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Experimental Studies on the Concentrations of Subjectively Equivalent Irritation for Odorants

By

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Abstract

Discussion of non-olfactory "irritation", which is caused by the excitement of the trigeminal nerve, has important implications for odor pollution. The main purpose of this study is to work out the mechanisms for the generation of non-olfactory irritation, and the sensory characteristics of the perceived irritation. Concentrations of subjectively equivalent irritation (C_E) of 16 odorous compounds were measured by the sensory test. The relations between the C_E and the physicochemical properties of the compounds were shown and discussed. It was found that the C_E of dissociated compounds is much lower than that of undissociated compounds. And among the dissociated compounds, the acidic ones have a lower C_E than the basic compounds. The C_E of acrolein, which is an unsaturated compound, was the lowest among all the compounds used in this experiment. The delay time, i.e. time until the sensation of irritation after inhaling, was longer for acrolein gas than for other gases. Therefore, the mechanism for generating sensory irritation by the acrolein gas may be different from that of other gases. From analysis on the basis of relation to some physicochemical properties, it was concluded that the electrophilic reactivity of the acrolein molecule may be related to the generation of sensory irritation. In the case of dissociated compounds, the C_E is related to the dissociation constants of the compounds. In other words, the larger the acidic and basic dissociation constants (K_a and K_b) are, the lower the C_E becomes.

Key Words: Sensory test, Odorous irritant, Concentration for subjectively equivalent irritation, Physicochemical properties, Dissociation constant

Introduction

Olfactory irritation is caused by the reception of odorants by the olfactory nerve. On the other hand, non-olfactory irritation is caused by the reception of odorants by the tip of the trigeminal nerve, and it is usually described as an irritation which assails human's nostrils[1]. It is necessary to study olfactory irritation as a

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step towards solving the problem of odor pollution, because olfactory irritation depends on the type and concentration of odorants which are dominant factors in the degree of discomfort an odorant causes.

Cain[2] mentioned that the odor degree is related to irritation and when the degree of odor is divided into olfactory stimulation and non-olfactory stimulation, an additivity is found between them[3]. On the other hand, Engen[4] pointed out that olfactory stimulation dominant with lower concentrations of an odorant, and non-olfactory stimulation with higher concentrations. However, studies about the sensory properties of irritation using psychophysical experiments are scarce and no histological study on the mechanism of odorant reception by the tip of the trigeminal nerve has ever been reported.

Tucker[5] measured the response of the trigeminal nerves of a turtle and a rabbit electro-physiologically, and showed a threshold value of 170 ppm for amyl acetate. Kulle and Cooper[6] determined the threshold values obtained from an experiment on a rat as 0.25 ppm for formaldehyde, 5.0 ppm for ozone, 0.3 ppm for amyl alcohol. Silver[7] also made a similar experiment.

Thus, the properties of the trigeminal nerve as chemical sense receptor have been examined by measuring its sensitivity to different chemicals. However, a clue clarifying the mechanism of odorant reception by the tip of the trigeminal nerve has not been worked out yet.

The purpose of this paper is to clarify the mechanism for the occurrence of non-olfactory trigeminal nervous irritation and its sensory properties. Firstly, the relationship between the irritative sensation of odorous materials and the concentration of materials were studied experimentally. In this regard, the concentrations of materials (concentration for subjectively equivalent irritation or C_E) have been measured for 16 kinds of odorants by the sensory test. Furthermore, some studies have been carried out to investigate the C_E for the difference between each odorant and also the physicochemical properties of odorants at molecular levels.

Experiment and Analytical Method

1. Selection of materials

Generally, the threshold values of odorants are higher for materials having lower molecular weights. We[8] have already pointed out the possibility that this tendency is related to the absorption of odorants by the lipid wall of the olfactory cell membrane and to its dissolution capacity. Since the threshold value of the trigeminal nervous stimulation is generally higher than that of olfactory stimulation[9], it is supposed that the threshold value of an odorant obtained by a sensory test corresponds to the concentration when the electric potential of olfactory receptor cell occurs, namely the threshold value of olfactory stimulation. Therefore, the sensory degree of high molecular-weight materials is usually higher than that of low molecular-weight materials when their concentrations are equal. This is because the threshold values of high molecular-weight materials are lower than those of low molecular-weight materials compared. For example, when methyl alcohol (with a threshold value of 21 ppm[8]) and octyl alcohol (0.0021 ppm[8]) are both 20 ppm, the former has almost no smell because its concentration is close to its threshold value, but the latter gives off a strong smell. Therefore, it is necessary to reduce the effect of olfactory stimulation when the sensory properties of trigeminal nervous stimulation, i.e. irritability, are compared. It is supposed that the use of low molecular-weight materials for such comparative measurements is effective.

Since it is supposed that the potency to irritate of an odorant depends on the structural properties of its constituent molecules, especially the type of functional groups, the materials for this study were selected from each group with different functional groups.

The subjects of this experiment are shown in Table 1. The total number of the materials are 16, as follows: Saturated solutions–four kinds of fatty acids (formic acid being the standard odor), four kinds of amines, two kinds of esters, one alcohol, one aldehyde, one ketone, two kinds of non-saturated solution, and hydrogen chloride. The molecular weights of these materials are 36–88 (with atomic numbers of 4–14). In Table 1, their chemical formulae, molecular weights, and threshold concentrations[8][10][11] are also presented.

2. Selection of the panel

For a person with little experience, it is difficult to recognize the difference in the sensation between trigeminal nervous stimulation and olfactory stimulation. Such a person especially tends to judge the case as a strong irritation when the odor is merely strong or the discomfort is of high degree. Therefore, we selected a panel who had had experience in sensory testing of odors for more than a year, and had enough physiological and chemical knowledge of odorants. The number of panel members was limited to three, which is less than the number for common odor concentration measurements[12], but the reason for our decision was to avoid any risk of the occurrence of olfactory disorder if something went wrong with preparing the concentrations of odorants which might have caused severe discomfort through inhaling. These three examinees were all male aged 23–27 years old. Their olfactory ability was tested by the T&T olfactory test[13] and accepted as normal.

Table 1. Compounds as subjects of this experiment.

Name of Compound	Formula	M.W.	Threshold (ppm)*
Formic acid	$HCOOH$	46.02	0.46
Acetic acid	CH_3COOH	60.05	0.021
Propionic acid	C_2H_5COOH	74.08	0.016
Isobutyric acid	$(CH_3)_2CHCOOH$	88.10	0.00012
Ammonia	NH_3	17.03	1.2
Methylamine	CH_3NH_2	31.06	0.025
Dimethylamine	$(CH_3)_2NH$	45.08	0.022
Trimethylamine	$(CH_3)_3N$	59.11	0.00040
Methyl formate	$HCOOCH_3$	60.05	31
Ethyl formate	$HCOOC_2H_5$	74.08	3.9
Methyl alcohol	CH_3OH	32.04	21
Acetaldehyde	CH_3CHO	44.05	0.023
Acetone	CH_3COCH_3	58.08	32
Acrolein	CH_2CHCHO	56.06	0.0036**
Acrylic acid	$CH_2CHCOOH$	72.06	—
Hydrogen chloride	HCl	36.46	10***

*Values cited from reference 8)
**Values cited from reference 10)
***Values cited from reference 11)
M.W. = molecular weight

3. Training of the panel

The purpose of this experiment was to decide the concentrations of odorants indicating sensory irritation (i.e., concentration for subjectively equivalent irritation or C_E), equal to the standard odor. The panel members were trained for this experiment because it is not easy for an unexperienced person to distinguish between a sensory irritation, which "assails one's nostrils," and an olfactory stimulation.

When the C_E was determined by comparing the subject odors with the standard odor, it was necessary to take into account two properties of the olfactory sense, namely that the memory of smell is not as clear as that of other senses[4)], and that the olfactory sense tends to get adapted (fatigued) in a very short time[4)]. These properties are contradictory when odors are compared with each other. That is to say, the adaptation of the olfactory sense affects the judgment within the short time of comparison while the memory is still fresh. When the comparison takes enough time to avoid the influence of olfactory adaptation, the first memory of subject odor becomes weak and a reliable rating (judgment) value is not obtained. Therefore, we studied the shortest time interval during which the olfactory adaptation will not affect the judgment.

(i) Procedure of training the panel

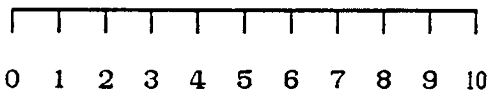
The training of the panel was carried out employing formic acid as follows:

Training 1: There were three concentrations of formic acid, i.e. 10 ppm, 30 ppm, and 90 ppm (three-fold series). One of the concentrations was randomly chosen as a sample and was given to the panel every five minutes to have them respond to its irritation compared to the Sensory Scale of Irritation (Fig. 1). In this way, dispersion of the rating of irritation could be known. Odor bags (3L each) were used as sample containers[14].

Training 2: The procedure for this training was the same as that of Training 1, but the three concentrations were made in 15 ppm, 30 ppm, and 60 ppm (i.e. two-fold series), and the gaps between the concentrations were made shorter than those in Training 1. Thus, changes in the capability of sensory distinction could be investigated when the concentration gap was shortened. Moreover, when the range of odor concentration and its number of steps were decided, the results obtained could be utilized for the measurement of the C_E (which will be mentioned later).

Training 3: One of the samples in the three concentrations (15 ppm, 30 ppm, 60 ppm, i.e. two-fold series) was given to the panel every five minutes. The panel were told that the 30 ppm sample would be given at first as a standard odor, and that it would be provided every twenty minutes again, i.e. every fourth step. In this way, the panel could correct their criterion concept every twenty minutes by comparing it with the irritation of the standard odor which had been rated initially.

In this experiment, it was clarified that the correction of the panel's criterion



0：刺激性は全く感じない。

10：想像しうる限りで最も強い刺激性。

※刺激性は"鼻につん"とくる感覚である。

Fig. 1 Sensory scale of irritation (in Japanese) concept with reference to the standard odor provided in between the experimental trials affected the dispersion of the rating of irritation, and in order to rate the C_E, it was found to be necessary to compare the panel's criterion concept with the standard odor. Trainings 1, 2, and 3 were each carried out on a different day.

(ii) Determining the time intervals for comparative rating of odor irritation (i.e. the relation between time intervals for rating and olfactory adaptation).

In order to decide the shortest time interval for rating the irritation which would not be affected by the adaptation of the olfactory sense, we asked the panel to rate the irritation for formic acid (30 ppm) at fixed time intervals, and measured the changes in the irritation with the passage of time. The time intervals were set at 40 sec, 30 sec, 20 sec, and 15 sec. For rating the odor irritation, the panel pressed the odor bags lightly with their hands and inhaled twice in ordinary breathing.

4. Procedure for the measurement of the C_E

The C_E of the subject materials was measured with reference to formic acid (30 ppm) as the standard odor. In a preparatory experiment, the range of concentrations of each odorous compound which produced almost the same level of irritation as that of the standard odor was decided, and within this range the samples having four different concentrations (dilutions of two-fold series) were iven to the panel along with the standard odor.

At first, the panel inhaled the standard odor and recognized the irritation. Then, 20 sec later, they smelled the lowest concentration of the subject odor and compared its level of irritation with that of the standard odor. In this way, the panel smelled the standard as well as the subject odors in turn at intervals of 20 sec, and then selected an odor concentration which had the same or higher level of irritation as that of the standard odor.

The reason for comparing the subject odors with the standard odor in parallel and then rating the irritation was that the dispersion of rating values is reduced by repeated recognition of the irritation caused by the standard odor; this fact was known from the results of Trainings 1–3. In order to maintain the memory of irritation, the panel were given a Sensory Scale of Irritation (Fig. 1) so as to check the potency to irritate of the odor samples.

5. Measurement of odorant concentration

The concentration of each odorant was adjusted as follows. Clean air, which was passed through an active carbon layer, was introduced into a polyester bag (1L) and a certain amount of each odorant in liquid state was poured into the bag by micro-syringe. This was kept undisturbed for 1–2 hours until the air and the liquid inside the bag reached almost a stable condition and became a saturated gas of high concentration. The concentration of the saturated gas inside the bag was measured as follows. Ammonia and hydrogen chloride were measured by the detector tube method, and the concentration of other constituents were measured by the gas chromatography method (FID). Table 2 presents the analytical conditions. The concentration of odor in the bags which were given to the panel, was obtained from the concentration of the saturated gas and the dilution rate.

Table 2. Experimental conditions for measuring the concentrations of odorants by gas chromatography.

	Alcohol Ketone Aldehyde Ester	Acid	Amine
Detector	Flame Ionization Detector (FID: YANACO G-2800)		
Column	PEG-HT 60/80 Glass $3\phi \times 2$ m	OV-17 60/80 Glass $3\phi \times 2$ m	Amipack 124 60/80 Glass $3\phi \times 2$ m
Column Temp. (°C)	100	150	70
Inject Temp. (°C)	120	180	100
Detect Temp. (°C)	120	180	100
H_2 (kg/cm^2)	1.0	1.0	1.0
Air (kg/cm^2)	1.0	1.0	1.0
He (kg/cm^2)	0.8	0.8	0.8

6. Selection of physicochemical parameters of gas molecules and method of calculation

In the molecular index, which determines physiological activity, there are many kinds of physicochemical and geometrical parameters. It is usually thought that a higher degree of chemical reactivity is found among the materials which cause irritation, and there are many dissociated materials which cause irritation. Therefore, in this study we studied the relationship of irritation to other factors as follows: the orbital energy of the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO)[15] as a general reactive index determined by the quantum-mechanical method; S_r as reactive index in proportion to Fukui's Superdelocalizability[15]; and acid and base dissociation constants. The HOMO and LUMO are called "frontier molecular orbitals," and it is known that they play very important roles in chemical reactions[15][16]. The HOMO is related to nucleophilic reactivity, and the LUMO to electrophilic reactivity. In simple terms, the higher the orbital energy of the HOMO is and also the lower the orbital energy of the LUMO is, the more unstable both orbitals are. Then there is a greater tendency for electrons to transfer, and reaction thus takes place faster. S_r is a reactive index which is shown in the following equations which also shows a tendency for nucleophilicity ($S_r^{(E)}$) and electrophilicity ($S_r^{(N)}$) to be reactive in the molecule.

$$S_r^{(E)} = 2 \sum_i^{\text{occu}} \frac{(C_r^i)^2}{-\varepsilon_i} \quad (1)$$

$$S_r^{(N)} = 2 \sum_i^{\text{unocc}} \frac{(C_r^i)^2}{\varepsilon_i} \quad (2)$$

where C_r^i is the element of eigenvector; $(C_r^i)^2$ shows the density of electrons in molecular orbital i, which is in atom r.

ε_i is a eigenvalue showing the orbital energy (eV) of a molecular orbital i.

If ε_i in equations (1) and (2) is replaced by λ_i according to the following equation, S_r will have the same value as that of Fukui's Superdelocalizability.

$$\lambda_i = (\varepsilon_i - \alpha)/\beta \quad (3)$$

where α is the Coulombic integral (about -3eV), and β is the resonance integral (about -7eV).

Since superdelocalizability is a very good index for the comparison of degrees of reaction between molecules, S_r may also be regarded as a reactive index. However, superdelocalizability is usually calculated for all the π electrons according to Huckel's method[15]. In this study, we took account of the possibility that σ electrons might be related to chemical reaction, and it was calculated for the σ electrons and the π electrons which were in the p orbital.

For the calculation of the orbital energy of the HOMO, the LUMO and density of electrons, which were required for calculating S_r, the MNDO method[17] was used, which is a semi-empirical molecular orbital method. In addition, MATERIA (TEIJIN system technology) was used for the calculation program of the MNDO method. The values of acid and base dissociation constants (K_a and K_b, respectively) were reported values[18].

Experimental Results

1. Training of the panel

(i) Training 1

Fig. 2 shows the rating values for irritation by formic acid with concentrations of 10 ppm, 30 ppm, and 90 ppm (three-fold series). The rating values for formic acid at 30 ppm are 3–5 as judged by panel A and 3–7 by panel B, which shows a slight dispersion. The standard deviations are 0.75 and 1.41, respectively.

(ii) Training 2

Fig. 3 shows the rating values for irritation by formic acid at 15 ppm, 30 ppm, and 60 ppm (two-fold series). The rating values for formic acid at 30 ppm are 3–7 in the case of the panel A and 2–6 for panel B, which again shows a slight dispersion. The standard deviations are 1.35 and 1.72, respectively.

(iii) Training 3

Fig. 4 shows the rating values for irritation by formic acid at 15 ppm, 30 ppm, and 60 ppm (two-fold series). The rating values for formic acid at 30 ppm are 4–6 for panel A and 4–5 for panel B. The standard deviations are 0.75 and 0.49, respectively.

2. Relationship between sensory adaptation and time intervals in the rating of the odor irritation

Table 3 shows the changes in the rating values for odor irritation when the

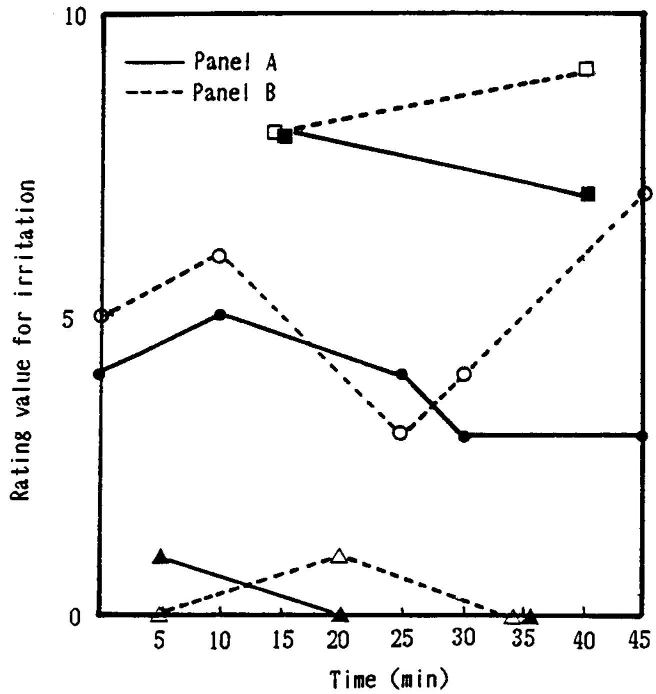


Fig. 2 Variation in the rating values of irritation for each concentration. Triangles, circles and squares represent the values for 10 ppm, 30 ppm and 90 ppm respectively.

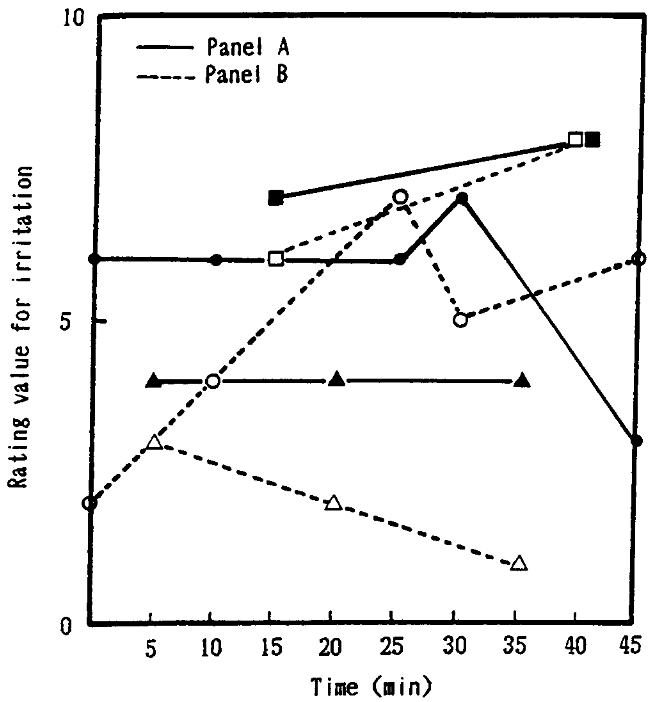


Fig. 3 Changes in the rating values of irritation for each concentration. Triangles, circles and squares represent the values for 15 ppm, 30 ppm and 60 ppm respectively.

panel kept on smelling 30 ppm formic acid at fixed time intervals. The rating was carried out ten times. It was found that the rating values decreased slightly for panel A in the latter half of rating, but there was no decrease in the rating values judged by panel B.

3. Results of the measurement of the C_E

Table 4 shows the average values measured for the C_E of subject materials, which had the same level of irritation as that of the standard odor (formic acid, 30 ppm). In calculating the average values, when the rating values for the irritation of subject odor were higher than the standard odor, the data were dealt with as follows. Assuming that odor irritation is proportional to the logarithm of the concentration of odorant, the average of the logarithmic values of the concentration of subject odor (C_2), which seemed to be higher than the standard smell, and the concentration one order lower (C_1), were taken as representative values. The equation for the calculation is as follows:

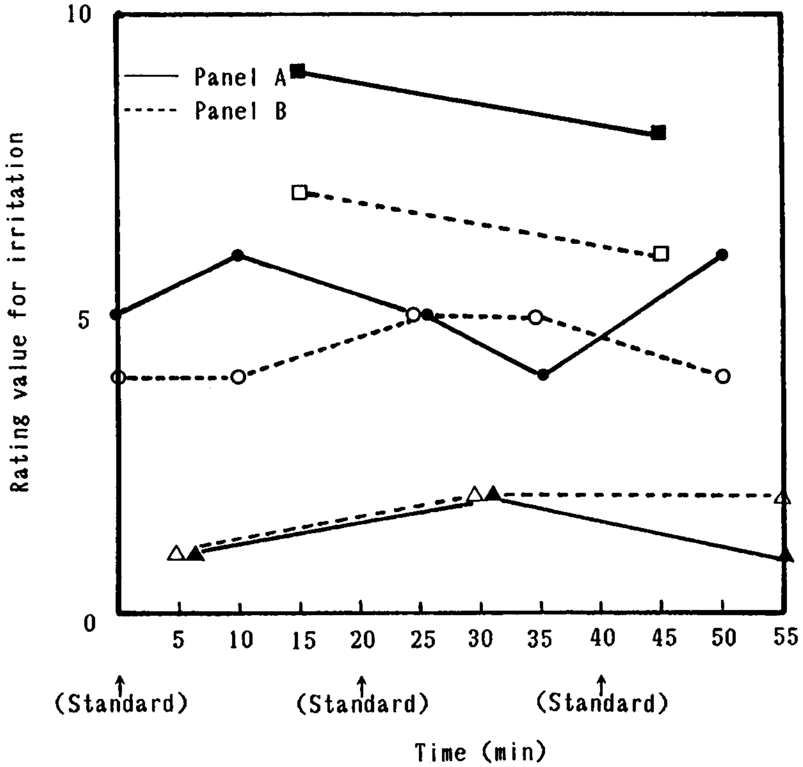


Fig. 4 Variation of rating value for irritation in each concentration in case of smelling the standard odor at every twenty minutes. Triangles, circles and squares represent the values for 15 ppm, 30 ppm and 60 ppm respectively.

Table 3. Time course of rating value for irritation. Rating values in the table were estimated on the basis of the sensory scale in Fig. 1.

Interval	Panel	1	2	3	4	5	6	7	8	9	10
40 sec	A	5	4	4	4	5	4	4	4	3	3
	B	3	4	4	3	3	3	4	4	4	4
30 sec	A	5	4	4	4	4	3	4	2	4	3
	B	4	4	5	4	4	3	4	4	3	4
20 sec	A	4	4	3	3	3	4	3	3	3	3
	B	3	4	4	3	3	3	4	4	4	4
15 sec	A	4	4	3	3	4	3	4	3	3	3
	B	3	4	4	4	4	4	4	4	3	3

Table 4. Concentrations for subjectively equivalent irritation (C_E) of 16 compounds.

Name of Compound	C_E (ppm)	Homologous series
Formic acid (Standard)	30	
Acetic acid	43	Carbonic
Propionic acid	38	acid
Isobutyric acid	41	
Ammonia	150	
Methylamine	93	Amine
Dimethylamine	110	
Trimethylamine*	290	
Methyl formate*	3.9×10^4	Ester
Ethyl formate*	1.9×10^4	
Methyl alcohol	2.0×10^4	Alcohol
Acetaldehyde*	$>1.2 \times 10^4$**	Aldehyde
Acetone*	2.3×10^4	Ketone
Acrolein	6.5	Unsaturated
Acrylic acid	45	compound
Hydrogen chloride	8.0	Acid

*Measured for one subject.
**Measurement of the concentration was not feasible more than 1.2×10^4 ppm.

$$M = (\log C_1 + \log C_2)/2 \tag{4}$$

$$(\text{representative value}) = 10^M \tag{5}$$

In Table 4, although the C_E of materials having the same level of irritation as that of the standard odor (formic acid, 30 ppm) shows the same level of odor among the same group of fatty acids, it differs greatly from other groups. The C_E of ester, alcohol and acetone are all a few percents and they show the same level of odor, but their concentrations are greater by two orders or more as compared with fatty acids and amine, which are dissociated materials. Among the dissociated materials, the

C_E of acids is lower than that of basic materials, especially hydrogen chloride which has a very low concentration (8.0 ppm).

Acrolein, which is an unsaturated aldehyde, produced the strongest irritation among subject materials. When a part of a chemical bonding of saturated aldehyde becomes a double bond and turns the substance into an unsaturated aldehyde, it will cause extremely strong irritation. However, acrylic acid, which is an unsaturated fatty acid, has the same level of irritation as that of the saturated fatty acid, and shows no clear tendency for irritation as the unsaturated aldehyde does.

Besides, it became clear that the olfactory irritation caused by acrolein occurred slightly late as compared to other materials. In other words, the sensory irritation which assails humans' nostrils occurred soon after inhaling each one of the materials except for acrolein. In the case of acrolein, the sensory irritation occurred after a little passage of time from inhalation and then the sensation was kept for a while. The panel measured the time gap between inhalation and occurrence of irritation by stopwatch, and the result was 1.4 sec on the average. Therefore, it is thought that acrolein is different from other materials in the kind of sensory irritation and the mechanism of its occurrence.

A comparison can be made between the measurements of the C_E of subject materials, and in the case of health problems the resultant values shown as the relationship with Dose-Response[19][20][21]. It is known that hydrogen chloride at 0.5 ppm-1 ppm gives off a slightly irritating odor, and at 10 ppm, the irritation to nostrils is strong. Therefore, the concentration value (8.0 ppm) obtained in this experiment is accurate. Also it is known that ammonia at 100–500 ppm causes irritation of the throat, nose, and eyes, and this is consistent with the result of this experiment (150 ppm). It is known that the exposure to acrolein at 1 ppm causes tears and irritation to eyes, nose and throat within five minutes, and at 5.68 ppm it is accompanied by strong symptoms of irritation, and the maximum time to tolerate the irritation is one minute. In this experiment, although the concentration value (6.5 ppm) is slightly larger, it is almost consistent. Regarding other kinds of materials, we could not make comparative studies on them because there were no reported cases illustrating their effects on the olfactory sense.

Considering the reported studies[20][21], there was an anxiety over the concentrations of subject materials of this experiment in that inappropriate levels or long exposures might cause health problems. However, there was no problem about inhalation twice. No experiment was carried out on methyl formate, acetaldehyde, and acetone because their concentrations were high and their olfactory smell was too strong. Measurement of acetaldehyde with concentrations of greater than 1.2×10^4 ppm was considered to be dangerous.

Discussion

1. Effect of the training of the panel

When the rating values for irritation by formic acid (30 ppm) in Training 1 and Training 2 are compared with each other, both panels A and B show a large dispersion of rating values in Training 2 in which there was a small gap in the odor concentrations. It indicates that the capacity for recognition of odor and the conception of the sensory scale becomes unstable when the gap between each odor concentration is small.

Since the panel did not know how the three odor concentrations were adjusted, it was difficult for them to rate the gap exactly when it was so small. However, they knew that the level of odor concentration generally corresponded to the level of irritation.

On the other hand, when the results of Training 2 and Training 3, in which the three odor concentrations were identical for both, are compared with each other, dispersion in the rating of irritation of formic acid (30 ppm) becomes small in the case where the standard odor was provided to the panel every fourth step. Since the panel were able to rate the irritation with reference to the standard odor provided to them during the rating of irritation, their rating values and thus the results of the experiment were consistent (stable).

Compared with the results of Training 1–3, Training 2, in which the three odor concentrations were of two-fold dilution series, yielded a large dispersion in the rating values, whereas Training 3 yielded consistent (stable) rating values due to the comparison of the irritation of the odor sample with that of the standard odor and the corrections made with reference to the sensory scale. The standard smell (formic acid, 30 ppm) and two-fold dilution series of samples were used as a pair in a comparison method to measure the C_E. That is to say, the panel smelled the standard odor in each comparison step, and rated both of their irritations to see which of them was stronger.

2. Time interval for rating irritation

Changes in the rating values with the passage of time were measured using formic acid (30 ppm) and setting the time intervals for the rating of irritation as 40 sec, 30 sec, 20 sec, and 15 sec. Since a clear phenomenon of adaptation was not found, the C_E was measured at 20 sec time intervals. When the panel were exposed to a constant strength of odor, their olfactory adaptation occurred within several minutes, but when the exposure was stopped, their olfactory sense recovered within the same time as it took for the adaptation[9]. Therefore in this experiment, where the rating of irritation was carried out by inhaling the subject odor twice at 15–40 sec intervals, it was assumed that the olfactory sense was sufficiently recovered between each rating.

In the case of panel A, the irritation decreased slightly in the latter half of rating at 30 sec-40 sec intervals. The reason for this was probably not the accumulation of olfactory adaptation but rather habituation[4] against irritation. This is a human's sensory property in which one has a strong impression and feeling against an odd irritation but as one gets used to it, the odor is hardly remembered or it is weakly perceived[4]. This is a psychological phenomenon in a human being as one's sensory scale changes.

A further study was carried out to see whether the memory of irritation by standard smells becomes weak and whether this affects the rating of irritation when a comparison is made between the irritation of a standard odor and that of a subject odor at 20 sec intervals. This experiment is described below. In Training 3, when the panel responded to the irritation every five minutes, the rating values were found to be stable. Therefore, it is believed that there was no such memory problem during the comparison of odor irritation at 20 sec intervals.

Provided that the perceived irritation in Training 3 was in good agreement with the Sensory Scale of odor irritation (with eleven grades from 0 through 10), the irritation by the standard odor and by subject odors in the case of C_E was also measured by the same scale. This was intended to improve the capacity for maintenance of sensory memory, as well as to improve the stability (consistenty) of rating according to the quantitative recognition of irritation, by employing the same scale.

3. Relationship between each material's C_E and physicochemical parameter

When the obtained C_E values in Table 4 are compared with each physicochemical parameter in Table 5, no clear relationship between the C_E and the reactivity indicator is found, and the value of C_E is at least two orders lower in dissociated materials than in undissociated materials. In other words, there was no tendency which could reasonably explain the reactivity indicator at the ground state. However, in acrolein, which is an unsaturated compound, the orbital energy of the LUMO is extremely low and the electrophilic reactivity is greater than that of other materials, and this is supposed to be the reason for the lower value of the C_E in acrolein.

Since the olfactory irritation caused by acrolein occurs a little late after the inhalation of odor gas and has somewhat constant properties, it is supposed that the

Table 5. Indicator of reactivity and dissication constant of each compound. HOMO and LUMO are the orbital energy of the highest occupied molecular orbital and that of the lowest unoccupied one respectively. $S_r^{(E)}$ and $S_r^{(N)}$ are indicators of nucleophilic and electrophilic reactivity. K_a and K_b are an acidic and a basic dissociation constant respectively. $pK_a = -\log(K_a)$.

Name of Compound	HOMO (eV)	LUMO (eV)	$S_r^{(E)}$	$S_r^{(N)}$	pK_a or pK_b
Formic acid	−11.69	0.93	−0.16	0.909	3.76 (K_a)
Acetic acid	−11.53	0.82	−0.166	0.954	4.76 (K_a)
Propionic acid	−11.52	0.88	−0.164	0.906	4.87 (K_a)
Isobutyric acid	−11.43	0.94	−0.164	0.877	4.85 (K_a)
Ammonia	−11.11	4.26	−0.130	0.219	4.74 (K_b)
Methylamine	−10.56	3.71	−0.132	0.394	3.35 (K_b)
Dimethylamine	−10.02	3.31	−0.138	0.400	3.27 (K_b)
Trimethylamine	−9.57	2.93	−0.145	0.407	4.22 (K_b)
Methyl formate	−11.32	1.03	−0.163	0.840	
Ethyl formate	−11.56	1.05	−0.163	0.833	
Methyl alcohol	−11.41	3.78	−0.157	0.397	
Acetaldehyde	−10.89	0.67	−0.160	1.146	
Acetone	−10.76	0.59	−0.162	1.202	
Acrolein	−10.58	−0.12	−0.160	—	
Acrylic acid	−10.70	0.42	−0.164	1.507	4.26 (K_a)
Hydrogen chloride	−12.99	0.87	−0.189	0.687	−7 (K_a)*

*estimated value

olfactory irritation and the mechanism for its occurrence in the case of acrolein is different from that of other materials. That is to say, the olfactory irritation of acrolein as evidenced from its properties is a sensation which occurs with electrophilic reactivity, and it is thus necessary to distinguish between this and the olfactory irritation of other materials.

The relationship between the dissociation constant of acids (K_a) and that of bases (K_b) was studied as follows. Among the acid compounds studied, the C_E of hydrogen chloride (with a $pK_a = -\log_{10} K_a$) is extremely small (i.e. the K_a is large), and also formic acid, which is a fatty acid and has a small pK_a, has a lower C_E

value. Although it is not clear why several ppm statistical variations exist between the C_E values of fatty acids, it can be thought that C_E has almost the same trend as mentioned above if the pK_a is kept at the same level.

Also among basic materials, the C_E values of methylamine and dimethylamine (whose pK_b is small) are lower. However, in the case of ammonia and trimethylamine, the C_E of trimethylamine (whose pK_b is small) is twice that of ammonia. This is contrary to the relationship in which the C_E is proportional to pK_b,the reason for which may be the suppression effect of olfactory stimulation on the trigeminal nerves, or a masking effect. Cain[22] measured the sensation of high concentrations of carbon dioxide, which is an irritating material, for trigeminal nerves and for a compound of amyl butyrate which causes no irritation. He found that the sensation of amyl butyrate suppressed the irritating sensation caused by carbon dioxide. Therefore it is believed that in trimethylamine, whose threshold value (0.0004 ppm[8]) is extremely low compared with those of other materials, the olfactory sensation becomes very strong around the C_E value, and in order to feel the irritation at the same level of the standard odor, higher concentrations of trimethylamine are required because the trigeminal nervous sensation is suppressed by the olfactory sensation. Such suppressive effect on the nerves is also evidenced from physiological experiments which show the existence of the negative-feedback circuit by the function of the suppressed neurons[23][24][25].

Cain's experiment on carbon dioxide was made using very high concentrations of the gas (10–40%) which caused irritation. Carbon dioxide is a dissociated material and its value of pK_b (6.35) is extremely large as compared with that of the acids used in this experiment, which are not easily dissociated. In other words, in the case of carbon dioxide, its potency to irritate, which is caused by its concentration, is also determined by the degree of dissociation constant. It is thus inferred that the values of the C_E in dissociated materials are proportional to the values of pK_a and pK_b; i.e. the C_E values of the materials with large dissociation constants tend to be lower. This further means that the number of ions in mucus is proportional to the occurrence of irritation when dissociated materials are dissolved into the mucus membrane of the trigeminal nerve.

However, since in this experiment the acidic and basic materials were studied separately, it is not possible to explain the difference of the C_E between these two kinds of materials at the same level. Moreover, although the C_E of non-dissociated materials was found to be extremely high, furher studies are needed to resolve whether the same mechanism is responsible for the irritation caused by dissociated materials and non-dissociated materials. If the mechanism is the same and it depends on reactivity, it is then necessary to study the reactivity indicator of dissociated materials not only in the ground state but also in the dissociated state.

It is known that there is a voltage receptor site to respond to electric stimulation in the nerve membranes[26][27], and that the change in the membrane potential of dissociated materials in mucus by ionization may stimulate the trigeminal nerve. Therefore, some other methods may be involved. One way is to presuppose the change of membrane potential during the dissolution of dissociated materials according to the relationship between the outer fluid of the nerve membrane and the composition of ions, and another way is to carry out physiological experiments.

We are planning to extend this study to other kinds of materials. However, the development of model experiments using an artificial membrane is a problem yet to be resolved because subject materials are limited from the standpoint of the safety of the panel.

Conclusions

This research attempted to study the mechanisms for the occurrence of irritation and the sensory properties of the trigeminal nervous stimulation,, which is a non-olfactory stimulation. This is important for the measurement and estimation of odor pollution. Sixteen kinds of odorous concentrations having the same sensory irritation (C_E) were studied by the odor sensory test. Moreover, some studies on the differences in the C_E between odorants, and the physicochemical properties of their molecules, were carried out. The results obtained are summarized as follows:

1) On the basis of the measurement of the C_E for different materials, it was found that the C_E values of dissociated materials are extremely low as compared with those of non-dissociated materials. Among the dissociated materials, the C_E of acids is lower than that of basic materials. Furthermore, among the materials whose C_E was compared with each other, the C_E of acrolein, which is an unsaturated aldehyde, was the lowest.

2) There was a delay in the sensory irritation of acrolein and it is thus thought that acrolein has a different mechanism for the generation of sensory irritation. It is also suggested that the electrophilic reactivity of acrolein molecules is related to its different mechanism.

3) It was found that the C_E of dissociated materials was related to the dissociation constant, and acid and base-dissociation constants were inversely related to the value of C_E, i.e., the materials which are readily dissociated also tend to have lower values of C_E.

Since chemicals with an "irritating odor," or "mucous membrane irritation" as described in physicochemical dictionaries, are mostly found in high-vapor pressure, they are categorized as irritating materials which are exposed mostly at high concentrations[28]. This research study showed that calculation based on such descriptions is not reasonable because the documented description of irritation is bsed on very subjective standards. From this standpoint, it is necessary to categorize irritating materials according to their sensory properties and the physiological mechanism for the generation of sensory irritation.

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