Estimation of α -pinene absorption in the Japanese resident based on toxicokinetic analysis in rats by inhalation exposure

Toshiaki YOSHIDA

Osaka Prefectural Institute of Public Health, 1-3-69, Nakamichi, Higashinari-ku, Osaka 537-0025, Japan

日本の住宅内での α -ピネン吸収量の推定
—吸入曝露ラットにおける体内動態の薬物動力学解析から—

吉田俊明

大阪府立公衆衛生研究所 〒537-0025 大阪市東成区中道1-3-69

Abstract

α -Pinene is a major compound contributing to indoor air pollution in Japanese residences together with many aliphatic or aromatic hydrocarbons. In the present study, the amounts of each enantiomer of α -pinene ((+)- and (-)- α -pinene) absorbed by a resident in a Japanese home were estimated by evaluating their inhalation toxicokinetics in rats. Measured amounts of the substances were injected into a closed chamber system in which a rat had been placed, and the concentration changes in the chamber were examined. The toxicokinetics of the substances were evaluated based on concentration-time courses using a nonlinear compartment model. The absorption amounts per unit time in rats exposed to the substances at constant concentration were simulated using the estimated values of the toxicokinetic parameters. The estimated amounts for the two enantiomers showed approximate agreement. When the values were compared with those for aliphatic or aromatic hydrocarbons examined in our previous studies, α -pinene was estimated to be absorbed more easily by inhalation than *n*-hexane, *n*-heptane, *n*-nonane, *n*-decane, toluene, xylenes, ethylbenzene and styrene, and to be absorbed about the same level as 1,2,4-trimethylbenzene. Their absorption amounts in human residents were extrapolated from the results for rats and the residential concentrations found in our previous study. The total amount of the two enantiomers absorbed was estimated to be 31 $\mu\text{g}/60\text{ kg}$ of human body-weight while at home for 16 h (residential concentration: 4.4 $\mu\text{g}/\text{m}^3$ as median value). The value was the highest after that for toluene. Similarly, in a residence where air pollution was marked, the absorption amount of α -pinene (13 mg for 16 h in a residence with an indoor maximum concentration of 1.8 mg/m^3) was estimated to be much more than those of other substances. The value (13 mg) was the same level as the tolerable daily intake (TDI) calculated from the No Observed Adverse Effect Level (NOAEL) proposed by the Environmental Protection Agency (EPA).

要 旨

α -ピネンは、多くの脂肪族及び芳香族炭化水素と同様に日本の住宅内の空気汚染に関与する主要な化学物質である。本研究では、 α -ピネンの2つの異性体(+)-及び(-)- α -ピネンのラットにおける体内動態をそれぞれ薬物動力学的に解析し、ヒトにおける経気道吸収量を外挿した。ラットを入れた閉鎖系曝露装置内に一定量の α -ピネンを注入後気化させ、ラットへの吸入による装置内濃度推移を調べ、薬物動力学的に解析した。得られた結果から、一定濃度の α -ピネンに一定時間曝露されたラットにおける吸収量を推定したところ、異性体間で差は認められなかった。ラットにおける炭化水素類の経気道吸収量について過去に我々が得た結果と比較すると、同一の曝露濃度下において α -ピネンは*n*-ヘキサン、*n*-デカン、トルエン、キシレン、エチルベンゼン、スチレンなどよりも吸収されやすく、1,2,4-トリメチルベンゼンと同程度であると推定された。ラットから得た結果及び日本の住宅における各物質の室内濃度に関する過去の調査結果をもとに、居住者(体重60 kg)における α -ピネンおよび各炭化水素類の吸収量を推定した。16時間の在宅時間中の α -ピネン吸収量(住宅内濃度中央値4.4 $\mu\text{g}/\text{m}^3$ において31 μg)は、トルエンに次いで多かった。また、各物質による空気汚染の著しい住宅居住者の α -ピネン吸収量(住宅内濃度1.8 mg/m^3 において13 mg)は他の物質の吸収量よりもはるかに多く、米国環境保護庁(EPA)の提案する α -ピネンの無毒性量(NOAEL)から算出した耐容一日摂取量(TDI)と同レベルであった。

Key words: α -pinene(α -ピネン), indoor air pollution(室内空気汚染), extrapolation(外挿), closed chamber system(閉鎖系曝露装置), residence(住宅), toxicokinetics(毒物動力学)

受付: 2010年4月12日 (Received: 12 April 2010)

受理: 2010年8月17日 (Accepted: 17 August 2010)

1. INTRODUCTION

The indoor air of residences is contaminated by a great variety of chemical compounds diffusing from building products and interior materials. Adverse effects on human health due to exposure to these airborne substances in residences are of concern because much time is spent at home. The average person in Japan is estimated spend about 16 hours a day at home¹⁾. α -Pinene, as well as *p*-dichlorobenzene and hydrocarbons such as toluene, was demonstrated to be a ubiquitous compound in residential air and to be a major compound contributing to indoor air pollution in Japan²⁻⁵⁾. In some buildings, marked indoor air pollution due to α -pinene has been suggested to contribute to the sick building syndrome (SBS)⁶⁻⁸⁾. In addition, α -pinene was shown to be significantly related to SBS in newly built Japanese dwellings^{9,10)}. Our previous study, which examined indoor air pollution in 105 residences of Osaka prefecture, found significantly higher concentrations of α -pinene in wooden houses than in steel-framed houses and in houses of up to 2 years after completion than in those of more than 3 years³⁾. Therefore, building products were considered to be the major source of indoor air pollution by α -pinene, although α -pinene is also a major component of turpentine oil used as solvents for paints and varnishes. We found a maximum α -pinene concentration of 1.8 mg/m³ as the average concentration over 24 h under usual living conditions among 79 volatile compounds determined for all residences examined.

The guideline value for indoor α -pinene is not given by the Japanese government or the World Health Organization (WHO), although Seifert¹¹⁾ proposed a guideline value of 30 μ g/m³ as the maximum indoor concentration of the sum of individual terpenes under normal conditions, not special circumstances. α -Pinene, which occurs naturally in pine, fir and spruce trees¹²⁾, is one of the monoterpenes and is a mixture of two enantiomers ((+)- and (-)- α -pinene). α -Pinene causes irritation of the eyes, nose and throat, palpitation, dizziness, nervous disturbances, chest pain and nephritis^{13,14)} and has been shown to be a tumor promoter in animal studies^{15,16)}. (+)- α -Pinene has been demonstrated to be more potent as a sensory irritant than (-)- α -pinene^{17,18)}.

Knowledge about daily absorption amounts of airborne α -pinene in humans while at home is very important to evaluate the adverse effects on health due to exposure to it. However, there has been no report in which the absorption amounts of α -pinene in the residents were discussed. In general, the amounts of volatile substances absorbed by inhalation are obtained by multiplying their exposure concentrations measured by respiratory minute volumes (pulmonary ventilation) cited from previous references. However, the absorption amounts per unit time of the substances by inhalation depend on their affinities for tissues and clearances from the body, that is, the absorption amounts are larger for substances that disappear rapidly by hepatic metabolism and renal elimination, and also for lipophilic substances with high affinities for tissues¹⁹⁻²¹⁾. Therefore, understanding the toxicokinetics for each substance is essential for accurate estimation of their absorption amounts. However, knowledge of the inhalation toxicokinetics for α -pinene in humans is limited¹³⁾, though there have been several reports on exposure to terpenes in workers handling wood²²⁻²⁴⁾. Falk *et al.*¹³⁾ examined the uptake, distribution and elimination of (+)- or (-)- α -pinene in human volunteers subjected to inhalation exposure (10, 225 and 450 mg/m³ during light physical exercise for 2 h). The pulmonary uptake rates of α -pinene were calculated as the difference between its amount in inhaled and exhaled air, and the mean value of the results measured periodically four times during 2 h was used to calculate the total α -pinene uptake during the entire exposure period. Although the uptake rates were presumed to vary markedly with time immediately after the start of the inhalation as the rates were considered to change with the amount of α -pinene accumulated in the body by inhalation, this matter was not considered in the study by Falk *et al.*¹³⁾. In addition, the values of the uptake rates determined by them varied markedly from 40 to 60% among exposure concentrations. Therefore, the knowledge obtained from the study was insufficient to accurately estimate the amounts absorbed in residents exposed to α -pinene. Although it was advisable to examine in human volunteers for the estimation, it was difficult to structure an exposure system in which the desired exposure

dose could be adjusted for the volunteers and the animal experiment is necessary. There also has been no report on the inhalation toxicokinetics of α -pinene for animals that would allow extrapolation of the absorption amounts for humans.

In previous studies, we established a method for estimating the amounts of volatile organic compounds, aliphatic or aromatic hydrocarbons, diffusing from the interior materials, absorbed by a driver in an automobile cabin by examining the inhalation toxicokinetics of the substances in rats, and verified the usefulness of the method^{19,25)}. In the present study, we used our method to estimate the amounts absorbed by humans exposed to each of the two enantiomers of α -pinene at home in order to obtain basic data for evaluating the health effects of exposure to α -pinene on Japanese residents under usual living conditions. The results were compared with the absorption amounts for human residents calculated for the above-mentioned aliphatic or aromatic hydrocarbons, which were among the major compounds contributing to residential air pollution, by using toxicokinetic data obtained previously. The absorption amounts could be more accurately compared among the substances by using the same method.

2. MATERIALS AND METHODS

2.1. Chemicals

(1R)-(+)- α -Pinene (CAS no.: 7785-70-8, purity: > 95%) and (1S)-(-)- α -pinene (7785-26-4, > 96.0%) were obtained from Tokyo Kasei Kogyo (Tokyo, Japan). Phosphoric acid was purchased from Wako Pure Chemical (Osaka, Japan), and Ascarite II (92% sodium hydroxide, 8-20 mesh) from Arthur H. Thomas (NJ, USA). The animals were euthanized using Somnopentyl, containing 64.8 mg/ml of pentobarbital sodium, which was purchased from Pitman-Moore (IL, USA). Gases (oxygen, nitrogen, hydrogen and air) were obtained from Naniwa Sanso (Osaka, Japan).

2.2. Equipment

The closed chamber system shown in Fig. 1 was set up in our laboratory. It was kept at room temperature of 25°C and used for repeated determination of the concentrations of (+)- and (-)- α -pinene in the chamber air.

The system consisted of a 2.7-L glass desiccator jar in which a rat was placed, a chilled vial (*ca.* 1°C) containing phosphoric acid as a trap for water from exhalation and ammonia from excrement by a cool stirrer (As-one model CPS-30, Osaka, Japan), a glass tube (300 × 21 mm i.d.) packed with Ascarite II to absorb the exhaled carbon dioxide, a 10-L Tedlar (polyvinyl fluoride) bag containing oxygen with a water trap to replenish oxygen decreased by respiration, a micro pump (flow rate 200 ml/min, reconstructed Enomoto model DM-403 ST, Tokyo, Japan) for circulation of the air in the chamber, and an electromagnetic-switching six-way valve equipped with a sampling loop of 1.0 ml (Shimadzu, Kyoto, Japan) for sending gaseous samples from the system into a gas chromatograph. The components were connected by Teflon (tetrafluoroethylene) tubes. A thin layer of high vacuum grease containing silicone (Dow Corning Toray, Tokyo, Japan) was used as a sealant for the ground glass joints. The total gas volume of the system was 2.8 L. No pressure decrease occurred in the chamber, as the loss of exhaled carbon dioxide absorbed by Ascarite II was automatically compensated for by oxygen. The gaseous samples from this system were analyzed using a Shimadzu model GC-17A gas chromatograph. The start of analysis, the pausing of the pump for gas sampling, and the switching of the sampling valve were controlled by a Shimadzu model Chromatopac C-R7A Plus integrator connected to a Shimadzu model PRG-102A auto analysis system controller.

2.3. Animals

Male Sprague-Dawley rats were purchased from Nippon Clea (Osaka, Japan). The animals, weighing 290-340 g, were used when they were 8 or 9 weeks old. Food (Laboratory Chow MF, Oriental Yeast, Japan) and water were available *ad libitum*, and the environment was maintained at a constant temperature (23°C) and under a light-dark cycle (light period; 8:00 to 18:00 h) before and after experimentation. During the exposure to the chemicals, both food and water were withheld. All studies were carried out according to the guidelines of the Animal Use and Care Committee of the Osaka Prefectural Institute of Public Health.

Fig.1 Closed chamber system used for repeated determination of concentrations of α -pinene in the chamber air. The total gas volume in the system was 2.8 L.

2.4. Analysis of α -Pinene in a Closed System

2.4.1. Estimation of toxicokinetic parameters for inhalation, exhalation, distribution and metabolic elimination of α -pinene for rats:

A rat was placed on a stainless-steel wire net in the glass desiccator jar, and immediately after the system was closed and the pump started, the circulation of air in the system was begun. The rat was allowed about 20 min for acclimation to the system. Measured amounts of (+)- or (-)- α -pinene were injected into the system through the sample injection port (Fig. 1) using a microsyringe (Hamilton, NV, USA). The liquid substances were allowed to evaporate immediately by warming the injection port with a hair dryer. For studies with one substance, inhalation runs were conducted with six different injection amounts (0.1, 0.3, 1.0, 3.0, 9.0 and 27 μ l). The different rats were used with each injection.

The gas in the system was sampled periodically by

switching the six-way valve equipped with a sampling loop, and the disappearance of the substances from the chamber air was monitored automatically by gas chromatography during the exposures. The rats were continuously exposed to the substances for about 2-6 hours. The gas chromatographic conditions were as follows: analytical column, capillary DB-5 (30 m $\times$ 0.53 mm i.d., 0.5 μ m film thickness, J&W Scientific, CA, USA); detector, flame ionization detector (FID; hydrogen, 50 ml/min; air, 500 ml/min; nitrogen as make-up gas, 30 ml/min); column oven temperature, 90°C; detector temperature, 200°C; carrier gas, nitrogen (flow rate, 10 ml/min); sampling interval, 5 min.

2.4.2. Estimation of rate constants for distribution and disappearance of α -pinene other than by inhalation by rats:

Airborne substances in a closed chamber system can disappear by reversible adsorption to the hair and skin of experimental animals and by adsorption to system

components²⁶⁾, and also by leakage due to repeated sampling for air analysis. To examine the disappearance due to factors other than by inhalation, we used rats with artificially induced respiratory arrest with a single intraperitoneal dose of about 1 ml of Somnopentyl as the control, and the carcass was placed in the glass desiccator jar. The disappearance of the substances from the chamber air was monitored for 6 h after their injection into the system in a similar manner to that described above to estimate their toxicokinetic parameters for the rats. For studies with one substance, the experiments were conducted with four different injection amounts (0.1, 0.7, 4.5 and 27 μ l).

2.5. Toxicokinetic Models

The rat body was considered to consist of two compartments (central and peripheral compartments), and the entire closed chamber system was regarded as having four compartments. The toxicokinetics of (+)- and (-)- α -pinene were evaluated using a nonlinear four-compartment (chamber air compartment, rat central compartment, rat peripheral compartment and adsorption space compartment) model with Michaelis-Menten disappearance kinetics as shown in Fig. 2. As the apparent volumetric flow rates between the two

Fig.2 Nonlinear four-compartment model for distribution and metabolism of α -pinene. V_{CA} , V_A , V_{R1} , and V_{R2} , apparent volumes of distribution for chamber air, adsorption space, and central and peripheral compartments in rat, respectively; k_1 , rate constant in the inhalation process into the central compartment of rat; k_2 , rate constant in the exhalation process from the central compartment; k_3 and k_4 , rate constants in distribution between central and peripheral compartments; k_5 and k_6 , rate constants in distribution between chamber air and adsorption space; k_7 , rate constant in the disappearance process from the chamber air; K_m , Michaelis constant; V_{max} , maximum rate of the metabolic elimination process.

compartments are the same, that is, $k_1 \times V_{CA} = k_2 \times V_{R1}$, $k_3 \times V_{R1} = k_4 \times V_{R2}$, and $k_5 \times V_{CA} = k_6 \times V_A$, the following simultaneous differential equations [Eqns (1 – 4)] can be derived:

$$dC_{CA}/dt = -(k_1 + k_5 + k_7) \times C_{CA} + k_1 \times C_{R1} + k_5 \times C_A \quad (1)$$

$$dC_{R1}/dt = k_2 \times C_{CA} - \{k_2 + k_3 + V_{max}/(K_m + C_{R1})\} \times C_{R1} + k_3 \times C_{R2} \quad (2)$$

$$dC_{R2}/dt = k_4 \times C_{R1} - k_4 \times C_{R2} \quad (3)$$

$$dC_A/dt = k_6 \times C_{CA} - k_6 \times C_A \quad (4)$$

where V_{CA} (2.8 L), V_A , V_{R1} and V_{R2} (L) are the apparent volumes of distribution for the chamber air, the adsorption space, and the central and the peripheral compartments in the rat, respectively; C_{CA} , C_A , C_{R1} and C_{R2} (μ g/L) are the concentrations of the substance in the chamber air, the adsorption space, and the central and the peripheral compartments in the rat, respectively; k_1 , k_2 , k_3 – k_6 and k_7 (1/h) are rate constants for inhalation, exhalation, distribution and disappearance processes, respectively; V_{max} (μ g/L/h) is the maximum rate of the metabolic elimination process; and K_m (μ g/L) is the Michaelis constant. The data of the concentrations in the chamber air for each exposure amount were fitted simultaneously to the model, and the toxicokinetic parameters (rate constants, V_{max} and K_m) for each substance were estimated by numerically solving the above differential equations with the initial conditions of $C_A = C_{R1} = C_{R2} = 0$ at time zero using a nonlinear least-squares program MULTI (RUNGE), programmed in BASIC for use on a personal computer (Epson Endeavor AT970, Nagano, Japan) by Yamaoka and Nakagawa²⁷⁾.

2.6. Simulation

2.6.1. Verification of estimated values for toxicokinetic parameters:

The time courses of the concentrations of the substances in the chamber air (C_{CA}) and the adsorption space (C_A) with the control (dead) rats, and in the chamber air (C_{CA}), and the central (C_{R1}) and the peripheral (C_{R2}) compartments with the live rats, were simulated using the values of the estimated toxicokinetic parameters in each exposure experiment for the substances by the

Runge-Kutta-Gill method using a program developed by Yamaoka²⁸⁾.

2.6.2. Estimation of absorption amounts of α -pinene in rats exposed to low-level concentrations:

The absorption amounts of the substances in rats exposed to constant low-level concentrations were simulated using the estimated values of the toxicokinetic parameters. The substances were apparently not eliminated by exhalation during exposure to their constant concentrations. Therefore, the apparent amounts of the substances absorbed by inhalation were approximately equivalent to the summation of the amounts of the substances that had disappeared from the body *via* metabolism by the liver and elimination by the kidney, and the amounts that had accumulated in the body during exposure. Little α -pinene was eliminated directly *via* the kidney^{13,29)}. The amounts of the substances that had accumulated in the central and peripheral compartments and the amounts metabolized during exposure to their constant concentrations were simulated using the estimated values of the toxicokinetic parameters for each substance by numerically solving the simultaneous differential equations [Eqns (5 – 7)] with the initial conditions of $X_{R1} = X_{R2} = X_M = 0$ at time zero using the Runge-Kutta-Gill method²⁸⁾ as follows:

$$dX_{R1}/dt = k_2 \times C \times V_{R1} - \{k_2 + k_3 + V_{max}/(K_m + X_{R1})\} \times X_{R1} + k_4 \times X_{R2} \quad (5)$$

$$dX_{R2}/dt = k_3 \times X_{R1} - k_4 \times X_{R2} \quad (6)$$

$$dX_M/dt = \{V_{max}/(K_m + X_{R1})\} \times X_{R1} \quad (7)$$

where X_{R1} , X_{R2} and X_M (μg) are the amounts of the substance in the central and the peripheral compartments in the rat and of the substances metabolized, respectively; C ($\mu\text{g/L}$) is the exposure concentration which should be stipulated; V_{max} ($\mu\text{g/h}$) is the maximum rate of the metabolic elimination process; and K_m (μg) is the Michaelis constant. The other abbreviations are as given above. The value of V_{R1} was calculated by $(k_1 \times V_{CA})/k_2$ where V_{CA} is 2.8 L. The values of V_{max} ($\mu\text{g/h}$) and K_m (μg) were obtained by multiplying these estimated values (V_{max} ($\mu\text{g/L/h}$) and K_m ($\mu\text{g/L}$)) by V_{R1} . The

absorption amount in the rat (A_R , μg) of a substance during exposure to its constant concentration (C , $\mu\text{g/L}$) was given by the following equation:

$$A_R = X_{R1} + X_{R2} + X_M \quad (8)$$

However, their cumulative amounts preexisted due to daily iterations of indoor (residential) and outdoor (atmospheric) exposures to the substances must also be considered to accurately evaluate the absorption attributed to the substances diffusing from interior materials and building products of the residence. Their cumulative amounts in the central and peripheral compartments of the rat immediately before renewed indoor-exposure to the substances (X_{R1-B} and X_{R2-B}) were estimated by repeatedly simulating their absorption for the rat exposed at definite time intervals to indoor and outdoor concentrations of the substances by applying the above method. The absorption amounts attributed to indoor exposure to their concentrations in the residence were simulated using the estimated amounts, X_{R1-B} and X_{R2-B} , as the initial values of X_{R1} and X_{R2} , respectively.

2.6.3. Extrapolation of absorption amounts in humans from results with rats:

Interspecies differences must be considered to extrapolate absorption in humans from the results obtained with the rats. There have been many studies for pharmaceuticals on allometric scaling of pharmacokinetic parameters from animals to humans³⁰⁻³⁵⁾. Caldwell *et al.*³¹⁾ found that the total systemic clearance (per unit body weight) of drugs in humans was *ca.* 16% of that for rats and the apparent volume of distribution (per unit body weight) in humans was *ca.* 67% of that for rats by analysis of previous pharmacokinetic data for more than 100 pharmaceuticals. Sawada *et al.*³³⁾ also showed that the metabolic clearance of drugs in humans was 13% of that for rats. Similarly, the results by Bachmann *et al.*³⁰⁾ show that the apparent volume of distribution of xenobiotics in humans was *ca.* 60% of that for rats. However, there was hardly any available data to compare the apparent volume of distribution and the clearance of the objective α -pinene, which were not taken up intentionally unlike pharmaceuticals, between humans and animals. As the volumes of

distribution of the substances absorbed are closely related to their lipophilicities, it was considered that the rates of the volumes in humans to those in rats would not differ much among chemical compounds. The olive oil/blood partition coefficient (190) of α -pinene³⁶⁾ agreed with that (183) for *n*-hexane³⁷⁾. The volume of distribution of *n*-hexane in humans was *ca.* 62% of that for rats³⁸⁾. This value agreed with those for the above pharmaceuticals and xenobiotics. In the present study, the result of Filser *et al.*³⁸⁾ was applied to the rat to human extrapolation of the apparent volume of distribution for α -pinene. The values of the systemic clearance in humans³⁹⁻⁴¹⁾ and rats^{42,43)} exposed to styrene were 1.43 (on average) and 11.7 L/h/kg, respectively, that is, the value in humans was *ca.* 12% of that for rats. The metabolic clearance of *n*-hexane in humans was 11% of that of rats³⁸⁾. The rates of the clearances in humans to those in rats for these lipophilic compounds as well as α -pinene approximately agreed with the rates for the drugs (13-16%) as mentioned above. The value (11%) for *n*-hexane was applied to the rat to human extrapolation of the clearance of α -pinene, because α -pinene consists of an isoprene which is one of the aliphatic hydrocarbons, such as *n*-hexane. The absorption amounts of (+)- and (-)- α -pinene diffusing from the interior materials and building products by a human while at home was calculated on the basis of the following three assumptions:

1. The apparent volume of distribution (per unit body weight) of the substances in humans is 62% of that of rats.
2. The metabolic clearance (per unit body weight) of α -pinene in humans is 11% of that of rats.
3. The physiological times in humans and rats are proportional to body-weight^{0.25-44)}.

Namely, the absorption amounts in humans (A_H , μg) of the substances were calculated by using the following equation:

$$A_H = (X_{R1} + X_{R2}) \times (W_H / W_R) \times 0.62 + X_M \times (W_H / W_R) \times 0.11 \quad (9)$$

where W_H and W_R (kg) are the body weights for humans and rats, respectively. X_{R1} and X_{R2} were the amounts of the substance in the central and the peripheral compartments at the rat's physiological time (T_R , h) converted to chronological time (T_H , h) for humans [$T_H = T_R \times (W_H / W_R)^{0.25-44)$].

3. RESULTS

3.1. Estimation of Toxicokinetic Parameters

The changes of concentrations of (+)- or (-)- α -pinene in the chamber air were monitored by gas chromatography after their injection into the system with the control rats in order to estimate the rate constants for their distribution to the adsorption space (inner surface of the system, body hair of the rat, *etc.*) and for their disappearance by dissolution into the phosphoric acid solution and by leakage from the system (k_5 , k_6 and k_7 in Fig. 2). The concentration-time courses of the substances in the chamber air are shown in Fig. 3 as semilogarithmic plots. The concentrations of both substances decreased exponentially after injection during circulation of the air in the system. The slopes of the curves at steady state after their injection appeared to be constant among the injection amounts. Their rates of decrease were proportional to their concentrations in the chamber air. Therefore, the processes of distribution and disappearance were considered to be linear, and the rate constants (k_5 , k_6 and k_7) for each substance were estimated by simultaneously fitting the concentration-time course data for each injection amount of a substance to a linear two-compartment model without the rat compartments in Fig. 2. The estimated values of k_5 , k_6 and k_7 for the substances, shown in Table 1, were used as constant values to estimate the other toxicokinetic parameters of the substances for the biotransformation in rats.

The concentration-time courses of the substances in the chamber air during exposure of the live rats are shown in Fig. 3. The slopes of the curves in steady state after injection of the substances into the closed system appeared to increase with lower exposure concentrations. The rates for disappearance of the substances were not proportional to their concentrations in the chamber air. The slopes of the curves were

(+)- α -Pinene **(-)- α -Pinene**

Fig.3 Concentration-time courses of α -pinene in the chamber air after injections into the closed system with a dead (left) or live (right) rat ($\circ$). The lines were generated by simulation with the toxicokinetic model using the values of the estimated kinetic parameters shown in Table 1; —, — — —, and ---- are concentrations of the substances in the chamber air (C_{CA}), the adsorption space (C_A), and the central (C_{R1}) and the peripheral (C_{R2}) compartments in rat, respectively.

The body weights for dead or live rats used were as follows: 321 ± 1.9 g (dead) and 313 ± 24 g (live) for (+)- α -pinene exposure, 316 ± 3.3 g (dead) and 306 ± 19 g (live) for (-)- α -pinene exposure. There were no significant differences between groups.

characteristic for saturation kinetics, namely the disappearance proceeded enzymatically. Furthermore, the substances studied were lipophilic. Therefore, the rat's body was postulated to consist of two compartments (two-compartment rat model) rather than one (one-compartment rat model), and the entire closed system was regarded as having four compartments for the substances. The toxicokinetics of the substances were evaluated using the nonlinear four-compartment model. In practice, the data fitted the two-compartment rat model more accurately than the one-compartment rat

model for both substances. The values of the estimated toxicokinetic parameters for each substance in rats are given in Table 1.

3.2. Simulation

The concentration-time courses of α -pinene in the chamber air, in the adsorption space, and in the central and peripheral compartments in rats were simulated using the values of the estimated toxicokinetic parameters shown in Table 1, and the results are presented in Fig. 3. An accurate fit can be seen for the simulation curves

for the substances in the chamber air to the experimental data. The absorption amounts of each substance were estimated in rats exposed to $50 \mu\text{g}/\text{m}^3$ for 12 h using the estimated values of the toxicokinetic parameters on the assumption that the compartment model used was also suitable outside the exposure concentration range tested in this study. The absorption amounts were estimated to be 38 and $40 \mu\text{g}$ per 1 kg of rat body weight for (+)- and (-)- α -pinene, respectively, namely showing approximate agreement between the enantiomers (Table 2).

3.3. Extrapolation

We had previously examined indoor air concentrations of volatile chemicals containing α -pinene in 105 Japanese residences under conventional living conditions³⁾. Their outdoor (atmospheric environment) concentrations had also been measured in our other study^{4,5)}. As the indoor and outdoor concentrations of α -pinene had been quantified in these studies as a mixture of the enantiomers, in the present study, the half values were assumed to be the concentrations of each enantiomer in both indoor and outdoor air; the values of indoor (median and maximum) and outdoor (median) concentrations are presented in Table 3. The

absorption amounts of α -pinene diffusing from the interior materials and building products in the residences under usual living conditions were estimated for humans with continuous exposure at home under these conditions of indoor and outdoor air pollution, and the results are shown in Table 3 with the values converted to the absorption amount per 60 kg of human body weight. The absorption amounts for 16 h, which is the average time spent at home by the Japanese¹⁾, were estimated to be $15 \mu\text{g}$ for (+)- α -pinene and $16 \mu\text{g}$ for (-)- α -pinene in a residence polluted by mid-level concentrations. A full-time housewife who spends a long time in a residence (20 h/day) was estimated to absorb about $38 \mu\text{g}$ or 16 mg as the total amount of α -pinene in a residence polluted by mid-level ($4.4 \mu\text{g}/\text{m}^3$) and high-level ($1.8 \text{ mg}/\text{m}^3$) concentrations, respectively.

4. DISCUSSION

The inhalation toxicokinetics of (+)- and (-)- α -pinene were evaluated for rats using a nonlinear four-compartment model, and the absorption amounts of the substances were extrapolated for humans exposed to their lower concentrations in residences on the basis of the results obtained with the rats. The absorption amounts of the two enantiomers were estimated to be

Table 1 Toxicokinetic parameters for transformation of α -pinene

	k_1 (1/h)	k_2 (1/h)	k_3 (1/h)	k_4 (1/h)	k_5 (1/h)	k_6 (1/h)	k_7 (1/h)	V_{max} ($\mu\text{g}/\text{L}/\text{h}$)	K_m ($\mu\text{g}/\text{L}$)
(+)- α -Pinene	8.18	7.42	4.92	0.150	2.68	3.00	0.142	245	5.31
(-)- α -Pinene	8.70	8.34	4.24	0.186	1.57	2.34	0.114	761	18.4

Table 2 Estimated amounts of α -pinene and typical indoor air contaminants absorbed in rats exposed to $50 \mu\text{g}/\text{m}^3$ for 12 h

(+)- α -Pinene	38	(-)- α -Pinene	40
<i>n</i> -Hexane	7.3 ^a	<i>o</i> -Xylene	22 ^b
<i>n</i> -Heptane	14 ^a	<i>m</i> -Xylene	21 ^b
<i>n</i> -Nonane	20 ^a	<i>p</i> -Xylene	32 ^b
<i>n</i> -Decane	22 ^a	Styrene	34 ^b
Toluene	25 ^b	1,2,4-Trimethylbenzene	39 ^b
Ethylbenzene	28 ^b		

The values were converted to the absorbed amount per 1 kg of rat body weight ($\mu\text{g}/\text{kg}$).

^a The values are cited from our previous paper¹⁹⁾.

^b The values are cited from our previous paper²⁵⁾.

approximately equal in rats exposed to concentrations of $50 \mu\text{g}/\text{m}^3$ for 12 h (Table 2). In the study with human volunteers by Falk *et al.*¹³⁾, no significant differences in uptake, distribution or elimination were found between the enantiomers. Therefore, the kinetics of α -pinene is likely to be the same between the enantiomers in mammals, although there is difference in their toxic activities^{17,18)}. The estimated amounts of absorbed α -pinene were compared with those for aliphatic or aromatic hydrocarbons examined by using the same experimental method in our previous studies (Table 2)^{19,25)}. In the studies, the absorption amounts of the substances had been suggested to show a tendency to increase with the number of carbon atoms in their chemical structures for each chemical class. α -Pinene was estimated to be absorbed more easily than the normal alkanes with C6-C10 or aromatics with C7-C8 which are typical indoor air contaminants, and to be absorbed nearly equally to 1,2,4-trimethylbenzene (C9), which is a more lipophilic aromatic.

The indoor and outdoor concentrations in Japan of the aliphatic or aromatic hydrocarbons as well as α -pinene were examined in our previous studies^{3,45)}. The absorption amounts of substances diffusing from the interior materials and building products in residences under usual living conditions were estimated for humans under these conditions of indoor and outdoor air pollution by using the values of their toxicokinetic parameters obtained by the same method as the present study^{19,25)}, and the results are shown in Table 4. Extrapolation of the absorption amount of α -pinene, $31 \mu\text{g}$ as the total amount for the two enantiomers during 16 h, shown in Table 3, gave the highest value next to toluene ($102 \mu\text{g}$) and gave the same level as the total amounts for three isomers of xylene for humans living in a Japanese residence in which the indoor air was polluted by mid-level concentrations of the substances. In a residence where air pollution was marked, the total amount of α -pinene (13 mg) absorbed over 16 h was much more than those of other substances (Table 3 and 4), and was *ca.* 10 times of that for *n*-nonane (1.4 mg) which was maximum among the absorption amounts of the hydrocarbons studied. Therefore, α -pinene was considered to be one of the volatile organic

compounds most abundantly absorbed in a Japanese residence. The Environmental Protection Agency (EPA)⁴⁶⁾ proposed No Observed Adverse Effect Level (NOAEL) of 21 (male) or 170 (female) mg/kg body weight per day for α -pinene by inhalation on the basis of results for rats tested in the National Toxicology Program⁴⁷⁾. As the values of Tolerable Daily Intake (TDI) of xenobiotics are usually calculated by $\text{NOAEL}/(10 \times 10)$, the values for α -pinene were extrapolated to be 13 mg (male) or 102 mg (female) per 60 kg of human body weight. The absorption amount of α -pinene by a human spending 16 h at home where air pollution was marked came to the same level as the TDI for males. Consequently, α -pinene was suggested to be the most noteworthy indoor air pollutant of Japanese residences, especially in new wooden residences³⁾, from the viewpoint of the effect on health.

The pulmonary uptake rate (U_H , %) of each enantiomer of α -pinene in a human while at home polluted by mid-level ($2.2 \mu\text{g}/\text{m}^3$) and high-level ($0.9 \text{ mg}/\text{m}^3$) concentrations of the substances was calculated from the results shown in Table 3 according to the following equations [Eqns (10, 11)]:

$$U_H = A_H / I_H \times 100 \quad (10)$$

$$I_H = C_E \times VP / 1000 \times t \quad (11)$$

where A_H is the absorption amount estimated for humans (μg); I_H is total pulmonary intake (μg); C_E is exposure concentration ($\mu\text{g}/\text{m}^3$); VP is pulmonary ventilation (L/min); t is duration of exposure (min). The values for A_H , C_E (indoor concentration) and t were from Table 3. The value of VP for a Japanese resident was assumed to be $12.5 \text{ L}/\text{min}$ ⁴⁸⁾. The values of the uptake rate for (+)- α -pinene were calculated to be 58% and 57% while at home (indoor concentration: $2.2 \mu\text{g}/\text{m}^3$) for 12 and 24 h, respectively, and similarly the values for (–)- α -pinene were 60% and 59% for 12 and 24 h, respectively. The values were calculated to be rarely different in the residences polluted by high-level ($0.9 \text{ mg}/\text{m}^3$) concentrations of both (+)- and (–)- α -pinene. The uptake rates for human volunteers exposed to (+)- α -pinene ($10, 225, 450 \text{ mg}/\text{m}^3$) or (–)- α -pinene ($450 \text{ mg}/\text{m}^3$) during light physical exercise (50 W) for 2 h

Table 3 Extrapolated amounts absorbed by a Japanese resident exposed to α -pinene diffusing from interior materials and building products in his/her residence

	Outdoor conc. (median, $\mu\text{g}/\text{m}^3$) ^a	Indoor conc. (median, $\mu\text{g}/\text{m}^3$) ^b	Absorption amounts (μg) ^c			Indoor conc. (max., $\mu\text{g}/\text{m}^3$) ^b	Absorption amounts (μg) ^c		
			Time at home				Time at home		
			12 h	16 h	20 h		12 h	16 h	20 h
(+)- α -Pinene	0.27	2.2	11	15	19	900	4737	6220	7643
(-)- α -Pinene	0.27	2.2	12	16	19	900	4923	6461	7938

As the indoor and outdoor concentrations of (+)- and (-)- α -pinene were quantified as a mixture, the half values were assumed to be the concentrations of each substance.

^a The values are cited from our previous paper⁴⁵⁾.

^b The values are cited from our previous paper³⁾.

^c The values are converted to the absorption amount per 60 kg of human body weight.

Table 4 Extrapolated amounts absorbed by a Japanese resident exposed to typical indoor air contaminants diffusing from interior materials and building products in his/her residence for 16 h

	Outdoor conc. (median, $\mu\text{g}/\text{m}^3$) ^b	Indoor conc. (median, $\mu\text{g}/\text{m}^3$) ^c	Absorption amounts (μg) ^d	Indoor conc. (max., $\mu\text{g}/\text{m}^3$) ^c	Absorption amounts (μg) ^d
<i>n</i> -Hexane	2.4	2.5	3.2	77	105
<i>n</i> -Heptane	1.0	2.2	5.7	194	510
<i>n</i> -Nonane	0.98	1.9	7.8	336	1412
<i>n</i> -Decane	1.6	2.8	12	217	981
Toluene	16	22	102	145	718
Ethylbenzene	2.7	4.7	24	50	267
<i>o</i> -Xylene	0.97	2.0	8.1	55	238
<i>m</i> -Xylene ^a	1.6	2.8	11	56	226
<i>p</i> -Xylene ^a	1.6	2.8	16	56	317
Styrene	0.028	0.11	0.72	22	145
1,2,4-Trimethylbenzene	1.5	2.5	18	69	515

^a As the indoor and outdoor concentrations of *m*- and *p*-xylene were quantified as a mixture, the half values were assumed to be the concentrations of each substance.

^b The values are cited from our previous paper⁴⁵⁾.

^c The values are cited from our previous paper³⁾.

^d The values are converted to absorption amount per 60 kg of human body weight.

had been as follows: 40% for 10 mg/m^3 of (+)- α -pinene, 60% for 225 mg/m^3 of (+)- α -pinene, 58% for 450 mg/m^3 of (+)- and (-)- α -pinene¹³⁾. The values for the two higher exposure concentrations are in agreement with our results.

No guideline value for indoor α -pinene concentration has yet been recommended in Japan. However, the absorption amount of α -pinene in a human in a Japanese residence, where air pollution was marked, was estimated to be the same level as the TDI calculated from the NOAEL proposed by the EPA⁴⁶⁾. Furthermore, α -pinene was clarified to be significantly related to SBS in newly built Japanese dwellings^{9,10)}. Therefore, it was

considered that the guideline value for indoor α -pinene should be established to prevent the adverse effects on health in the residents.

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