asthmatic children (aged 5-7 years), and observed significant decreases in PEF in the morning and evening (-2.36 L/min [95% CI: -3.86, -0.86] and -1.71 L/min [95% CI: -3.09, -0.34] respectively for a 10-$\mu g/m^3$ increase in the 24-h concentrations of $PM_{2.5}$). Many studies have also evaluated respiratory symptoms in asthmatics in relation to exposure to PM.[4-10,29,30] Romieu et al[9] reported that respiratory symptoms among asthmatic children were associated with $PM_{2.5}$ concentrations (OR = 1.08 [95% CI: 1.03, 1.14] for a 10-$\mu g/m^3$ increase in $PM_{2.5}$ on the same day). However, in their study, no significant association was observed between wheezing and exposure to PM.

Previous studies that found the significant effects of PM on respiratory health had been conducted in areas with high concentrations of air pollutants.[8,31] Some studies observed no significant associations between PM concentration and respiratory symptoms in areas with low levels of air pollution.[5,7,12] Romieu et al[32] reported that the effects of PM on changes in PEF in asthmatic children were not significant in an area with low ambient levels of PM. In our study, the concentrations of stationary-site $PM_{2.5}$ were not associated with changes in PEF in the asthmatic children, although they were weakly associated with wheezing. This study was conducted in a suburban city without major sources of air pollution, and the concentration of stationary-site $PM_{2.5}$ during the study period was considerably lower (average $PM_{2.5}$ concentration = 19.1 $\mu g/m^3$) than the $PM_{2.5}$ levels in areas where the significant effects of $PM_{2.5}$ were previously found. This may explain why we were unable to detect its effects on the changes in PEF.

The concentration of $PM_{2.5(LD)}$ at the entrance of the hospital was significantly associated with changes in PEF and wheezing, while the concentration of $PM_{2.5(LD)}$ in the hospital was more consistently and strongly associated with these symptoms. The maximum decreases in PEF in relation to a 10-$\mu g/m^3$ increase in the 24-h concentration of indoor $PM_{2.5(LD)}$ were -2.86 L/min in the morning and -3.59 L/min in the evening. These changes in PEF in relation to an increase in $PM_{2.5(LD)}$ concentration were greater than the changes observed in previous studies.[9,15] The prevalence of wheezing was also significantly associated with indoor $PM_{2.5(LD)}$ concentration, although the observed ORs for wheezing were considerably small (ORs in relation to a 10-$\mu g/m^3$ increase in the 24-h concentration of indoor $PM_{2.5(LD)}$ were 1.014 and 1.025 in the morning and evening, respectively). The concentrations of indoor and outdoor $PM_{2.5(LD)}$ varied considerably during the study period. The analyses using quartiles of each PM showed that the prevalence of wheezing increased in relation to exposure to high concentrations of indoor $PM_{2.5(LD)}$ or stationary-site $PM_{2.5}$.

It is difficult to compare the results of our study with those of other studies because of the differences with regard to various factors, such as race, age, severity of asthma, and concentrations of co-pollutants, which can influence PEF. In most of the previous studies, the data on air pollutants was collected at central regional sites, and all the subjects were usually assigned to uniform exposure.[6,12,14] Thus, exposure misclassification is expected to diminish the accuracy of exposure-response estimates, possibly leading to a null effect. We measured the concentrations of PM inside and outside the hospital in which the subjects stayed for a long period of time. Because the concentration of indoor PM was estimated from measurements taken at 3 sites in the hospital, it is conceivable that personal exposure to PM in the children has been evaluated much more accurately in the present study than in previous studies. In addition, daily measurements of PEF were conducted regularly using a spirometer, under the guidance of trained nurses, and wheezing was assessed based on auscultation. Therefore, we believe that our results reflect the actual exposure-response relationship.

In our study, the decreases in PEF and increases in wheezing in relation to increases in $PM_{2.5(LD)}$ concentration were more remarkable in the evening than in the morning. Roemer et al[14] found that decreases in PEF in relation to increases in PM concentration were larger in the evening than in the morning. However, other studies have reported that decreases in PEF associated with exposure to PM were greater in the morning than in the evening.[31,33] Thus, there have been no consistent findings on the difference in the effects of exposure to PM between morning and evening. PEF values in the evening appear to be affected by daily activities during the daytime. In this study, all the subjects were children who were hospitalized, and their habits were almost identical. We found that the effects of confounding factors other than air pollution were small, thereby allowing detection of the marked effects of indoor $PM_{2.5(LD)}$ concentration on PEF and wheezing.

To assess the temporality from exposure to PM to the changes in PEF and the occurrence of wheezing, the lag structure of the associations has been examined in many reports.[5,8,12,33,34] Decreases in PEF have been reported to be more relevant to the concentration of PM after a 1-d lag than that on the same day.[8] In a panel of asthmatic children in another study, a significant relationship between lower respiratory symptoms and the 5-d mean concentration of PM_{10} was found, but the associations for 0-2 d lags were not significant.[5] Desqueyroux et al[34] reported that asthma attacks in adults with severe asthma were associated with PM_{10} concentrations for 3-5 d lags, but such association for a 1- or 2-d lag was not significant.

In the present study, significant decreases in PEF were observed in relation to outdoor $PM_{2.5(LD)}$ concentration for 1- and 2-d lags in the morning and 0- and 1-d lags in the evening. Wheezing in the morning was related to outdoor $PM_{2.5(LD)}$ concentration only for 2-d lags. These findings are consistent with the results of previous reports,[5,8,34] which show that the effects of PM differ in relation to the number of lag days. The concentrations of indoor $PM_{2.5(LD)}$ for 0-3 d lags were consistently associated with both PEF and wheezing, and such

associations became gradually weaker as the number of lag days increased. Thus, lag periods from exposure to PM to observed effects were different for indoor and outdoor PM concentrations. These results suggest that indoor PM may affect asthmatic children more easily than outdoor PM. However, the concentrations of stationary-site $PM_{2.5}$ were not significantly related to PEF for upto 3-d lags.

Some researchers have shown that concentrations of indoor PM often differ from those found in outdoor air.[25,26] Long et al[24] assessed the *in vitro* toxicity of indoor and outdoor $PM_{2.5}$ collected in Boston-area homes, and suggested that indoor-generated particles may be more bioactive than outdoor particles. The differences may be driven by the types of materials used for building.[35] It was also reported that the concentrations of indoor PM were similar to the outdoor levels when air change was conducted frequently. However, indoor sources might seriously affect the concentrations of indoor PM.[36] In the present study, the mean concentration of indoor $PM_{2.5(LD)}$ was higher than that of outdoor $PM_{2.5(LD)}$. The concentration of indoor $PM_{2.5(LD)}$ did not correlate with the concentration of stationary-site $PM_{2.5}$, although it showed a weak correlation with outdoor $PM_{2.5(LD)}$ concentration. Moreover, indoor $PM_{2.5(LD)}$ reached a higher concentration during the nighttime than during daytime. These results suggest that the sources of indoor PM differ from those of outdoor PM. However, in the hospital, no typical sources of PM, such as smoking and cooking, were present, and we could not identify the major sources of indoor PM. In addition, the factors that account for high concentrations of indoor $PM_{2.5(LD)}$ during the nighttime remain unknown. Some allergens, such as house dust mites, might contaminate the indoor environment.[37] The origin and characteristics of PM in the hospital should be further evaluated.

With respect to the lag structure for every 12 h before PEF measurement, indoor $PM_{2.5(LD)}$ concentrations during both the daytime and nighttime were significantly associated with PEF in children. Compared to the nighttime concentrations, the concentrations of indoor $PM_{2.5(LD)}$ during the daytime were more strongly associated with changes in PEF. Similar results were observed with regard to the effects on wheezing. This may reflect the difference in the concentrations of indoor $PM_{2.5(LD)}$ between daytime and nighttime. Alternatively, the effects of nighttime PM concentration on children might be lesser than those of daytime PM concentration because they were asleep for most of the time during the night.

Several studies have used size-fractionated PM data to compare the effects of fine and coarse fraction particles. Schwartz et al[16] reported that $PM_{2.5}$ may have more adverse effects on respiratory symptoms and pulmonary functions among schoolchildren than $PM_{2.5-10}$. A study on Chinese schoolchildren reported that during the winter heating season, the effects of fine particles on pulmonary functions were greater than those of coarse particles.[38] With respect to the effects on PEF in asthmatics, Romieu et al[9] and Pekkanen et al[13] reported comparable results for $PM_{2.5}$ and PM_{10}, while Peters et al[15] found slightly greater effects for $PM_{2.5}$. In Japan, particulate air pollution is usually assessed based on the concentration of suspended particulate matter (SPM), which is the fraction of particles with diameters less than 10 μm. However, the method of measuring SPM is different from that of PM_{10} measurement in foreign countries, and the concentration of SPM cannot be regarded as that of coarse particles. Therefore, we did not consider the effects of coarse particles. An additional study is necessary to evaluate the effects of coarse particles in Japan.

Several previous studies have reported the relationships between air pollutants and medication use in asthmatic children.[4-7,39,40] In a panel of children, most of whom had asthma, Gielen et al[5] reported an association between PM concentration and medication use. All the subjects in our study had severe asthma and used maintenance medication, including ICS, daily. Therefore, in this study, we did not examine the associations with medication. The medication for asthma might obscure the effects of exposure to PM, although the measurements of PEF and the assessments of wheezing were conducted immediately before medication. Delfino et al[11] reported that associations between asthma symptoms and exposure to PM were significant in only the group of children who were not under anti-inflammatory medications. However, Gent et al[41] reported that children using maintenance medication were particularly vulnerable to ozone. In our study, the concentrations of indoor $PM_{2.5(LD)}$ were significantly associated with PEF and wheezing. These findings are compatible with the results by Gent et al.[40]

In conclusion, among children from this panel study, we found no obvious association between the concentrations of stationary-site $PM_{2.5}$ and PEF or wheezing. However, even at low levels of ambient air pollution, the concentrations of indoor and outdoor $PM_{2.5(LD)}$ were associated with PEF and wheezing among asthmatic children. The consistent and strong associations of PEF and wheezing with indoor $PM_{2.5(LD)}$ concentrations suggest that it is desirable to estimate exposure to PM in the environment where the subjects spend most of their time.

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〔Original Paper〕

Age-related changes in allergic symptoms and serum TARC concentration in school children

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SUMMARY

Background Thymus and activation-regulated chemokine (TARC) is a Th2-related chemokine that is associated with allergic diseases. We investigated growth-related changes in allergic symptoms and TARC concentration in serum during later childhood to examine how Th2-related factors are associated with allergic symptoms.

Methods This study was performed in 146 school children living in a suburban Japanese city. At the 1st grade in 1997 and the 5th grade in 2001, these children underwent questionnaire investigation on respiratory and allergic symptoms and blood collection for determining total IgE, mite IgE and TARC concentrations in serum.

Results TARC concentration in serum significantly decreased at the 5th grade (424.1 pg/mL) compared with at the 1st grade (489.2 pg/mL) in the entire child population. In children with no allergic symptom at either grade, serum TARC concentration was significantly decreased at the 5th grade (410.7 pg/mL) compared with at the 1st grade (521.4 pg/mL), while there was no decrease in the serum concentration of TARC in allergic children at either or both of the two grades. There was also a difference in the decrease of serum TARC concentration at the 5th grade according to total IgE and mite IgE in addition, the presence of allergic symptom affected the decrease of serum TARC concentration.

Conclusion Serum TARC concentration decreased in association with growth in children, suggesting possible age-related decrease of Th2 cell activity. TARC concentration in serum significantly decreased in children with no allergic symptom, but no significant decrease was observed in allergic children, showing that TARC is associated with the onset of allergic symptoms. It is also considered that serum TARC concentration had strong relation to especially atopic dermatitis.

Key words: thymus and activation-regulated chemokine (TARC), bronchial asthma, allergic rhinitis, atopic dermatitis, IgE

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平野好絵[1,3], 島　正之[2], 羽田　明[1], 栗山喬之[3]: 児童のアレルギー症状及び血清中TARC濃度の経年変化について.
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I. Introduction

The increased prevalence of allergic diseases such as bronchial asthma and allergic rhinitis has gathered the attention of researchers worldwide in recent years[1]. An epidemiological study is necessary to evaluate the incidence of allergic diseases with time for elucidation of changes in the prevalence of allergic symptoms and individual symptoms and associated factors[2]. It is known that cytokines that are released from Th2 cells play important roles in the onset of allergic diseases. Th2-type cytokines promote IgE production and affect the function and life of eosinophils[3]. It is pointed out that TARC, a CC chemokine, is relevant to allergic diseases because its receptor, CCR4, is specifically expressed in Th2 cells and involved in the activity of Th2 cytokines[4]. High blood TARC concentration has been reported in allergic diseases[5-7].

There have been few studies of successive follow-up of children with allergic diseases, and no paper is available concerning the follow-up investigation of allergy-related cytokines and chemokines. It is important for better understanding of the cause of allergic disorders to examine how Th2-related factors are associated with allergic symptoms in school age.

The present study investigated the prevalence of allergic symptoms in primary school children at the 1st and 5th grades, and determined serum total IgE, mite IgE and TARC concentrations. Based on these results, we examined growth-related changes in the prevalence of allergic symptoms in later childhood. We also studied changes in TARC concentration with time to examine how the changes were associated with allergic diseases.

II. Materials and methods

Subjects

This study was performed in children from three primary schools in a suburban city of Chiba Prefecture, Japan. All children were at the 1st grade (6 to 7 years old) in 1997 at any of the three schools and at the 5th grade (10 to 11 years old) in 2001 at the same school, and lived in a neighborhood of these schools. In the autumn of 1997 and 2001, a standardized respiratory symptom questionnaire (ATS-DLD-78-C)[8]and the International Study of Asthma and Allergies in Childhood (ISAAC)[9] programme-compatible questionnaire on allergic symptoms were administered to investigate the presence of symptoms of bronchial asthma, wheeze, allergic rhinitis and atopic dermatitis. Blood samples were collected at the same time points for serological investigation. Both parents of each child were well explained how to respond to the questionnaires and the details of blood sampling, and written informed consent was obtained. The study protocol was approved by the Ethics Committee of Graduate School of Medicine, Chiba University.

Definition of allergic symptoms

The presence of allergic symptoms was evaluated from responses to the questionnaires. Bronchial asthma, wheeze, allergic rhinitis, and atopic dermatitis were defined in the same fashion as in our previous investigation[10] and the ISAAC programme[2]. Briefly, a child had bronchial asthma if at least two events of wheezing and/or whistling in the chest and acute onset of breathing difficulty, and he/she was diagnosed with asthma by his/her physician at the time of onset; a child had wheeze if he/she presented with wheezing and/or whistling respiration on at least two occasions when he/she caught cold within 2 years; a child had allergic rhinitis if symptoms of sneezing, nasal

discharge and/or nasal obstruction occurred within 1 year even when he/she did not suffer from cold; and a child had atopic dermatitis if he/she had itchy eczema continuing for 6 months or longer and occurred in the elbow, knee, ankle, hip, neck, ear, and/or periocular area within 1 year. A child was defined as allergic if he/she corresponded to any of the above criteria, and not allergic if he/she did not correspond to any of the above criteria.

Sample collection and laboratory measurements

Children with findings indicative of acute infection on the day before or the day of the investigation were excluded and blood was collected from the remaining children. Blood samples were collected in the morning and centrifuged on the same day. Serum thus separated was analyzed for total IgE concentration according to the Latex-Nephelometry (Dade Behring, Marburg, Germany) and for mite IgE according to the Uni-CAP methods (Pharmacia, Uppsala, Sweden), and then kept frozen at −80℃. Frozen serum samples were subsequently analyzed for TARC concentration using a commercially available enzyme-linked immunosorbent assay (ELISA) kits (R&D systems, Minneapolis, MN, USA).

Data analysis

Serum TARC concentration data showed a distribution similar to the logarithmic normal type. These data thus underwent logarithmic transformation and were analyzed for geometric means and 95% confidence intervals to examine the presence of allergic symptoms and the relationship with total IgE and mite IgE concentrations in serum. Data on serum concentration of total IgE was classified into high and low groups of ≥250 IU/mL and <250 IU/mL in conformity with the clinical criteria in Japanese children[11]. Similarly, serum mite IgE concentration data were classified into high and low groups of ≥0.35 U_A/mL and <0.35 U_A/mL, respectively. Serum TARC concentration was compared between the 1st and 5th grades within each child for the time profile. Children were divided into four groups according to the presence of symptoms at the 1st and 5th grades, and the relationship with changes in serum TARC concentration were examined. Children were also classified into groups according to high and low total IgE and mite IgE concentrations in serum at the 1st and 5th grades, and the relationship with changes in serum TARC concentrations was examined. In addition, children were divided into groups according to allergic symptoms at the 1st and 5th grades for cross-sectional comparison of serum TARC concentration.

The Stat View (SAS Institute, Cary, NC, USA) was used for statistical procedures.

Ⅲ. Results

It was possible to administer the questionnaires and hematological examination in 146 children at both the 1st and 5th grades. Table 1 shows symptoms in the 146 children. Of males and females combined, at least one allergic symptom was observed in 34.9% at the 1st grade and 34.2% at the 5th grade. The prevalence of allergic symptoms was significantly higher in males than in females at the 1st grade. The most frequent allergic symptom was rhinitis in both males and females at both the 1st and 5th grades.

As for the relationship between allergic symptoms and total IgE, the percentage of allergic children was significantly higher in the high total IgE group compared with the low group at both the 1st and 5th grades. Similarly, children were allergic more frequently in the high mite IgE group than in the low group at both the 1st and 5th grades (Table 2).

Table 1 Allergic characteristics in study subjects

		male (%) (n = 82)	female (%) (n = 64)	total (%) (n = 146)
1st grade	not allergic	57.3	75.0	65.1
	allergic	42.7*	25.0	34.9
	asthma	3.6	3.1	3.4
	wheeze	14.6	4.7	10.3
	rhinitis	28.0	20.3	24.7
	A.D.	10.9	3.1	7.5
5th grade	not allergic	64.6	67.2	65.8
	allergic	35.4	32.8	34.2
	asthma	4.9	3.1	4.1
	wheeze	3.7	3.1	3.4
	rhinitis	31.7	31.3	31.5
	A.D.	7.3	3.1	5.4

A.D.; atopic dermaitits
*p = 0.025 compared with females

Table 2 Serum concentrations of total IgE and mite IgE in children

			n	allergic (%)	p value
1st grade	total IgE	250≧	114	28.1	
	(IU/mL)	250<	32	59.4	0.001
	mite IgE	0.35≧	97	24.7	
	(U_A/mL)	0.35<	49	55.1	<0.001
5th grade	total IgE	250≧	96	21.9	
	(IU/mL)	250<	50	58.0	0.018
	mite IgE	0.35≧	77	20.8	
	(U_A/mL)	0.35<	69	50.7	<0.001

IgE; Immunoglobin E
IU; International unit

Table 3 Allergic symptoms at the 1st and 5th grades

		1st grade not allergic	1st grade allergic	total
5th grade	not allergic	78 (53.4%)	18 (12.3%)	96
	allergic	17 (11.6%)	33 (22.6%)	50
	total	95	51	

Table 3 shows changes in allergic symptoms between the 1st and 5th grades. Of all children, 53.4% were not allergic either at the 1st or 5th grade, 22.6% were allergic at the 1st and 5th grades, 12.3% were allergic only at the 1st grade, and 11.6% were allergic only at the 5th grade.

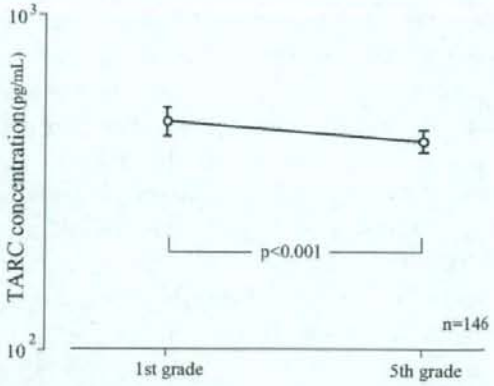

Fig. 1 Changes in serum TARC concentration in all children.

TARC; thymus and activation-regulated chemokine

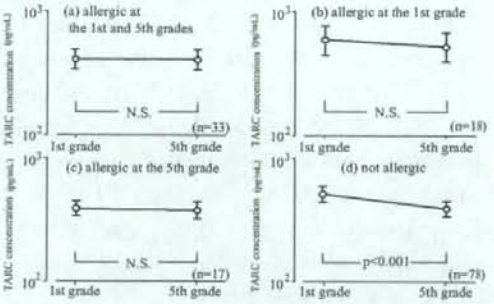

Fig. 2 Changes in serum TARC concentration according to the presence of allergic symptoms. Changes in serum TARC concentration are compared among four groups of allergic children at both the 1st and 5th grades, allergic children at the 1st grade, allergic children at the 5th grade, and children who were not allergic at either of the 1st or 5th grade.

N.S.; not significant

As for changes in serum TARC concentration from the 1st to 5th grades, it was decreased at the 5th grade compared with at the 1st grade within each child (Fig. 1). According to the presence of allergic symptoms, TARC concentration in serum decreased only in children who were not allergic either at the 1st or 5th grade. Serum TARC concentration did not significantly decrease in children who were allergic at both the 1st and 5th grades or only at the 1st or 5th grade (Fig. 2), and the almost

same results were obtained when we studied about each allergic disease.

According to total IgE concentration in serum, serum TARC concentration showed no significant decrease in the group with high total IgE only at the 5th grade (23 children). Serum TARC concentration significantly decreased, however, in the groups with high total IgE at the 1st and 5th grades (27 children) and low total IgE at the 1st and 5th grades (91 children) (Fig. 3-a). There were only 5 subjects whose total IgE were high at the 1st grade and low at the 5th grade, and they were thus excluded from comparison.

According to mite IgE concentration in serum, serum TARC concentration did not significantly decreased in the group of high mite IgE at both the 1st and 5th grades (47 children). Serum TARC concentration was significantly decreased, however, in the group of low mite IgE at both the 1st and 5th grades (75 children) and the high mite IgE group only at the 5th grade (22 children) (Fig. 3-b). There were only 2 subjects whose mite IgE were high at the 1st grade and low at the 5th grade, and they were thus excluded from comparison.

The above groups were further examined according to the presence of allergic symptoms. In children with high total IgE at both the 1st and 5th grades, TARC concentration in serum showed no significant decrease either in the asymptomatic group, the group of symptomatic relief and onset, or the persistently

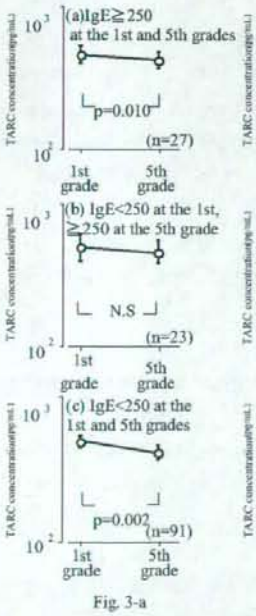

Fig. 3-a

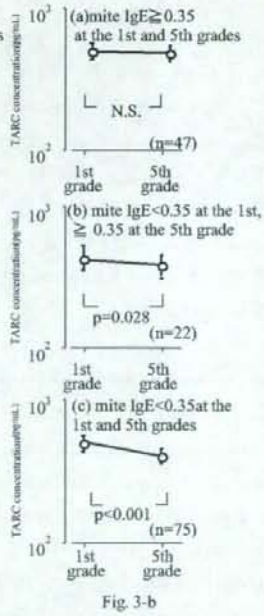

Fig. 3-b

Fig. 3-a Comparison of changes in serum TARC concentration according to total IgE concentration (IU/mL).

3-b Comparison of changes in serum TARC concentration according to mite IgE concentration (U_A/mL).

IgE: Immunogloblin E
N.S.: not significant

Table 4-a Changes in serum TARC concentration in children with high serum total IgE at the 1st and 5th grade

allergic symptoms	n	1st grade GM	1st grade 95%CI	5th grade GM	5th grade 95%CI	p value
none	7	455.5	358.1-579.5	405.0	313.4-523.5	N.S.
both or either grades	20	492.5	409.4-592.4	449.1	383.2-526.4	N.S.

The group of children with total IgE concentration of ≧250 IU/ml at both the 1st and 5th grades is divided into subgroups of children who were not allergic at the either grade and children who were allergic at the 1st and/or 5th grades for comparison of TARC changes.

Table 4-b Changes in serum TARC concentration in children with high serum mite IgE at the 5th grade

allergic symptoms	n	1st grade GM	1st grade 95%CI	5th grade GM	5th grade 95%CI	p value
none	4	406.1	269.6-611.6	343.2	242.1-486.6	0.039
both or either grades	13	425.4	325.5-556.0	397.1	318.4-495.2	N.S.

GM: geometric mean
CI: confidence interval

The group of children with serum mite IgE of ≧0.35U_A/mL at the 5th grade is divided into subgroups of children who were not allergic at the either grade and children who were allergic at the 1st and/or 5th grades for comparison of TARC changes.

symptomatic group (Table 4-a). In children with high mite IgE only at the 5th grade, serum TARC concentration significantly decreased in the asymptomatic group, with no significant decrease in the group of symptomatic relief and onset or the persistently symptomatic group (Table 4-b).

In the cross-sectional investigation of serum TARC concentration at the 1st and 5th grade, there was no difference in the decrease of TARC concentration in serum according to the presence of allergic symptoms. As for individual allergic symptoms, serum TARC concentration was significantly higher in 5th grade children with atopic dermatitis compared with non-allergic children or children with other allergic symptoms. There was no difference in serum TARC concentration in terms of other allergic symptoms (Table 5).

Table 5 Serum TARC concentration at the 1st and 5th grades

			TARC concentration (pg/mL)	
		n	GM	95%CI
1st grade	not allergic	95	500.3	452.8-552.7
	all allergic students	51	469.4	405.1-543.8
	asthma	5	463.0	414.3-517.4
	allergic without asthma	46	470.1	399.4-553.3
	wheeze	15	420.6	318.2-555.9
	allergic without wheeze	36	491.3	413.1-584.4
	rhinitis	36	451.1	381.0-534.2
	allergic without rhinitis	15	516.2	383.6-694.6
	A.D.	11	546.7	416.3-718.0
	allergic without A.D.	40	450.1	379.2-534.2
5th grade	not allergic	96	432.4	391.9-477.1
	all allergic students	50	408.7	355.4-470.1
	asthma	6	447.7	342.9-584.4
	allergic without asthma	44	403.7	345.7-471.4
	wheeze	5	567.4	339.0-949.7
	allergic without wheeze	45	394.1	341.7-454.5
	rhinitis	46	389.5	341.3-444.5
	allergic without rhinitis	4	711.3	338.1-1496.4
	A.D.	8	604.9*	361.3-1012.9#
	allergic without A.D.	42	379.3	334.4-430.3

A.D.: atopic dermatitis
GM: geometric mean
CI: confidnece interval
#p = 0.048 compared allergic without A. D.
*p = 0.007 compared with not allergic

Ⅳ. Discussion

The prevalence of allergic diseases such as bronchial asthma and allergic rhinitis is increasing in recent years[1,2]. In Sweden, the number of pediatric allergic patients has approximately doubled during the last 12 years [12]. Such increase in the number of allergic children is of global concern. Various research institutions and other organizations conducted other epidemiological studies, many of which were cross-sectional targeting single regions and populations[13,14]. Few follow-up investigations of the same individuals are available except for a study of Kulig et al.[15]. In the present study, we investigated the same individuals twice with a 4-year interval for allergic symptoms and determined serum concentrations of total IgE, mite IgE and the allergy-related chemokine TARC. There is no other study where any chemokine was determined at multiple time points with intervals of several years during the later childhood where allergic symptoms are apt to change.

TARC is a CC chemokine. CC chemokines chemically induce eosinophils, monocytes and T lymphocytes in asthmatic patients and are thus indicated to play important roles in the pathogenesis of bronchial asthma[16,17]. In addition, the preferential expression of CC chemokine receptors in Th2 cells gathers attention of researchers for the relationship with allergic disorders[5,6]. Serum TARC is thus expected to be a novel potential marker for allergic disorders.

It is reported that the overexpression of Th2 cells leads to the occurrence of allergic disorders, and imbalance of Th1 and Th2 cells plays a partial role[1,18-20]. Th2 cells are predominant

in newborns[1]because antenatal predominance of Th2 cells is preferable for pregnancy[21,22]. It has been indicated that, in individuals without hereditary atopic predisposition and people with predominant presence of Th1 cells due to such reasons as infection, Th2 cell activity subsides and allergic diseases are less likely to occur[23,24]. A research article describes that children have the same balance of Th1 and Th2 cells as adults within 5 years after birth[25]. It has also been reported that imbalance of Th1 and Th2 cells in infants will specify the onset of allergic symptoms[26].

In the present study in school children that examined changes in serum TARC concentration between 6 and 11 years of age, the activity of Th2 chemokine decreased with age. This finding suggests that TARC may induce changes in Th2 cell activity even during the later childhood. In addition, Th2 chemokines such as TARC might be a cause of age-related changes in allergic symptoms in school children. Specifically, in children with persistent allergic symptoms, high TARC activity continued even at the 5th grade, suggesting that TARC may play a role in the onset and persistence of allergic symptoms. It is necessary to further study other cytokines and chemokines that are associated with allergic diseases.

We also investigated the relationships of serum TARC concentration with total IgE and mite IgE concentrations in serum. Decrease of serum TARC concentration depended not only on serum IgE concentration but also on the presence of allergic symptoms. In the group of high serum concentration of total IgE at both the 1st and 5th grades, there was no significant difference in the decrease of serum TARC concentration in asymptomatic children. This was probably due to the small number of such subjects. Generally, there is a strong association between serum IgE concentration and allergic diseases[27], but serum IgE is not enough to explain some allergic disorders[28]. Chemokines such as TARC regulate IgE production by activating Th2 cells via B cells in allergic diseases[18]. It is considered from the results of the present study that TARC may be involved in the occurrence of allergic symptoms while this chemokine directly affected IgE production.

In this cross-sectional investigation, there was no difference in serum TARC concentration other than the difference according to the presence of allergic symptoms at either the 1st or 5th grade. This was probably due to the involvement of various cytokines and chemokines in addition to TARC in the onset of allergy. In children with atopic dermatitis at the 5th grade, serum TARC concentration was significantly higher than in non-allergic children, and the concentration was also higher compared with children with other allergic diseases. It has been reported that TARC concentration in serum differs according to the severity of atopic dermatitis with higher serum TARC concentration in severer cases[5], showing that the serum concentration of TARC may be higher even in milder cases of atopic dermatitis compared with other allergic diseases. Subjects of the present study, even allergic children, can perform the normal activities of daily living, and this may explain the non-significant difference in children with allergic symptoms except for atopic dermatitis in contrast to a previous report[6]. It is necessary to continue this type of follow-up investigation in a wider range and increased number of subjects.

In conclusion, we determined serum concentrations of total IgE, mite IgE and TARC and investigated their time profiles in primary school children in the present study. It is considered from the results of the present study that changes in serum TARC concentration may alter the balance of Th1 and Th2 cells even during the later childhood. Changes in serum TARC concentration differed according to the

presence of allergic symptoms, suggesting that there may be a close relationship between TARC and allergic symptoms. It is also considered that serum TARC concentration had strong relation to especially atopic dermatitis.

Th2 cell activation by chemokines other than TARC and cytokines should be further examined in future research.

要　旨

【目的】Thymus and activation-regulated chemokine（TARC）はアレルギー疾患に関与するTh2関連ケモカインの一つである。学童期におけるアレルギー症状と血清TARC濃度の成長に伴う変化を調査し，Th2関連因子がどのようにアレルギー症状に関連しているかを検討した。

【方法】日本の地方都市に在住する学童146人を対象として，小学校1年生時と5年生時の2回，呼吸器及びアレルギー症状に関する質問紙調査と共に，採血を行って血清総IgE，ダニ特異IgE及びTARCの血清中濃度を測定した。

【結果】対象児童全体において血清TARC濃度は1年時（489.2pg/ml）に比べ，5年時（424.1pg/ml）には，有意に低下していた。いずれの学年でもアレルギー症状がなかった児童では血清TARC濃度は1年時（521.4pg/ml）に比べ，5年時（410.7pg/ml）で有意に低下していたが，いずれかの学年または両学年でアレルギー症状があった児童では血清TARC濃度の低下が見られなかった。総IgE値及び，ダニ特異IgE値によっても血清TARC濃度の低下の差が認められたが，これらに加えアレルギー症状の有無が更に血清TARC濃度低下に影響していた。

【結論】小児の血清TARC濃度は成長に伴い低下することから，Th2細胞の活性は年齢と共に低下する可能性が示唆された。アレルギー症状の無い児童ではTARC濃度の低下が有意であったのに対し，アレルギー症状のある児童では有意な低下は見られず，TARCがアレルギー症状の発現に関与しているものと考えられた。

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DETERMINATION OF GASEOUS ORGANIC COMPOUNDS IN HYOGO PREFECTURE, JAPAN

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Abstract. The aim of this study was to analyze gaseous organic chemicals (GOCs) of high traffic (Nishinomiya City: 979,987 vehicles/day) and low traffic areas (Miki City: 29,338 vehicles/day) by gas chromatography-mass spectrometry (GC-MS) and to evaluate general environment exposure by PAHs in GOCs. After air sampling using an OMNIPORE membrane filter (< 0.45 μm) and Porapak-QS, sorbents were extracted with solvent (dichloromethane: acetone (4:1 v/v), and analysis was carried out by GC-MS. Oxidative derivatives of diethylbenzene, such as diacethylbenzene and ethylacetophenone, were detected in GOCs. PAHs and phthalates in GOCs were measured. Pyrene, benz[a]anthracene, benzo[a]pyrene and benzo[ghi]perylene level were significantly higher in high traffic areas. The geometric mean of pyrene was 0.76 ng/m^3 for low traffic areas and 1.96 ng/m^3 for high traffic areas; benz[a]anthracene was found at 0.72 ng/m^3 and 1.80 ng/m^3 in low and high traffic areas, respectively; benzo[a]pyrene was found at 0.87 ng/m^3 and 3.60 ng/m^3 in low and high traffic areas, respectively and benzo[ghi]perylene was found at 0.57 ng/m^3 and 3.04 ng/m^3 in low and high traffic areas, respectively. The bis(2-ethylhexyl) phthalate (DEHP) level was the highest in the detected GOCs. The geometric mean of the DEHP levels in high traffic and low traffic areas were 484.85 and 387.26 ng/m^3, respectively. Adult and child DEHP exposure levels were 145.32 and 300.33 ng/kg/day, respectively, in high traffic areas. In low traffic areas, adult and child DEHP exposure levels were 116.18 and 240.10 ng/kg/day, respectively.

INTRODUCTION

Major air pollution incidents in the 1950s raised public awareness of the health hazards associated with deterioration in air quality. For Japan, the health hazards of sulfur oxides, nitrogen oxides and particulate matter (PM) also became a public concern. The sulfur oxide level has decreased following regulation of exhaust gas and the control of power plant fuel, except for nitrogen oxides. However, PM has not tended to decrease.

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Quinones present in PM can act as catalysts to produce reactive oxygen directly and may be key compounds in PM-based oxidative stress (Xia *et al*. 2004). Polycyclic aromatic hydrocarbons (PAHs) can induce oxidative stress indirectly, through biotransformation by cytochrome P450, expoxide hydrolase, and dihydrodiol dehydrogenase to generate redox active quinones (Penning *et al*. 1999). Therefore, many reports have considered the actions of the compounds contained in PM. It is well known that PM contains many PAHs and exposure to PAHs increases the risk of cancer in humans. The PAHs have received considerable attention as an important class of environmental organic pollutants. Epidemiological studies have suggested that there is a clear relationship between mortality and the

concentration of ambient PM with an aerodynamic diameter less than 10 μm (PM_{10}), and an increase in the PM_{10} level is associated not only with respiratory disorders but also with acute cardiovascular events (Dockery *et al*, 1993; Samet *et al*, 2000; De Leon *et al*, 2003). Recently, the focus has begun to shift to health effects arising from inhalation of fine particles. It has been reported that the association between $PM_{2.5}$ and cardiovascular disease mortality is strong and a 10 $\mu g/m^3$ increase in $PM_{2.5}$ from mobile sources accounts for a 3.4% increase in daily mortality (Laden *et al*, 2000; Pope *et al*, 2004). The air contains ultrafine particles, which are smaller than $PM_{2.5}$ and have unknown health effects. Further, the no bound-particle fraction of PAHs and other chemicals (gaseous organic compounds: GOCs) has the possibility to exist in ambient air. Numerous epidemiologic studies have demonstrated an association between elevated levels of ambient particles and morbidity or mortality. However, gas-phase ambient organic air contaminants have not been studied thoroughly. The aim of this study was to determine and compare GOCs in high and low traffic areas and evaluate PAHs exposure on the general population.

MATERIALS AND METHODS

Reagents

Dichloromethane and acetone were purchased from Wako Pure Chemical Industries, (Osaka, Japan). 1,2-Diacetylbenzene, 1,2-diethylbenzene, 1,3-diacetylbenzene, biphenyl, benzo[ghi]perylene, 1,4-diacetylbenzene and 1,4-diethylbenzene were purchased from Sigma-Aldrich (St Louis, USA). Benz[a] anthracene, acenaphthene, acenaphthyrene, benzo[a]pyrene and phenanthren were purchased from Tokyo Chemical Industry (Tokyo, Japan). Fluorene, pyrene, n-butyl benzyl phthalate and fluoranthene were purchased from Nakarai tesque (Kyoto, Japan). These compounds were used for mass spectra structure analysis. All reagents and solvents used were analytical grade.

Study sites

As shown in Fig 1, an area of high traffic and an area of low traffic in Hyogo Prefecture were selected. The distance between Nishinomiya City (representing the high traffic area) and Miki City (representing the low traffic area) is about 35 km (about 22 miles) in a straight line. Nishinomiya, with an approximate population of 446,500, has two highways: route 43 (67,138 vehicles/day) and Hanshin Expressway (912,849 vehicles/day) through the city, with a total of 979,987vehicles/day. Miki, with an approximate population of 75,000 people, has route 175 (29,338 vehicles /day).

GOC collection sites and number of samples

GOCs were collected from low traffic and high traffic areas in Hyogo Prefecture, Japan. The sampling period was October 2005 to December 2006. The total number of collected samples were 14 (low traffic area was 6 samples and high traffic area was 8 samples) and the sampling time was from two to four weeks (about 20 to 40 m^3). The sampling periods in the low traffic and high traffic areas overlapped.

Collection of GOCs

The GOC collecting system was composed of a MP-Σ3 pump (Sibata, Tokyo, Japan), a 100 mm x 22 mm (length x diameter) Allihn type funnel with fused-in fritted glass disc (100-160 mesh), OMNIPORE membrane filter (0.45 μm Millipore, USA) and Porapak-QS polymer beads (50-80 mesh). Before use, the Porapak-QS polymer beads were washed with methanol followed by dichloromethane to remove organic contaminants. To prevent adsorption of impurities from the air, polymer beads were stored in a methanol solvent until use. The Allihn type funnel was packed with Porapak-QS (5 g) and the inlet was closed with an OMNIPORE membrane filter, which was

wound with Tephrone tape. The outlet of the Allihn type funnel was connected to the MP-Σ3 pump. GOCs were collected on the Porapak-QS at a flow rate of 1 liter/minute. The Allihn type funnels were protected against light during and after collection by wrapping in aluminium foil.

Preparation of collected GOCs

The GOC was collected on Porapak-QS and then extracted with 250 ml of mixed solvent [dichloromethane:acetone (4:1 v/v)] in a soxhlet apparatus for 24 hours. The extraction solvent was evaporated to 1.0 ml in a KD-condenser.

GC-MS analysis and calibration procedure

GC-MS measurements were performed in a model QP-5000 (Shimadzu, Kyoto, Japan) connected to a CG-17A gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a SLB-5ms fused silica capillary column (30 m x 0.25 mm i.d. with 0.25 μm film thickness) (SUPELCO, Bellefonte, PA) with helium as the carrier gas.

Injection was performed in split less mode (spilt opening after 4 minutes, column head pressure 150 kPa, injector 250°C). The oven temperature program was as follows: 80°C for 10 minutes, then increased at a rate of 10°C/minute up to 350°C, and holding at 350°C for 8 minutes. The mass spectrum scan mode and the selected ion monitor (SIM) were operated in electron impact mode at 70 eV. At least five different concentrations across the working range were measured in duplicate. Calibration curves were calculated by the least-squares method.

Calculation of phthalate esters and PAH exposure

We estimated phthalate and PAH exposure with the following equation (On-line reference):

Inhalation exposure (ng/kg/day) = $C_n \times V_i \div Bw_i$

C_n= mean concentration of phthalates or PAHs in GOCs (ng/m^3)

The value of this C_n is the mean concentration of phthalates and PAHs as shown in Table 2.

V_i = Respiration volume (adults: 15 m^3/day, children: 9.3 m^3/day)

Bw_i = body weight (adults: 50 kg, children: 15 kg).

Statistical analysis

The data were analyzed with the Mann-Whitney *U* test with a level of significance set at $p<0.05$.

RESULTS

The GC-MS total ion chromatograms (TIC) of GOCs for Nishinomiya and Miki City are shown in Fig 2. The TIC of Nishinomiya was different from Miki. Many peaks were observed after 25 minutes in the TIC for Nishinomiya (Fig 2A). These peaks may be diesel engine discharge compounds (> C_{22}).

The mass spectra of the congener compounds in the GOCs are shown in Fig 3. These mass spectra were observed for TIC of GOCs at retention times from 5 to 15 minutes. The mass spectrum in Fig 3A indicates 1,2-diethylbenzene (1,2-DEB). The mass number at m/z 134 appeared to be a molecular ion (M^+). The fragment ion m/z 105 appeared to be $(C_2H_5C_6H_4)^+$. The mass spectrum in Fig 3B indicates 2'-ethylacetophenone (2-EAP). The mass number at m/z 148 appeared to be M^+. The fragment ions m/z 133 and m/z 105 appeared to be $(M\text{-}CH_3)^+$ and $(C_2H_5C_6H_4)^+$, respectively. DEB has three structural isomers, and these hydroxyl derivatives of DEB had an asymmetric carbon. The mass spectrum in Fig 3C shows presumably one of 1,2-EPE or 1,2-EPEi. The mass number m/z 222 appeared to be M^+. The fragment ions m/z 207 and m/z 132 appeared to be $(M\text{-}CH_3)^+$ and $(C_2H_5C_6H_4CH{=}CH_2)^+$, respectively. The six

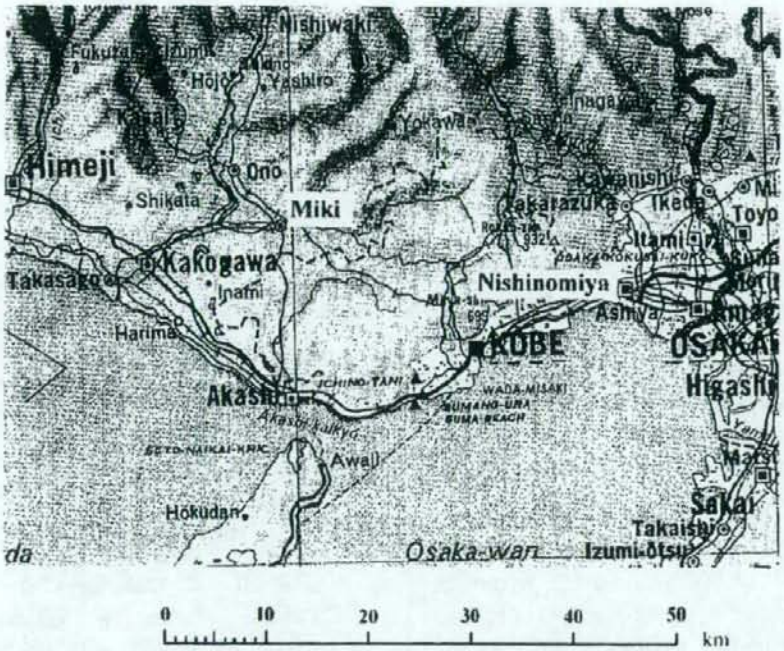

Fig 1–The geographical surroundings of the sampling points. Nishinomiya (high traffic area 979.987 traffic/day) has route 43 and the Hanshine expressway in the southern of the city and is located of Amagasaki City. Miki (low traffic area 29,338 traffic/day) is surrounded by mountains and is located north of Akashi City. The distance between Nishinomiya and Miki is about 35 km (about 22 miles) in a straight line.

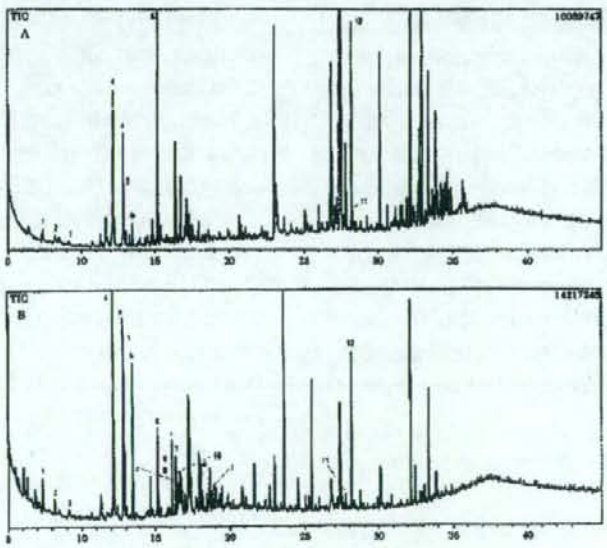

Fig 2–Total ion chromatogram (TIC) pattern of PM in Nishinomiya, and GOCs in Nishinomiya and Miki City. The peak labels correspond to those appeared in Table 1. A: TIC pattern of GOCs in Nishinomiya City. B: TIC pattern of GOCs in Miki City. The oven temperature program was as follows: 80°C for 10 minutes; increase at a rate of 10°C/minute up to 350°C, and holding at 350°C for 8 minutes.

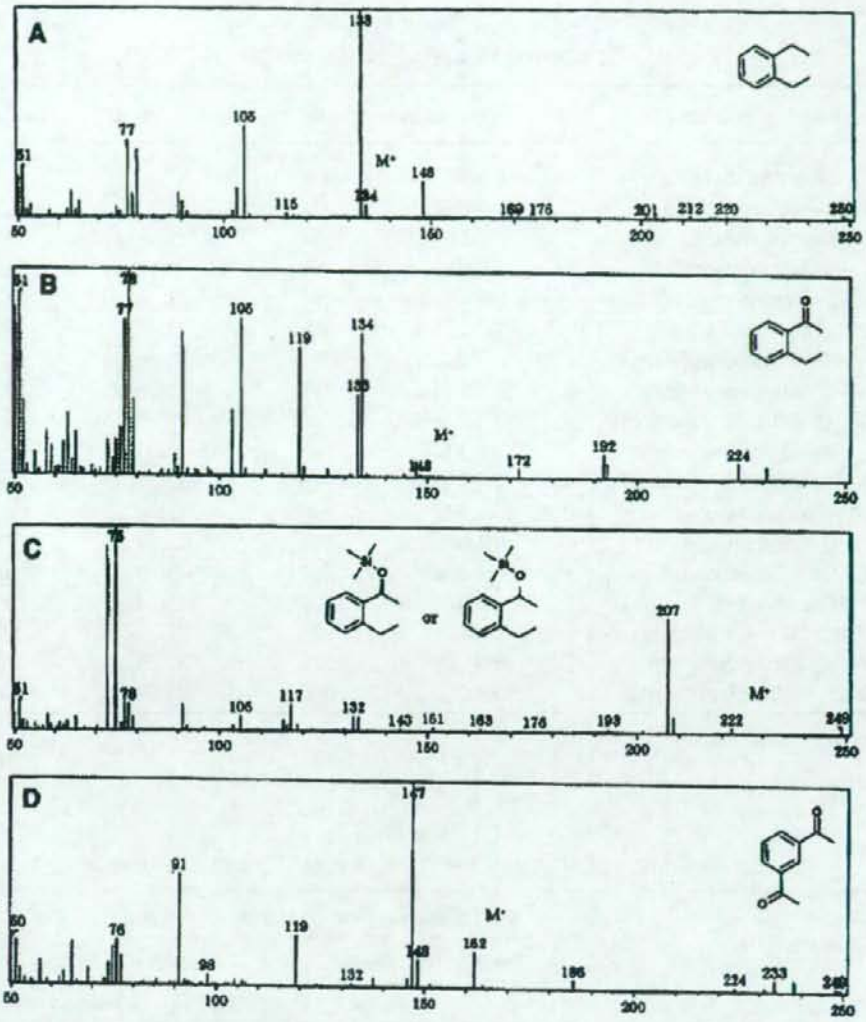

Fig 3–Mass spectra of compounds in gaseous organic chemicals.

A 1,2-Diethylbenzene; B 2'-Ethylacetophenone; C 1-(2'-ethylphenyl)ethanol or its symmetrical carbon containing isomer; D 1,3-Diacetylbenzen

peaks of the mass number m/z 207 were observed in GOCs. The fragment ion m/z 117 appeared to be $(CH_2C_6H_4CH{=}CH_2)^+$. The mass spectrum in Fig 3D is 1,3-Diacetylbenzene (1,3-DAB). The mass number m/z 162 appeared to be M^+. The fragment ion m/z 147 and m/z 119 appeared to be $(M\text{-}CH_3)^+$ and $(C_6H_4COCH_3)^+$, respectively.

The detected GOC compounds are shown in Table 1. All detected compounds had mass spectra and retention times of authentic compounds, except for six EPEs. Many detected compounds were related to DEB, except for BIP, DBBQ, BBP and DEHP. The levels of phthalate esters and PAHs in the GOCs are shown in Table 2. Pyrene, benz[a]anthracene, benzo[a]

Table 1
Compounds found in the air samples.

Detected compound	Abbreviation	MW	Formula	Peak label
1,2-Diethylbenzene	1,2-DEB	134	$C_{10}H_{14}$	1
1,3-Diethylbenzene	1,3-DEB	134	$C_{10}H_{14}$	2
1,4-Diethylbenzene	1,4-DEB	134	$C_{10}H_{14}$	3
2'-Ethylacetophenone	2-EAP	148	$C_{10}H_{12}O$	4
4'-Ethylacetophenone	4-EAP	148	$C_{10}H_{12}O$	5
Biphenyl	BIP	154	$C_{12}H_{10}$	6
D-1-(2'-ethylphenyl)ethanol	D-1,2-EPE	148	$C_{10}H_{12}O$	a
L-1-(2'-ethylphenyl)ethanol	L-1,2-EPE	148	$C_{10}H_{12}O$	b
D-1-(3'-ethylphenyl)ethanol	D-1,3-EPE	148	$C_{10}H_{12}O$	c
L-1-(3'-ethylphenyl)ethanol	L-1,3-EPE	148	$C_{10}H_{12}O$	d
D-1-(4'-ethylphenyl)ethanol	D-1,4-EPE	148	$C_{10}H_{12}O$	e
L-1-(4'-ethylphenyl)ethanol	L-1,4-EPE	148	$C_{10}H_{12}O$	f
1,2-Diacetylbenzene	1,2-DAB	162	$C_{10}H_{10}O_2$	7
1,3-Diacetylbenzene	1,3-DAB	162	$C_{10}H_{10}O_2$	8
1,4-Diacetylbenzene	1,4-DAB	162	$C_{10}H_{10}O_2$	9
Di-tert-butyl-1,4-benzoquinone	DBBQ	220	$C_{14}H_{20}O_2$	10
n-Butyl benzyl phthalate	BBP	312	$C_{19}H_{20}O_4$	11
Bis(2-ethylhexyl) phthalate	DEHP	390	$C_{24}H_{38}O_4$	12

MW=molecular weight

Table 2
Concentration of phthalates and PAHs in urban and rural GOCs.

Phthalate and PAHs	SIM	Gaseous organic chemicals (< 0.45 μm)				Mann -Whitney
		Nishinomiya (*N*=8)		Miki (*N*=6)		
		GM ng/m³	Range ng/m³	GM ng/m³	Range ng/m³	*U* test p
n-Butyl benzyl phthalate	m/z 149	15.86	2.15 - 59.64	16.69	4.47 - 89.49	0.897
Bis(2-ethylhexyl)phthalate	m/z 149	484.58	183.72 - 1,002.86	387.26	138.5 - 1,024.40	0.606
Acenaphthylene	m/z 154	85.25	4.61 - 1,010.99	53.06	5.19 - 609.96	0.606
Acenaphthene	m/z 153	9.84	3.93 - 24.44	7.71	1.18 - 163.23	0.606
Biphenyl	m/z 154	243.21	69.35 - 1,998.34	175.58	49.93 - 868.71	0.439
Fluorene	m/z 165	4.40	0.17 - 26.12	6.12	1.19 - 27.39	0.699
Phenanthrene	m/z 178	11.07	2.56 - 31.48	12.64	4.35 - 40.77	0.846
Fluoranthene	m/z 202	3.23	0.62 - 28.02	1.45	0.23 - 5.12	0.245
Pyrene	m/z 202	1.96[a]	0.74 - 4.97	0.76	0.39 - 1.62	0.020
Benz[a]anthracene	m/z 228	1.80[a]	0.41 - 3.50	0.72	0.17 - 2.00	0.039
Benzo[a]pyrene	m/z 252	3.60[a]	1.10 - 9.39	0.87	0.04 - 4.13	0.024
Benzo[ghi]perylene	m/z 276	3.04[a]	0.09 - 18.07	0.57	0.16 - 1.33	0.020

[a]$p<0.05$

Table 3
Estimated average inhalation exposure (ng/kg/day) of phthalate esters, biphenyl and PAHs.

Phthalate and PAHs	Nishinomiya		Miki	
	Adults	Children	Adults	Children
n-Butyl benzyl phthalate	4.76	9.83	5.01	10.35
Bis(2-ethylhexyl)phthalate	145.32	300.33	116.18	240.10
Acenaphthylene	25.58	52.86	15.92	32.90
Acenaphthene	2.95	6.10	2.31	4.78
Biphenyl	72.96	150.79	52.67	108.86
Fluorene	1.32	2.73	1.84	3.79
Phenanthrene	3.32	6.86	3.79	7.84
Fluoranthene	0.97	2.00	0.44	0.90
Pyrene	0.59	1.22	0.23	0.47
Benz[a]anthracene	0.54	1.12	0.22	0.45
Benzo[a]pyrene	1.08	2.23	0.26	0.54
Benzo[ghi]perylene	0.91	1.88	0.17	0.35

Adults: Respiration volume 15 m^3/day, Body weight 50 kg
Children: Respiration volume 9.3 m^3/day, Body weight 15 kg
(The respiration volume per day and the weight were the Japanese mean when the exposure of dioxin was estimated by the Japan Ministry of the Environment in 2001).

pyrene and benzo[ghi]perylene levels for Nishinomiya were significantly higher than that for Miki. As for other compounds Nishinomiya had higher levels than Miki except for fluorene.

Based on our measured phthalate and PAH levels, the inhalation exposure was calculated using the respiration volume per day and the weight (the respiration volume per day and the weight were the Japanese mean, when the Japan Ministry of the Environment estimated the exposure of dioxin in 2001). The estimated inhalation average exposure of phthalate esters and PAHs are shown in Table 3. The bis(2-ethylhexyl)phthalate (DEHP) level was the highest in the detected GOCs; adult and child DEHP exposures were 145.32 and 300.33 ng/kg/day, respectively, in Nishinomiya. In Miki, the adult and child DEHP exposures were 116.18 and 240.10 ng/kg/day, respectively. Phthalate and PAH exposures for children were higher than for adults. Pyrene, benz[a]anthracene, benzo[a]pyrene and benzo[ghi]perylene were significantly greater in adults and children in high traffic areas (Nishinomiya).

DISCUSSION

Many collection devices and sorbents have been employed to evaluate air pollution (Pellizzari *et al*, 1975). It has been reported that a good recovery pattern has been obtained for PAH on quartz micro-fiber, polyurethane foam (PUF), and XAD-2 (Iavicoli *et al*, 2006). Their results showed that pyrene, and benz[a]anthracene are adsorbed on quartz filter (at 0.3 and 2.3 ng/m^3, respectively), and naphthalene, and biphenyl are adsorbed on PUF (at 1,820 and 1,562 ng/m^3, respectively). In general, most PAHs are adsorbed or condensed onto airborne particles at high concentrations and in elevated temperatures present in smoke or exhaust pipes leading from the combustion processes in which these agents are generated. It has been reported