpropyl)-Nonane	64
19 (302)	2-Ethyl-1-hexanol	302
20	Propylene glycol	748
(3,016)	2-Ethyl-1-hexanol	572
	α-Dimethyl-α-benzenemethanol	328
	4-Methyl-1-undecene	92
	2-Propyl-1-heptanol	75

表 4　カーペットから放散される 12SVOCs の放散速度

($\mu g/m^2/h$)

Sample ID No.	TEP	DEP	TBP	DiBA	TCEP	DBA	DBP	BBP	DOA	TPhP	TEHP	DEHP
01	N.D.	N.D.	0.43	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
02	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.71	N.D.	0.43	N.D.	0.65	N.D.
03	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
04	N.D.	N.D.	N.D.	N.D.	N.D.	0.82	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
05	N.D.	N.D.	N.D.	N.D.	N.D.	0.20	0.60	N.D.	N.D.	N.D.	N.D.	N.D.
06	N.D.	N.D.	0.41	N.D.	N.D.	0.41	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
07	N.D.	N.D.	1.1	N.D.	N.D.	0.91	N.D.	N.D.	N.D.	N.D.	0.69	N.D.
08	N.D.	N.D.	N.D.	N.D.	N.D.	0.56	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
09	N.D.	N.D.	1.7	N.D.	N.D.	0.58	0.60	N.D.	0.45	N.D.	N.D.	N.D.
10	N.D.	N.D.	N.D.	N.D.	N.D.	0.41	0.59	N.D.	N.D.	N.D.	N.D.	6.2
11	N.D.	N.D.	N.D.	N.D.	N.D.	0.78	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
12	N.D.	N.D.	1.9	N.D.	N.D.	1.2	N.D.	N.D.	N.D.	N.D.	0.66	N.D.
13	N.D.	N.D.	2.9	N.D.	N.D.	0.41	1.4	N.D.	0.43	N.D.	N.D.	N.D.
14	N.D.	N.D.	0.41	N.D.	N.D.	N.D.	0.72	N.D.	N.D.	N.D.	N.D.	2.2
15	N.D.	0.97	N.D.	1.2	34	1.5	1.3	N.D.	N.D.	N.D.	N.D.	N.D.
16	N.D.	N.D.	N.D.	N.D.	N.D.	1.3	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
17	N.D.	N.D.	0.41	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
18	N.D.	N.D.	0.41	N.D.	N.D.	N.D.	1.3	N.D.	N.D.	N.D.	N.D.	N.D.
19	N.D.	1.0	N.D.	1.2	N.D.	218	1.3	N.D.	N.D.	N.D.	N.D.	N.D.
20	N.D.	N.D.	N.D.	N.D.	N.D.	1.9	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

N.D. : < 0.41 $\mu g/m^2/h$ (TEP, TBP, TCEP, DBA, BBP, DOA, TPhP, TEHP), < 0.56 $\mu g/m^2/h$ (DEP), < 0.91 $\mu g/m^2/h$ (DiBA), < 0.59 $\mu g/m^2/h$ (DBP), < 2.1 $\mu g/m^2/h$ (DEHP)



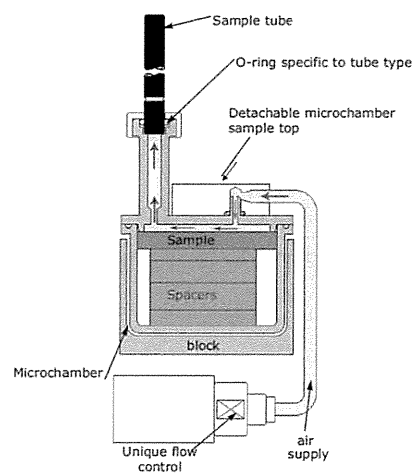
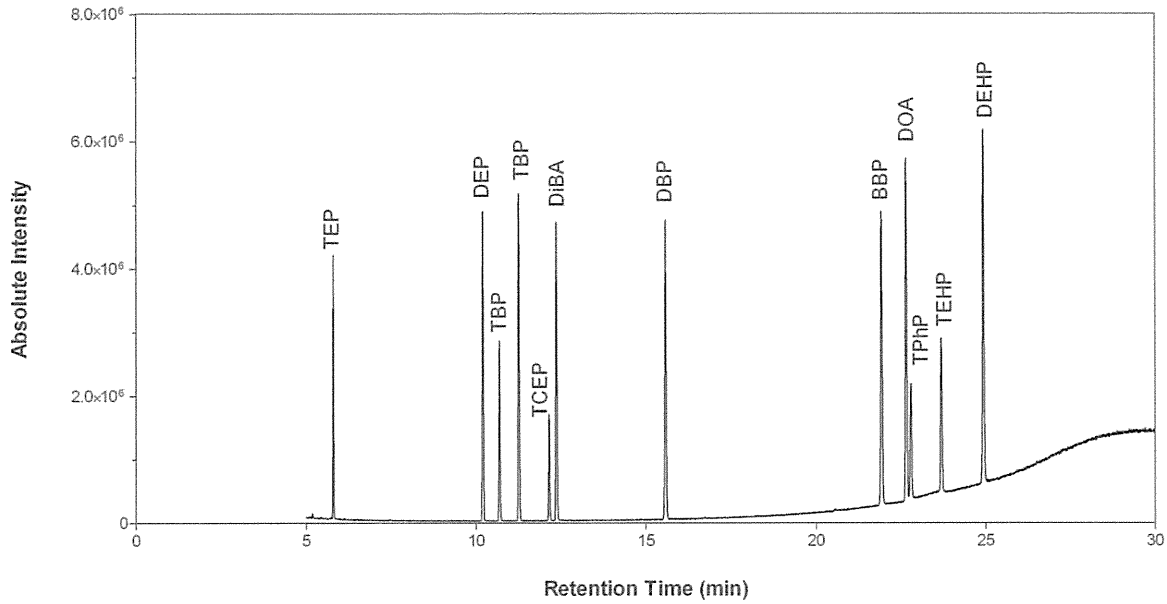


図 1　μ-CTE　250i の外観及び構造



Phthalates

DEP : Diethyl Phthalate, DBP : Dibutyl Phthalate, BBP : Benzyl Butyl ,

DEHP : bis(2-Ethylhexyl) Phthalates

Adipates

DiBA : Disiobutyl Adipate, DBA : Dibutyl Adipate, DOA : Dioctyl Adipate

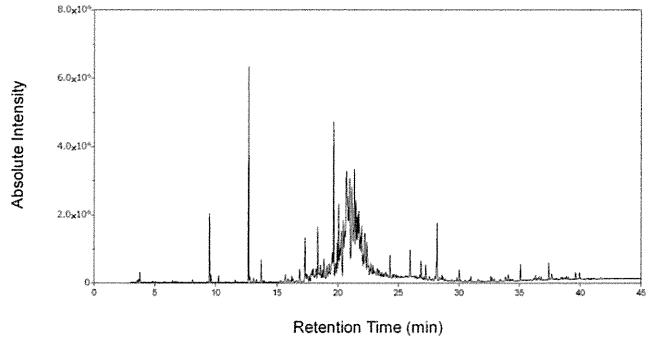
Phosphate Triesters

TEP : Triethyl Phosphate, TBP : Tributyl Phosphate,

TCEP : tris(2-Chloroethyl) Phosphate , TPhP : Triphenyl Phosphate,

TEHP : tris(2-Eethylhexyl) Phosphates

図 2　準揮発性有機化合物 12 標準物質の GC/MS 分析によるとクロマトグラム



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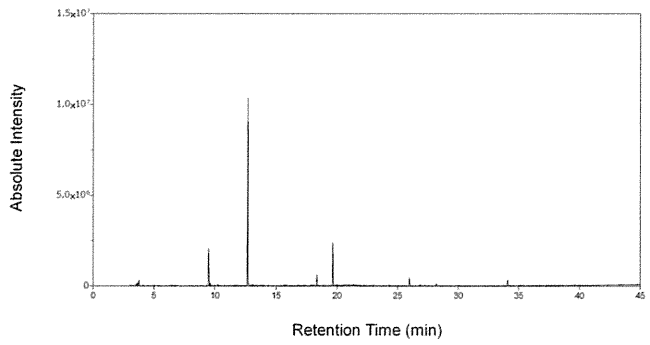

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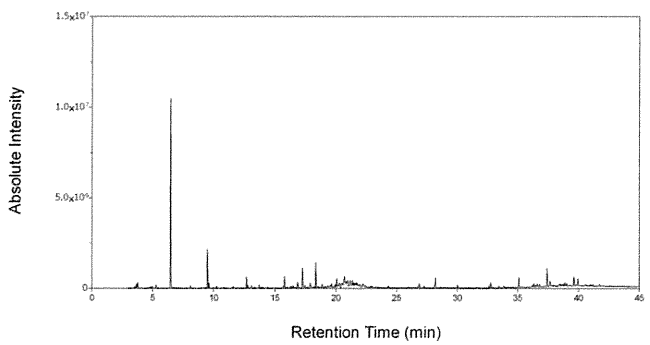

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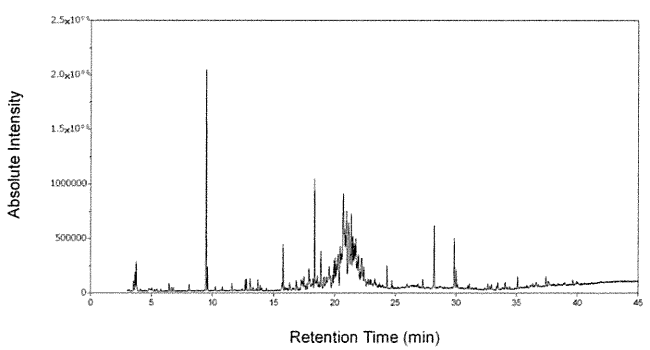
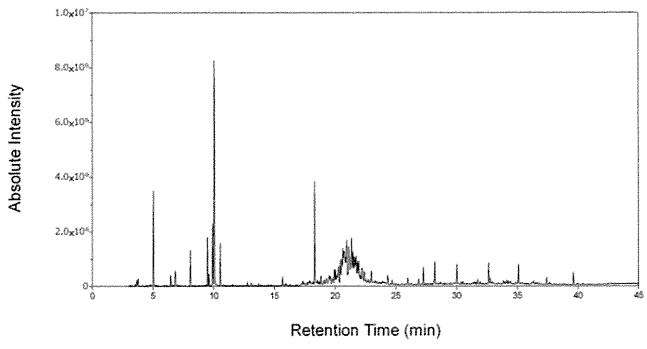


ID No. 4

図 3　　GC/MS 分析によるカーペットサンプルの
トータルイオンクロマトグラム（ウール製）



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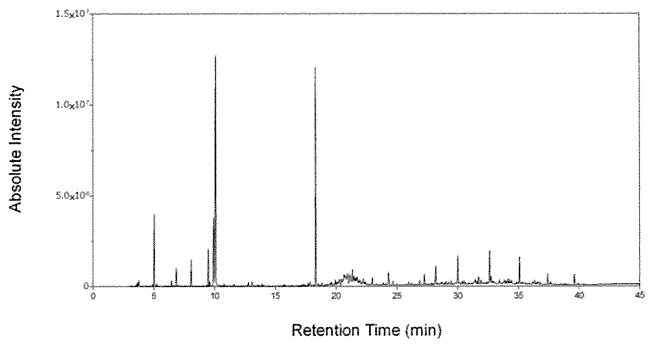

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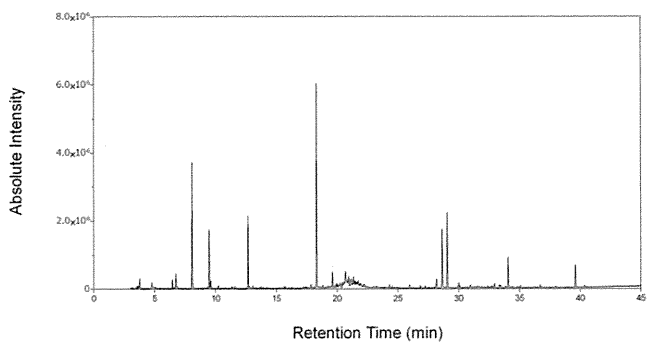

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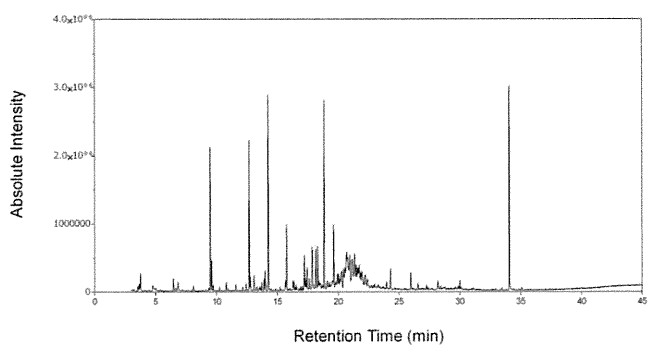
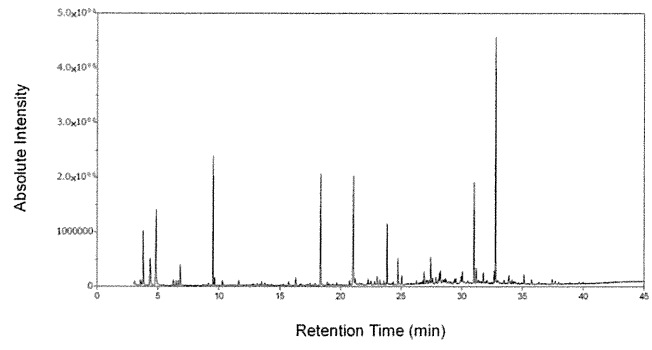


ID No. 8

図 4　　GC/MS 分析によるカーペットサンプルの
トータルイオンクロマトグラム（ナイロン製）



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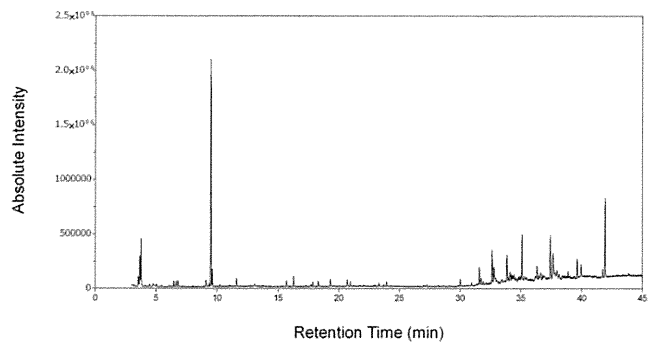

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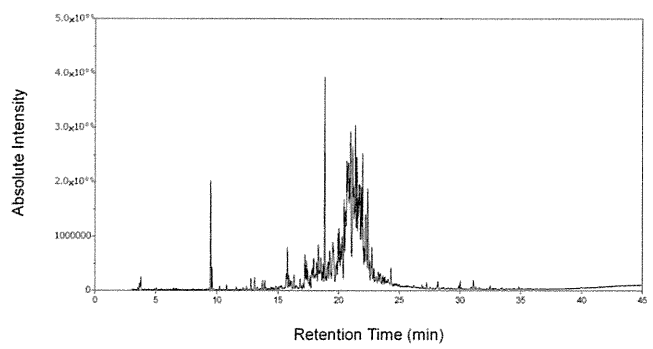

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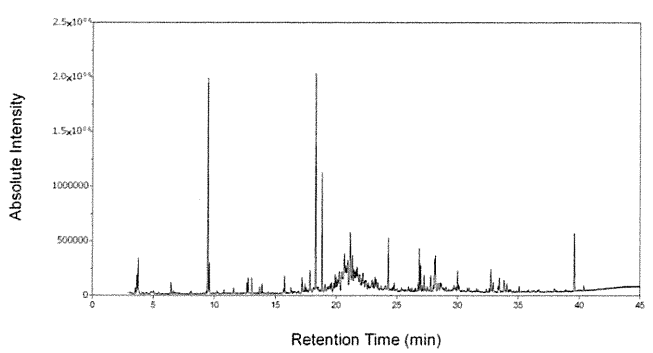
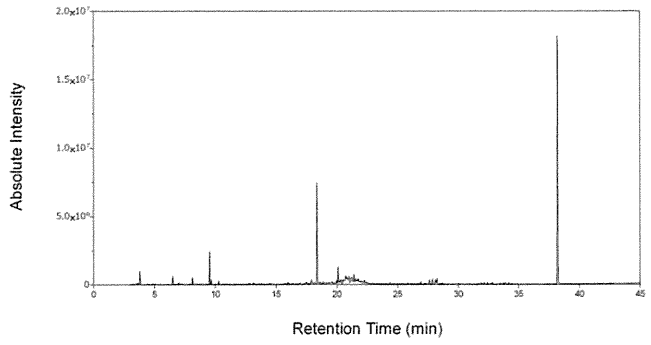


ID No. 12

図 5　GC/MS 分析によるカーペットサンプルの
トータルイオンクロマトグラム（ポリエステル製）



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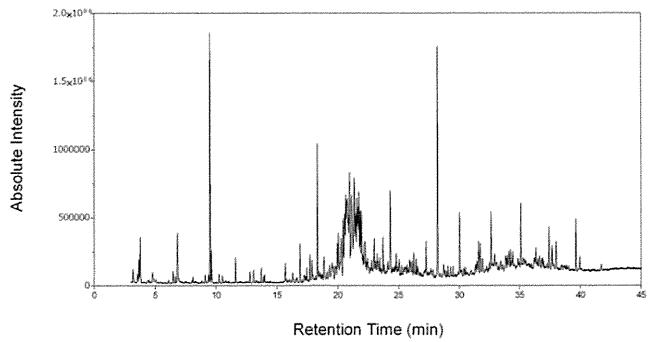

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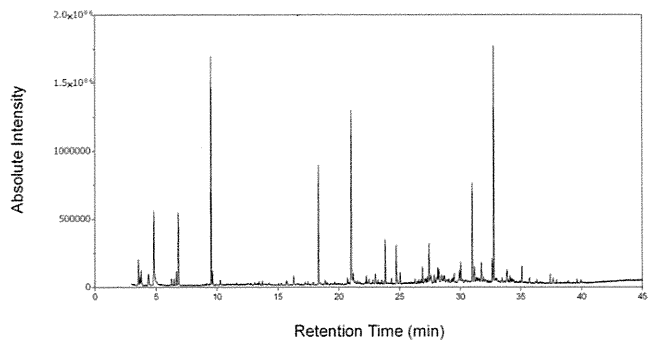

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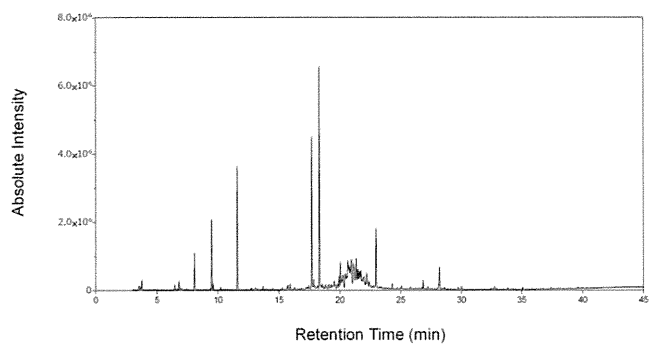

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図 6　GC/MS 分析によるカーペットサンプルの
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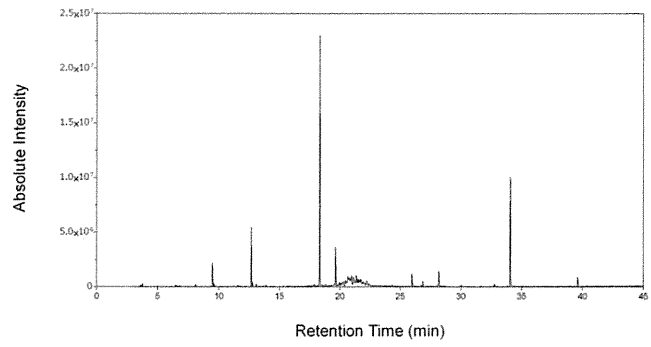

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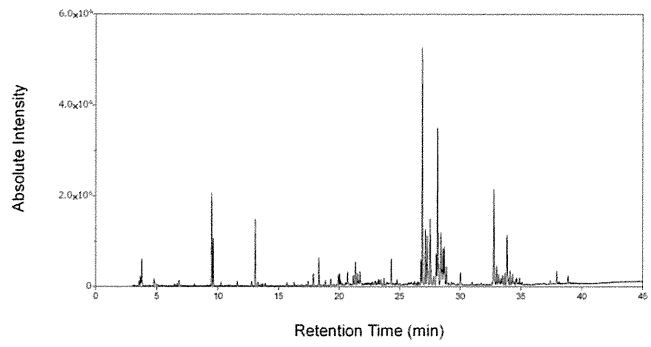

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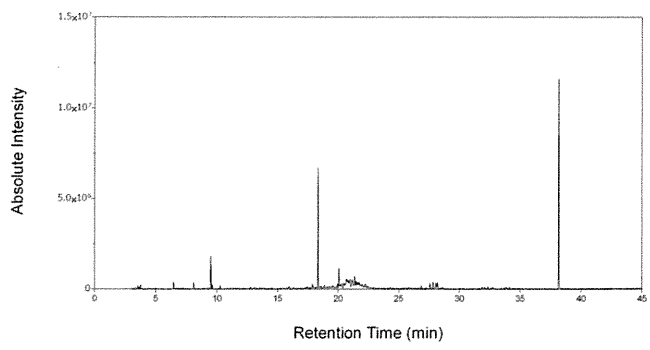

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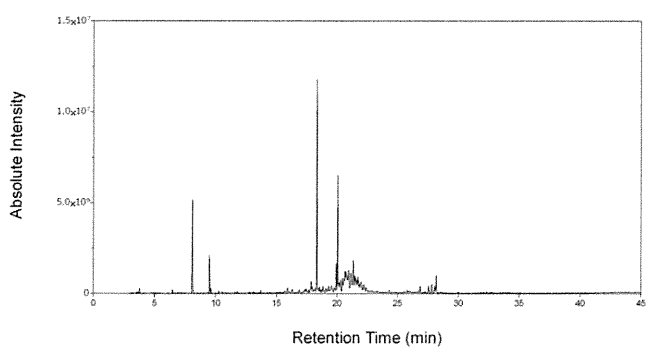
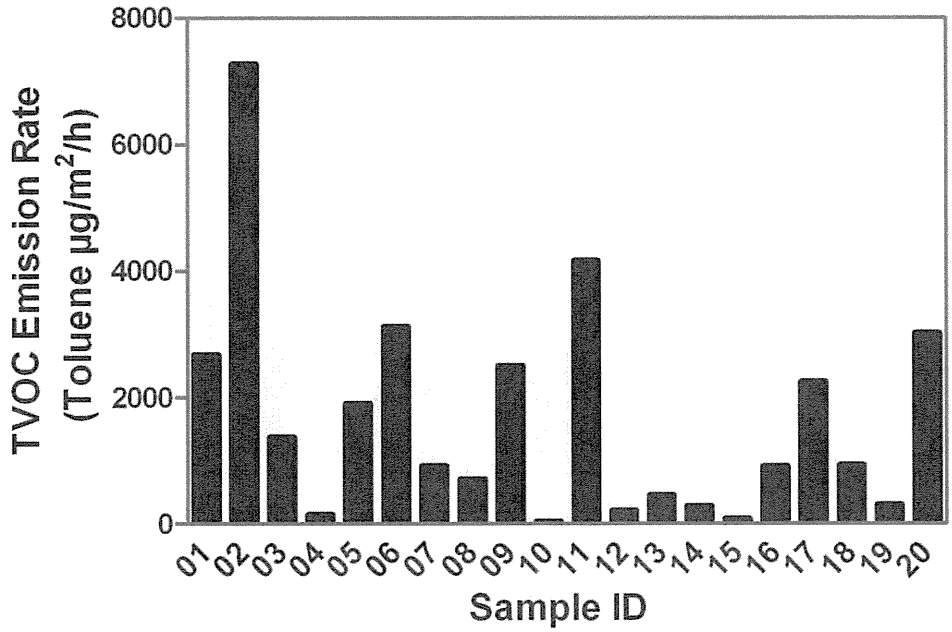


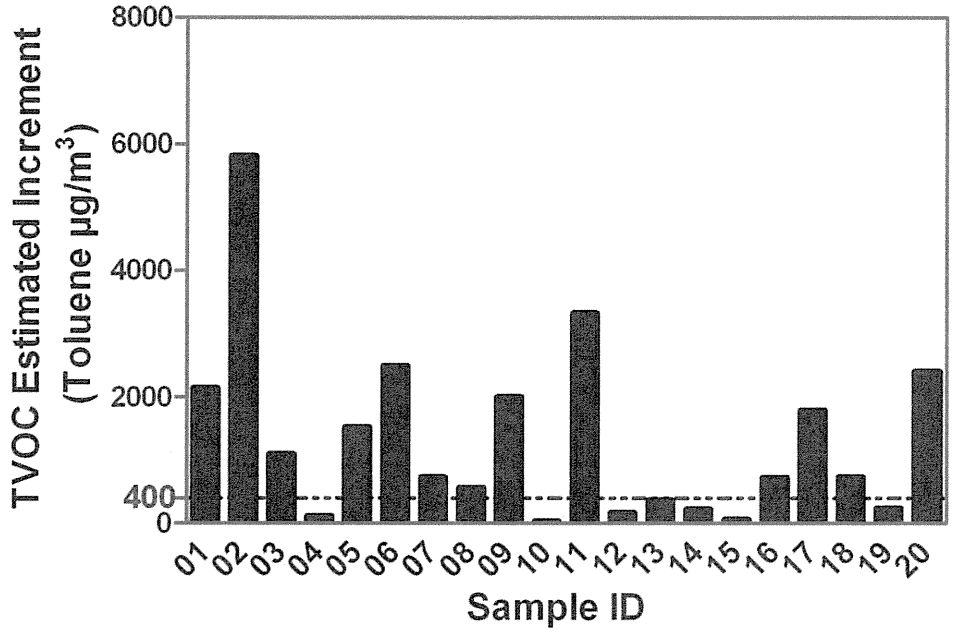
ID No. 20

図 7　GC/MS 分析によるカーペットサンプルの
トータルイオンクロマトグラム（ポリプロピレン製）



Number of values	20
Minimum	40.00
25% Percentile	285.5
Median	920.5
75% Percentile	2638
Maximum	7269
5% Percentile	41.85
95% Percentile	7114
Mean	1663
Std. Deviation	1795
Std. Error	401.4
Geometric mean	828.6

図 8　　カーペットから放散される TVOC の放散速度



Number of values	20
Minimum	32.00
25% Percentile	228.3
Median	736.5
75% Percentile	2110
Maximum	5815
5% Percentile	33.50
95% Percentile	5691
Mean	1331
Std. Deviation	1436
Std. Error	321.1
Geometric mean	663.1

図 9　　カーペットから放散される TVOC の室内空気中濃度増分予測値

赤字：TVOC 暫定目標値　400 g/m^3

III. 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

発表者氏名	論文タイトル名	発表誌名	巻号	頁	出版年
Takayama N, Iwamoto N, Sumi D, Shinkai Y, Tanaka-Kagawa T, Jinno H, Kumagai Y.	Peroxiredoxin 6 is a molecular target for 1,2-naphthoquinone, an atmospheric electrophile, in human pulmonary epithelial A549 cells.	*J Toxicol Sci.*	36(6)	817-821	2011

IV. 研究成果の刊行物・別刷

The Journal of Toxicological Sciences (J. Toxicol. Sci.)
Vol.36, No.6, 817-821, 2011

Letter

Peroxiredoxin 6 is a molecular target for 1,2-naphthoquinone, an atmospheric electrophile, in human pulmonary epithelial A549 cells

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ABSTRACT — 1,2-Naphthoquinone (1,2-NQ) is an electrophile found in the atmosphere, which reacts readily with protein nucleophiles to form a stable protein adduct. Peroxiredoxin 6 (Prdx6) is predominantly expressed in lung tissue and functions in antioxidant defense by facilitating the repair of damaged cell membranes via reduction of peroxidized phospholipids. In the present study, human A549 pulmonary epithelial cells were exposed to 1,2-NQ to explore whether 1,2-NQ can bind covalently to Prdx6, thereby disrupting its catalytic activity. Two-dimensional SDS/PAGE followed by western blot analysis with a specific antibody against 1,2-NQ showed that Prdx6 was covalently modified by 1,2-NQ. Using purified human Prdx6, it was found that 1,2-NQ bound covalently to Prdx6 through Cys47, Lys144 and Cys91, resulting in a significant reduction in phospholipase A_2 activity. These results suggest that arylation of Prdx6 by 1,2-NQ may, at least in part, be involved in the cellular toxicity induced by 1,2-NQ.

Key words: 1,2-Napthoquinone, Peroxiredoxin 6, Phospholipases A_2

INTRODUCTION

There are a huge number of electrophiles in the atmosphere that can covalently modify cellular proteins to form stable adducts (Iwamoto *et al.*, 2010). Of these, we have focused on 1,2-naphthoquinone (1,2-NQ) (Cho *et al.*, 2004). Accumulating evidence indicates that 1,2-NQ reacts with protein thiols via the Michael reaction to form 1,2-dihydroxynaphthalene-protein adducts, which are rapidly auto-oxidized to 1,2-NQ-protein adducts (Fig. 1) (Kumagai *et al.*, 2012; Miura *et al.*, 2011a). For example, sensor proteins containing reactive nucleophiles undergo arylation by 1,2-NQ, thereby disrupting their function (Endo *et al.*, 2011, 2007; Iwamoto *et al.*, 2007; Miura *et al.*, 2011a, 2011b; Sumi *et al.*, 2010). In a previous study, we found that several unidentified proteins expressed by human pulmonary epithelial A549 cells are modified after exposure to 1,2-NQ (Miura *et al.*, 2011b). This suggests that the cellular cytotoxicity mediated by 1,2-NQ may be caused by reduction and/or loss of function of essential proteins.

Peroxiredoxin 6 (Prdx6), a bifunctional 25 kDa protein with both glutathione peroxidase and phospholipase A_2 (PLA_2) activity, is the only mammalian 1-Cys member of the Prdx superfamily. It is expressed in all major organs, with particularly high levels being observed in lung tissue (Manevich and Fisher, 2005). Prdx6, when stably overexpressed in cells, protects against oxidative stress, whereas antisense treatment results in oxidative stress and apoptosis. Also, inhibition of Prdx6-catalyzed PLA_2 activity results in alterations in lung surfactant phospholipid synthesis and turnover (Manevich and Fisher, 2005). These observations suggest that 1,2-NQ may modify Prdx6 via reactive nucleophiles, resulting in a decrease in catalytic activity. To address this issue, we conducted experiments using A549 cells, and cell-free studies using purified human Prdx6.

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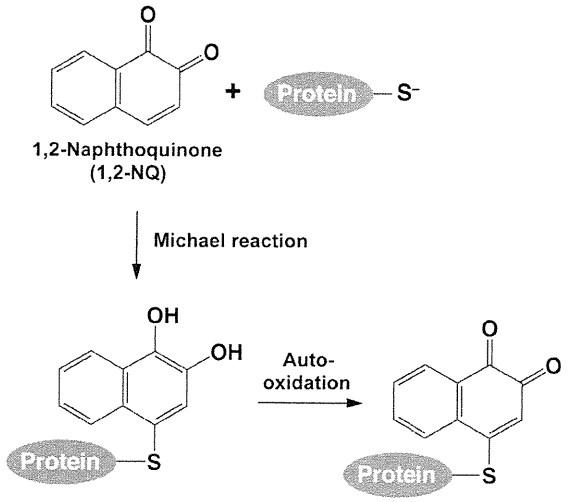

Fig. 1. Covalent modification of 1,2-NQ to protein thiols.

MATERIALS AND METHODS

Materials

1,2-NQ was purchased from Tokyo Kasei Industries, Ltd. (Tokyo, Japan). A specific antibody against 1,2-NQ was prepared as previously described (Iwamoto *et al.*, 2007). All other reagents used were of the highest purity available.

Cell culture

The human lung epithelial cell line, A549 (ATCC, Manassas, VA, USA) was grown in Dulbecco's modified Eagle's medium (DMEM; Wako, Osaka, Japan) supplemented with 10% fetal bovine serum, 2 mM Gluta Max-1 and antibiotics (100 units/ml penicillin, 100 μg/ml streptomycin) at 37°C in a humidified atmosphere containing 5% CO_2.

2-Dimensional SDS-polyacrylamide gel electrophoresis (2D-SDS/PAGE)

Sample preparation and the gel composition used for the second-dimension SDS-PAGE were as outlined in "2-D Electrophoresis PRINCIPLES and METHODS" (GE Healthcare, Bio-Sciences Corp., Piscataway, NJ, USA). Cells were extracted with lysis buffer (6 M urea, 1 M thiourea and 3% CHAPS) and the lysates were sonicated in short bursts to avoid heating. IPG strips (7 cm; pH interval: 3-10) were rehydrated prior to isoelectric focusing (IEF) in a solution containing 8 M urea, 2% CHAPS, a small amount of SDS, 0.3% dithiothreitol (DTT) and 0.5% IPG buffer for 10 hr. The rehydrated strips are then placed onto the cooling plate of an electrofocusing chamber and IEF was performed at a voltage of 3,500 V (Multiphor II, GE healthcare). After IEF, the IPG strips are equilibrated in the presence of SDS, DTT, urea, glycerol and iodoacetamide, and then placed on top of a vertical SDS gel for the second dimension.

Preparation of Prdx6

The expression vector encoding Prdx6 (Prdx6-pET21b) was kindly provided by Dr. Aron B. Fisher, University of Pennsylvania (PA, USA). The plasmid was transformed into *E.coli* BL21 cells for protein expression. Bacterial cultures were grown to 0.6 absorbance units at 600 nm in LB broth at 37°C with shaking at 120 rpm (Taitec, Saitama, Japan). The cultures were then induced by the addition of 1 mM IPTG and grown for an additional 12 hr at 37°C. Prdx6 was purified using Ni-IDA ProBond (Invitrogen, Carlsbad, CA, USA) as described previously (Iwamoto *et al.*, 2007). The purity of the Prdx6 preparation, as assessed by SDS-PAGE, was > 90%. Thiol groups oxidized during purification were reduced by incubation with 10 mM DTT for 1 hr and the DTT was removed on an Econo-Pac 10 DG column. Each enzyme preparation was stored at -80°C in 50 mM potassium phosphate buffer (pH 7.0) before use.

Western blotting

Cells were extracted with RIPA buffer (50 mM Tris-HCl (pH 7.5) containing 150 mM NaCl, 0.5% deoxycholate, 0.1% SDS, 1 mM ethyleneglycoltetraacetic acid (EGTA), 0.4 mM ethylenediaminetetraacetic acid (EDTA) and a protease inhibitor cocktail). SDS-PAGE and subsequent immunoblot analysis were performed as reported previously (Miura *et al.*, 2011a). The protein concentrations were determined using the Bio-Rad Protein assay kit (Bio-Rad, Richmond, CA, USA) or the bicinchoninic acid (BCA) protein assay reagent (Pierce Biotechnology Inc., Rockford, IL, USA), incorporating bovine serum albumin as a standard. Western blotting with a specific antibody against 1,2-NQ was used to detect Prdx6 bound to 1,2-NQ according to the method of Miura and Kumagai (2010).

Immunoprecipitation

Cell lysates containing 1 mg of cellular protein from A549 cells exposed to 1,2-NQ were incubated overnight at 4°C with an anti-Prdx6 antibody (Lab Frontier, Seoul, Korea) with constant shaking. Protein A sepharose CL-4B beads (GE Healthcare) were then added to the samples and incubated with rocking for 3 hr at 4°C. The beads were rinsed three times with RIPA buffer and pelleted by centrifugation