TOXICOLOGY AND APPLIED PHARMACOLOGY 24, 279–297 (1973)

Sensory Irritation of the Upper Airways by Airborne Chemicals

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Received May 1, 1972

Sensory Irritation of the Upper Airways by Airborne Chemicals. ALARIE, Y. (1973). *Toxicol. Appl. Pharmacol.* **24**, 279–297. Twenty-seven chemicals all containing the >C=C< group have been tested for their potential sensory irritation properties to the upper respiratory tract when administered in an aerosol form to mice. An analytical model for the data is suggested for comparison of the activity of these compounds, and the essential molecular features for sensory irritant activity in these compounds are delineated. Correlation between the biological and chemical reactivity data suggests that these compounds initiate the sensory irritation reaction by associating with SH groups of a receptor molecule on the free nerve endings of the afferent trigeminal which are located at the surface of the nasal mucosa.

Sensory irritation of the mucous membranes by chemicals has been termed the common chemical sense (Parker, 1912) to differentiate this phenomenon from the special chemical senses of taste and smell. Sensory irritation from exposure of the upper respiratory tract and the eyes to airborne chemicals can provoke sternutation, lachrymation and several respiratory and cardiovascular reactions in man and animals (Kratschmer, 1870; Alarie, 1966). No specialized organ for sensory irritation has been demonstrated and the free nerve endings from the afferent branches of the trigeminal located diffusely at the surface of the nasal passages and the cornea are probably acting as the chemoreceptors (Beidler, 1965; Cauna *et al.* 1969; Ulrich *et al.*, 1972). The types of chemicals capable of stimulating these free nerve endings vary widely (Moncrief, 1944). In general, it is accepted that aldehydes are capable of stimulating these chemoreceptors and that the level of irritation increases with α,β unsaturation such as in acrolein (Moncrief, 1944). Also, >C=C< groups, and C=O, particularly with the presence of an halogen such as in chloracetophenone, confer irritating properties to an organic molecule (Dixon and Needham, 1946).

The mechanisms by which chemicals evoke sensory irritation are still undefined. A theory has been proposed suggesting that chemicals capable of forming an addition complex with SH or NH_2 groups of proteins or enzymes present in membranes lining the nasal passages and the eyes would be capable of inducing an irritation response (Dixon and Needham, 1946; Dixon, 1948; Holmes, 1962). Following these suggestions, it has been shown that several chemicals considered to be sensory irritants are capable of forming complexes with *n*-butylamine or have been shown to inhibit the action of enzymes by selectively blocking SH groups (Dixon and Needham, 1946; Mackworth, 1948; Lough and Currie, 1966; Silver *et al.*, 1967).

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In this article, the results obtained on the ability of a series of closely related chemicals to initiate sensory irritation of the upper respiratory tract in mice are presented. Also, correlation of the sensory irritation activity obtained with the chemical reactivity of these compounds toward 2-diethylaminoethanethiol is presented (Tarantino and Sass, 1969; personal communication). By correlating certain features of the chemicals tested with their potency as sensory irritants, an attempt is made to delineate the essential molecular arrangement needed for stimulation of the chemoreceptors related to the common chemical sense. Also, an analytical model for evaluation of the data obtained on sensory irritation is presented.

METHODS

Evaluation of the Degree of Irritation

The method used to assess the degree of sensory irritation to the upper respiratory tract by airborne chemical irritants has been previously described (Alarie, 1966) and was used without any substantial modification. This method consists of evaluation of the decrease in respiratory rate during the exposure to an aerosol of the material to be tested. A dose-response curve is obtained by plotting the percent decrease in respiratory rate from normal control conditions against the logarithm of the concentration of the airborne chemicals. From such plots, the RD50, or the concentration capable of decreasing the respiratory rate by 50%, is obtained. The activity of several chemicals can be compared. In this report the analysis of the data was modified from the previous publication (Alarie, 1966), and the method of analysis is fully described. All materials were tested for a period of 3 min instead of 1 min (Alarie, 1966) in order to obtain a plateau response which was used for all chemicals, rather than peak response or mean response as previously used (Alarie, 1966). Extension of the exposure from 1 to 3 min was important to obtain a definite plateau response with certain chemicals because of slow onset of the reaction, particularly at low concentrations.

Chemicals Tested

The chemicals tested are presented in Tables 1–4. The majority were obtained from Aldrich Chemical Company, Inc. of New Jersey. All chemicals were solubilized or suspended in polyethylene glycol-200. Aerosols were prepared with a Dautrebande generator as previously described (Alarie, 1966).

Analysis of the Data

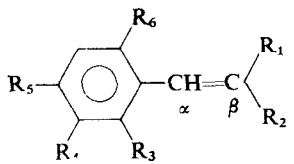
Basis of the analysis. The analytical model for the data obtained is similar to the model proposed by Beidler (1961) for stimulation of taste receptors and has been extended to include onset and recovery along with the plateau response.

Consider the following as essentially irreversible reactions, where (A) represents the association of the irritant molecule with a receptor thus producing a response and (B) represents the breakdown of the irritant-receptor conjugate, either by simple dissociation or by subsequent reaction:

$$\overset{\text{(A)}}{\text{I} + \text{F}} \rightarrow \overset{\text{(B)}}{\text{Z}} \rightarrow \text{F} \qquad (1)$$

TABLE 1

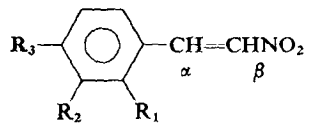
SERIES A: ACTIVITY OF CHEMICALS RELATED TO STYRENE WITH SUBSTITUTION ON β CARBON AND ON BENZENE RING



Chemical number and name	General structure						Activity				
	R_1	R_2	R_3	R_4	R_5	R_6	RD50 (μg/l)	95% confidence limits	R_s (%)	K (l/μmol)	R_s50 (μmol/l)
I Styrene	H	H	H	H	H	H	666	574–758	83	0.238	4.20
II 4-Chloro-β,β-dimethylstyrene	CH_3	CH_3	Cl	H	H	H	253	216–290	92	0.77	1.29
III 3-Chlorostyrene	H	H	H	Cl	H	H	213	165–261	73	1.40	0.714
IV 2,6-Dichlorostyrene	H	H	Cl	H	H	Cl	106	85–128	80	2.69	0.373
V Cinnamonitrile	H	CN	H	H	H	H	—	—	40	2.80	0.357
VI Diethylbenzalmalonate	$CO_2C_2H_5$	$CO_2C_2H_5$	H	H	H	H	16.5	14–19	78	26.9	0.037
VII Benzalmalononitrile	CN	CN	H	H	H	H	11.09	10.5–11.6	105	12.7	0.079
VIII Nitropropenylbenzene	CH_3	NO_2	H	H	H	H	8.99	7.5–10.5	93	21.3	0.0047
IX β-Nitrostyrene	H	NO_2	H	H	H	H	1.83	1.5–2.2	87	109.6	0.0009

TABLE 2

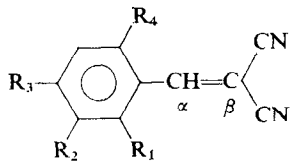
SERIES B: ACTIVITY OF CHEMICALS RELATED TO β-NITROSTYRENE WITH SUBSTITUTION ON BENZENE RING



Chemical number and name	General structure			Activity				
	R_1	R_2	R_3	RD50 (μg/l)	95% confidence limits	R_s (%)	K (l/μmol)	R_s50 (μmol/l)
IX β-Nitrostyrene	H	H	H	1.83	1.5–2.2	87	109.6	0.0009
X 2-Chloro-3,4-dimethoxy-β-nitrostyrene	Cl	OCH_3	OCH_3	4.32	2–6	87	78	0.0052
XI 2,4-Dimethoxy-β-nitrostyrene	OCH_3	H	OCH_3	7.88	6.21–9.56	80	45	0.0222
XII 3,4-Dimethoxy-β-nitrostyrene	H	OCH_3	OCH_3	53.5	31–76	53	75	0.0134
XIII 3-Methoxy-4-hydroxy-β-nitrostyrene	H	OCH_3	OH	—	—	59	—	—

TABLE 3

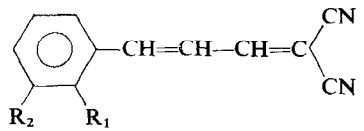
SERIES C: ACTIVITY OF CHEMICALS RELATED TO BENZALMALONONITRILE WITH SUBSTITUTION ON BENZENE RING



Chemical number and name	General structure				Activity				
	R_1	R_2	R_3	R_4	RD50 (μg/l)	95% confidence limits	R_s (%)	K (l/μmol)	R_s50 (μmol/l)
VII Benzalmalononitrile	H	H	H	H	11.1	10.5–11.6	105	13	0.079
XIV 2-Chloro-6-hydroxybenzalmalononitrile	Cl	H	H	OH	25.5	20.8–30.1	81	13	0.0759
XV 4-Chlorobenzalmalononitrile	H	H	Cl	H	12.7	11–14.6	76	28	0.0358
XVI 2-Chlorobenzalmalononitrile	Cl	H	H	H	3.27	2.98–3.56	87	67	0.0149
XVII 2-Fluorobenzalmalononitrile	F	H	H	H	3.20	2.90–3.50	88	70	0.0140
XVIII 2-Trifluoromethylbenzalmalononitrile	CF_3	H	H	H	2.86	2.22–3.51	87	101	0.0099
XIX 2-Chloro-6-fluorobenzalmalononitrile	Cl	H	H	F	2.16	1.44–2.87	83	146	0.0069
XX 2,6-Dichlorobenzalmalononitrile	Cl	H	H	Cl	2.10	1.63–2.57	88	142	0.0071

TABLE 4

SERIES D: ACTIVITY OF CHEMICALS RELATED TO CINNAMALMALONONITRILE WITH SUBSTITUTION ON BENZENE RING



Chemical number and name	General structure		Activity				
	R_1	R_2	RD50 (μg/l)	95% confidence limits	R_s (%)	K (l/μmol)	R_s50 (μmol/l)
XXI Cinnamalmalonic acid[a]	H	H	—	—	38	42.4	0.236
XXII Cinnamalmalononitrile	H	H	8.53	7.4–9.7	89	27.2	0.368
XXIII 2-Iodocinnamalmalononitrile	I	H	5.23	3.2–7.2	84	85.3	0.117
XXIV 3-Nitrocinnamalmalononitrile	H	NO_2	5.12	1.1–9.2	61	208	0.0048
XXV 2-Nitrocinnamalmalononitrile	NO_2	H	4.24	0–11.9	61	251	0.0040
XXVI 2-Chlorocinnamalmalononitrile	Cl	H	2.96	2.4–3.5	90	92.1	0.0109
XXVII 2-Fluorocinnamalmalononitrile	F	H	2.84	2.2–3.5	89	90.2	0.0111

[a] CN groups replaced by COOH.

where I = single irritant molecule; F = free receptor site; Z = irritant-receptor conjugate. These will be referred to in terms of total available quantities for reaction: I as C = concentration of irritant by aerosol, μg/liter of air or μmol/liter of air; F as the total number of free receptor sites, Z as the total number of conjugated receptor sites: $N = \mathrm{F} + \mathrm{Z}$ as the total number of receptor sites.

It is then assumed that all sites are equivalent in relation to the irritant molecules and to initiation of sensory irritant response.

The following definitions are made: k_1 = rate constant for association; k_2 = rate constant for conjugate breakdown; $p_z = \mathrm{Z}/N$ = fraction of conjugated receptors; $p_f = \mathrm{F}/N$ = fraction of free receptors = $1 - p_z$.

We assume that the response R is proportional to p_z such that when $p_z = 1$, the saturation of available receptors sites, R is equal to R_s, the maximum elicitable response. Thus,

$$R = (R_s)(p_z) \tag{2}$$

Response plateau during exposure. With this model, the plateau response is reached when a steady-state concentration of Z is obtained. The condition of equal formation and breakdown rates is

$$k_1(C)(\mathrm{F}) = k_2(\mathrm{Z}) \tag{3}$$

From the above assumptions it follows that

$$p_z = k_1(C)/(k_2 + k_1 C) \tag{4}$$

and

$$R = R_s k_1 C/(k_2 + k_1 C) \tag{5}$$

This concentration–response relation is particularly useful when rearranged to give the following form:

$$C/R = C/R_s + 1/KR_s \tag{6}$$

where $K = k_1/k_2$. This form of dose-response relation is typical of drug occupation theory models. Equation (6) is given in the same form as used by Beidler (1961).

Response as a function of time. The characteristic actions of irritating chemicals differ not only in the dose-response curves predicted from plateau response analysis, but also in the time required for the onset of reaction and the time required for recovery at the end of exposure.

Referring to Eq. (1), a differential equation for the overall reaction rate can be written as

$$-dZ/dt = -k_1 \mathrm{CF} + k_2 Z \tag{7}$$

$$-dp_z/dt = -k_1 C p_f + k_2 p_z \tag{8}$$

$$dp_z/dt = k_1 C(1 - p_z) - k_2 p_z \tag{9}$$

from which

$$d\ln(k_1 C - [k_1 C + k_2]p_z = -(k_1 C + k_2)\,dt \tag{10}$$

is obtained.

Subsequent integration and evaluation of the integration constant leads to the following relation which is presented in terms of response, R, using Eq. (2),

$$\ln(1 - [1 + 1/KC]\, R/R_s = -(k_1 C + k_2)\, t \tag{11}$$

$$R = (KC/[KC + 1])\, R_s(1 - \exp[-(k_1 C + k_2)\, t]) \tag{12}$$

Upon termination of exposure, C becomes zero and the only available reaction is breakdown of receptor conjugates as below

$$Z \rightarrow F \tag{13}$$

Applying first-order kinetics to the breakdown of those conjugates left at the end of exposure,

$$-dZ/dt = k_2 Z \tag{14}$$

Substituting and integrating,

$$\ln p_z = \ln p_z^0 - k_2 t \tag{15}$$

where p_z^0 is the fraction of conjugated sites at the end of exposure, now referred to as $t = 0$. It follows again with Eq. (2) that

$$\ln R = \ln R^0 - k_2 t \tag{16}$$

Equation (16) may be used as the basis for evaluation of the rate constant k_2 from appropriate recovery plots. Solution of Eq. (16) for R gives

$$R = R^0 e^{-k_2 t} \tag{17}$$

Given values for K and k_2, k_1 may be evaluated from the definition of K.

$$k_1 = (k_2)(K) \tag{18}$$

Example of the Results Obtained with the Model Described

Dose-response analysis. The data obtained for all the chemicals tested have been analyzed by the method described above. Calculations and statistical treatment are presented below for one of the chemicals tested, *o*-chlorobenzalmalononitrile, since a large amount of data was available for this chemical during 1-min exposure and comparison can be made with a previous report (Alarie, 1966). Table 5 presents the data for the concentrations tested, the response obtained and the C/R calculations. Figure 1 presents the plot of data as it appears on cartesian coordinates, a hyperbola which approaches a maximum response level asymptotically at high concentration. The data are also shown in Fig. 2 as the linear C/R against C plot. Assuming linearity of the C/R vs C plot as presented in Fig. 2, the data were used to find the best straight line, the slope and the intercept by the method of least squares (Draper and Smith, 1966), assuming variation in the ordinate only. The values of R_s and K may then be evaluated from the values of slope and intercept which describe the best fit line of the C/R vs C plot. From Eq. (6)

$$C/R = C/R_s + 1/KR_s$$

$$R_s = 1/\text{slope}$$

$$K = 1/R_s\,(\text{intercept})$$

$$= \text{slope/intercept}$$

TABLE 5

DATA OBTAINED DURING 1-min EXPOSURE TO *o*-CHLOROBENZALMALONONITRILE (MW 188.6)

Exposure concentration (C) (μg/l)	Response (R) (% decrease in respiratory rate from control, mean of 4 animals)	C/R (μg/l/% decrease)	C/R (μmol/l/% decrease)
4.6	32	0.14	0.00076
9.2	51	0.18	0.00096
15.0	61	0.25	0.00131
20.0	62	0.32	0.00172
25.0	69	0.36	0.00193
30.0	67	0.45	0.00238
35.0	77	0.45	0.00242
40.0	71	0.56	0.00300
53.0	81	0.65	0.00348
95.0	81	1.17	0.00624
132.0	79	1.67	0.00889

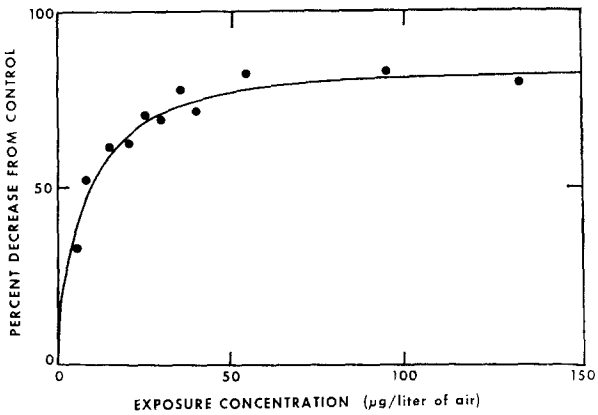


FIG. 1. Dose-response curve obtained with *o*-chlorobenzalmalononitrile.

The results from the data presented in Table 5, Fig. 2 and Fig. 3 are as follows:

$$\text{Slope} = 0.01188 \pm 0.48\% \text{ (SE)}$$

$$\text{Intercept} = 0.161 \times 10^{-9} \pm 4.4\% \text{ (SE)}$$

$$R_s = 87\% \text{ decrease}$$

$$K = 32 \pm 1.6 \text{ (SE) liter/}\mu\text{mol}$$

If the value for R_s is >50%, then the RD50 (concentration at which a 50% decrease in respiratory rate occurs) can be estimated as follows:

$$\text{RD50}/50 = \text{RD50}/R_s + 1/KR_s$$

$$\text{RD50} = (1/KR_s)/(1/50 - 1/R_s)$$

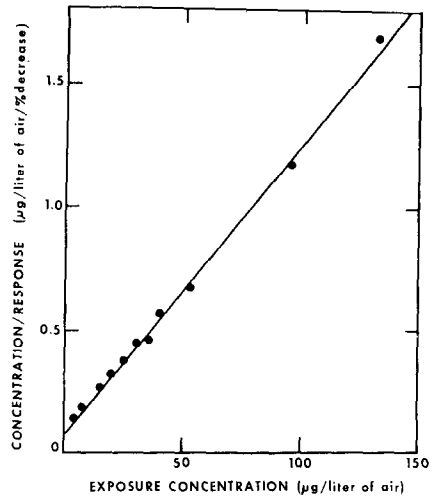


FIG. 2. Dose-response curve obtained with *o*-chlorobenzalmalononitrile using C/R vs. C transformation.

The 95% confidence limits for the RD50 are estimated from the error associated with the values determined for R_s and K. As the determination of intercept already includes that of the slope determination, the percent error in intercept determination (hence in the determination of K) is used to estimate the error in the RD50. The confidence interval (95%) is thus given

$$1 \pm \left(2\frac{\text{SE (intercept)}}{\text{intercept}}\right)\left(\frac{1}{KR_s}\right)\Big/\left(0.02 - \frac{1}{R_s}\right)$$

The RD50 found was 8.6 with 95% confidence limits of 7.8 and 9.4.

These derivations and calculations in the evaluation of the RD50 and other parameters have been made on consideration of the response having reached a plateau. To best accommodate the assumptions leading to the derivations of Eq. (6), an effort should be made to continue all exposures until the time of appearance of a pleateau decrease in respiratory rate. If this is not obtained, as with the 1-min exposure to

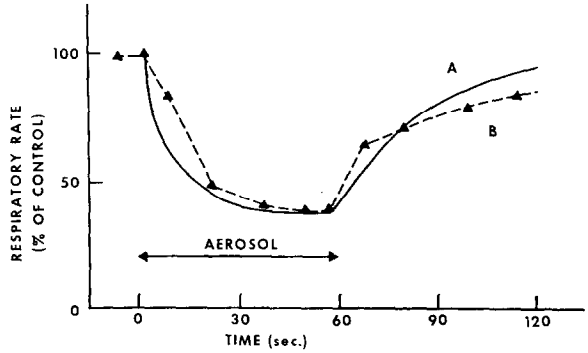


FIG. 3. Time-response curve obtained with *o*-chlorobenzalmalononitrile using Eqs. (12) and (17′) for curve A and experimental results in curve B.

o-chlorobenzalmalononitrile at low concentrations, discrepancies will occur between two series of experiments as can be observed when comparing results obtained with 1-min exposure and 3-min exposure. However, in every case inspected so far the plot of C/R vs C shows substantially lower dispersion of the points about the straight line than does the corresponding regression plot (Alarie, 1966) using the same data, whether or not the plateau response was reached.

Time-response analysis. Analysis of the recovery following exposure based on Eq. (16) may lead to the evaluation of the constant k_2. The constant k_1 may be obtained from k_2 and the value of K obtained in dose-response analysis. Equation (16) may be rearranged to facilitate evaluation of k_1 from available data for response as a function of time after exposure.

$$R = R^0 e^{-k_2 t} \tag{17}$$

$$-\ln(R/R^0) = k_2 t \tag{16'}$$

$$k_2 = 2.303 \log(R/R^0)/t \tag{19}$$

where R^0 is the response at the end of exposure.

TABLE 6

VALUES FOR k_2 (sec^{-1}) CALCULATED FROM MEASUREMENTS OF % DECREASE IN RESPIRATORY RATE DURING 1-min POST-EXPOSURE AFTER A 1-min EXPOSURE TO *o*-CHLOROBENZALMALONONITRILE

Exposure concentration (μg/l)	Post-exposure (sec)			
	7.5	22.5	37.5	52.5
4.6	0.0614[a]	0.0375	0.0265	0.0267
9.2	0.0725	0.0345	0.0396	0.0236
15.0	0.0492	0.0467	0.0614	0.0404
20.0	0.0329	0.0234	0.0251	0.0361
25.0	0.0232	0.0299	0.0312	0.0217
30.0	0.0384	0.0227	0.0339	0.0288
35.0	0.0594	0.0290	0.0321	0.0306

[a] The values are the calculated values of k_2 from Eq. (19) for each of four points with time post-exposure, for each concentration, and are the mean for 4 animals exposed at each concentration.

Table 6 presents values of k_2 calculated from measurements of respiratory rate in the first minute after exposure of mice to seven concentrations of *o*-chlorobenzalmalononitrile for 1 min. The mean value of k_2 was found to be 0.0364 $sec^{-1} \pm 0.0025$ (SE).

From $K = k_1/k_2$ we find

$$k_1 = 0.0364 \times 3.21 \times 10^7$$

$$= 1.17 \times 10^6 \text{ liter air/mol/sec} \pm 0.13 \times 10^6 \text{ (SE)}$$

The analysis of experimental results for 1-min exposure to *o*-chlorobenzalmalononitrile using the proposed analytical model has thus yielded the parameters listed in Table 7.

TABLE 7

RESULTS FROM ANALYSIS OF DATA FOR 1-MIN EXPOSURE TO *o*-CHLOROBENZALMALONONITRILE USING THE C/R VS C PLOT

Parameter		Value found	Standard error	Units
Definition	Abbreviation			
Maximum obtainable response or saturation	R_s	84.0	0.5	Percent decrease from control
Concentration required to decrease respiratory rate 50% from control	RD50	8.6	0.4	μg/liter
Concentration required to produce a decrease in respiratory rate equivalent to 50% of the maximum response	R_s50	0.031		μmol/liter
Constant for association	k_1	1.17	0.13	liter/μmol/sec
Constant for dissociation	k_2	0.0364	0.0025	sec^{-1}
Ratio of k_1/k_2	K	32.0	1.6	liter/μmol

To use the analytical model for the exposure and post-exposure recovery, Eq. (12) was applied for $t = 0$ to $t = 60$ sec, and Eq. (17) is used for the recovery period for $t > 60$ sec. In order to maintain a uniform time scale with onset of exposure as $t = 0$, Eq. (17) was modified to give

$$R = R^{0-k_2(t-60)} \tag{17'}$$

where R^0 is the respiratory rate at $t = 60$ or end of exposure. The close correlation between experimental results and the results predicted by the model is presented in Fig. 3. Also prediction from the model for R_s agrees closely with the experimental results. The R_s value for *o*-chlorobenzalmalononitrile was predicted to be 84% and found experimentally to be 83% with only a 6% increase in response over a 4-fold increase in concentration from 53 to 200 μg/l of air.

RESULTS

The results obtained for the chemicals tested are listed in Tables 1 to 4. The activity found for each material is presented with the chemical structures. The variation in activity within each group is demonstrated. The effects of various substituting groups for increasing the sensory irritation of these chemicals are elaborated upon in the discussion section.

DISCUSSION

The Trigeminal Reflex

The free nerve endings in the upper respiratory tract at the surface of the nasal epithelium can be regarded as chemoreceptors because they respond to changes in their natural chemical environment. Stimulation by specific chemicals is followed by an increase in activity in the afferent trigeminal nerve (Dawson, 1962; Beidler, 1965; Ulrich *et al.*, 1972). Signals are then received and integrated at the medulla; a decrease in respiratory rate is induced by inhibition of the phrenic nerve activity (Massion *et al.*, 1954) and a general reflex inhibition occurs (Massion *et al.*, 1954). Therefore, the reflex inhibition of respiratory activity is a defense mechanism; one of protecting

the lower airways from being exposed to irritating materials. This protective mechanism is schematically presented in Table 8. Also, many other reflex reactions are induced by the action of airborne chemicals; these are also summarized in Table 8.

Whether or not these free nerve endings can also be stimulated by other stimuli such as pure odorant devoid of irritating action or by tactile stimuli is still unclear (Tucker, 1962). In this report we are concerned only with sensory irritation since chemicals such as *o*-chlorobenzalmalononitrile and a large number of the chemicals tested are reported to be odorless but powerful irritants in man or at least if they have odor properties, the sensory irritation is so overpowering that the odor is not recognized (Owens and Punte, 1963, Punte *et al.* 1963).

Since we have demonstrated that in our series of tested compounds the irritation potential can change by a factor of 600 or more depending on specific chemical groups, it can be concluded that a rather specific receptor molecule is present and must be altered before effective trigeminal stimulation can occur.

Recognition of Sensory Irritation by Airborne Chemicals and Data Analysis

In 1955, Amdur and Mead recognized the usefulness of measurements of various parameters of the respiratory system as indices of either sensory or tissue irritation by low concentrations of airborne chemicals. The first dose-response relationship was obtained for sulfur dioxide, and then several chemicals were tested for their irritation potential to the respiratory tract (Amdur and Mead, 1955; Amdur, 1957; Amdur, 1959; Murphy *et al.*, 1963). With the exception of a series of nitro-olefins tested by Murphy *et al.* (1963), using respiratory resistance, respiratory rate and tidal volume as the criteria of response, there has been no systematic investigation to try to correlate chemical structure with irritating activity and thus gain a better understanding of the mechanism of action of sensory irritation.

In 1966, Alarie proposed measurement of the change in respiratory rate as an index of sensory irritation of the upper respiratory tract. It was found that as the concentration of airborne chemical irritants increases there is a proportional decrease in respiratory rate, and a dose-response relationship can be obtained. This decrease in respiratory rate was also recognized by Amdur and Mead (1955) during exposure to sulfur dioxide. Since measurement of respiratory rate is very simple and several animals can be exposed simultaneously, the proposed technique is particularly well suited to investigate series of chemicals and compare their activity. In this report we have presented the results obtained in a series of closely related chemicals and have been able to grade their sensory irritation potency by taking the amount of decrease in respiratory rate as the index of sensory irritation. Furthermore, a straight line relationship was found by plotting C/R vs C (see Fig. 2). The simple analytical model is useful and adequate to analyze the data and to make comparisons between chemicals. In keeping with Beidler (1961), who originally proposed this model for analysis of data on stimulation of taste receptors, we have kept the same notation for k_1 and k_2. In our work, they have different meanings since the response measured is removed from the action sites and undoubtedly other factors come into account (see Table 8). This analytical model, however, may be applied to data previously presented (Ulrich *et al.*, 1972) where the afferent neural activity of the trigeminal during stimulation by airborne chemical irritants was measured. This situation will be more comparable to the work of Beidler (1961). With the

TABLE 8

NASAL TRIGEMINAL STIMULATION AND REFLEXES[a]

Receptor	→	Afferent nerve	→	Processor	→	Efferent nerves	→	Effect on systems
Combination of specific chemicals with receptor molecule of free nerve endings of afferent trigeminal at surface of nasal epithelium		Increase impulse activity in the afferent trigeminal		Integration at CNS level: medulla		(a) Change in activity of phrenic nerve		(a) Decrease in respiratory rate
						(b) Change in activity of efferent vagus nerve		(b) Decrease in heart rate
						(c) Change in activity of sympathetic splanchnic		(c) Vasoconstriction and rise in blood pressure
								(d) Others: reflex bronchoconstriction, general reflex inhibition, bronchial spasm

[a] References: Massion *et al.* (1954), Dawson (1962), Tucker (1962), Widdicombe (1964), Alarie (1966), Cauna *et al.* (1969), Ulrich *et al.* (1972).

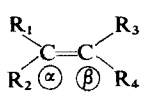
restrictions noted above, it can be concluded that taking the change in respiratory rate during exposure to airborne chemicals and analyzing the data as suggested has yielded useful information for comparison of series of chemicals on their sensory irritant properties.

Characteristics of Sensory Irritants

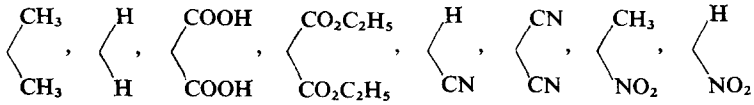
In 1948, Dixon pointed out that three types of organic chemicals can be recognized as sensory irritants. He defined them as lachrymators, sternutators or both. The first type containing halogen atoms, the second type containing $>C{=}C<$ groups, and the third type being some organo arsenicals. In the first type sensory irritation properties are associated with the presence in the molecule of an halogen such as in chloracetophenone. On this basis, Dixon and Needham (1946) report that Ford Moore obtained a good correlation between the chemical structure and the potency of chemicals as lachrymators based on the "positiveness" of the halogen atom in the molecules of chemicals of the first group. Groups which confer this "positiveness" include keto, aldehyde and ester. The compounds of this first group have also been reported to interact with SH groups in proteins and have been shown to be specific inhibitors of SH-containing enzymes by irreversible combination with the SH groups (Dixon and Needham, 1946; Dixon, 1948; Mackworth, 1948). The compounds of the second group, which are those tested and presented in this report, can add nucleophilic reagents such as SH-compounds or NH_2 compounds, particularly when a neighboring group has a polarizing effect on the double bond (Corson and Stoughton, 1928; Silver *et al.*, 1967; Tarantino and Sass, 1969; Tarantino and Sass, personal communication). Such neighboring groups can be keto, aldehyde, ester, nitro or nitrile. In this manner the compound containing the highest polarizing group should be the strongest irritant. The compounds of the third group are usually less irritating and take more time for the onset of the reaction, but their duration of action is more prolonged (Alarie, 1966). Some members of the third group are as potent as compounds of the first and second groups (Alarie, 1966) and are recognized as reacting reversibly with SH groups in proteins (Dixon and Needham, 1946, Dixon, 1948).

Although these three groups of chemicals have been recognized as sensory irritants, and a voluminous amount of data on irritating and pungent chemicals has been reported by Moncrief (1944), no overall concept of the mechanism by which these three classes of materials are irritating has been suggested. All three groups, as noted above, are capable of forming association complexes with SH groups in proteins. It should also be pointed out that there is a close resemblance between the reactions of α,β-unsaturated dinitriles such as in the series we tested and unsaturated aldehydes such as acrolein which is a potent sensory irritant or unsaturated ketones due to the presence of the reactive conjugated system $>C{=}C(CN)_2$ or $>C{=}C{-}C{=}O$ and both can add to SH or NH_2 groups in proteins (Corson and Stoughton, 1928; Schubert and Sanders, 1971).

The essential features of the organic chemicals in the series we investigated are:



where R_1 is H and R_2 is a phenyl group. R_1 must be H on the α carbon and is absolutely essential for the activity since replacement by such groups as phenyl, CH_3 or CN yield completely inactive compounds (Corson and Stoughton, 1928). With styrene, having H in R_3 and R_4, the activity is very low, but it rapidly increases with replacement by CN groups. Compounds such as the benzalmalononitriles with CN in both R_3 and R_4 are very active. Also, when R_3 and R_4 are H and NO_2 such as in β-nitrostyrene the activity is at the highest. This compound is the most potent sensory irritant tested, although the onset of the reaction is slower with β-nitrostyrene than with the benzalmalononitriles at low concentration. Increasing the chain length such as in the cinnamalmalononitriles does not increase the activity over the benzalmalononitriles, although the onset of the reaction and recovery time differ between the two series. In terms of the most effective groups in R_3 and R_4, the following, in order of increasing activity, would be indicated by the results obtained in our experiments:



These are essentially the groups which polarize the adjacent double bond so that nucleophilic reagents can be added with increasing rapidity. In general, substitution in the ortho position of the benzene ring by an halogen will increase the irritant activity of the benzalmalononitriles, cinnamalmalononitriles, and styrenes. The most effective substitution on the benzalmalononitriles has been found to be with Cl in both the 2 and 6 positions of the benzene ring. The activity conferred by a Cl in the 2 position is greatly inhibited when an OH group is added to the 6 position.

Mechanism of Sensory Irritation

Although the information about structure-activity of chemical irritants is still limited, and there is no information on the nature of the receptor molecule with which they interact, it seems appropriate to define some probable mechanism by which these chemicals induce a change in the environment of the trigeminal nerve endings yielding a painful sensation of sensory irritation and initiating reflex reactions. In their work on sensory irritants used as riot control agents, Tarantino and Sass (1969, personal communication) have hypothesized that these compounds induce sensory irritation by reacting with SH-groups in proteins. They used 2-diethylamino ethyl mercaptan (DEAEM) as the simplest model for a protein containing nucleophilic groups (SH, NH_2) as the reactant with benzalmalononitriles and several other related compounds. Taking their results on chemical reactivity toward DEAEM for compounds VII, XV, XVI, XVIII, XIX and XX, and plotting these data against our K values found in mice, taken as an intrinsic activity constant (K_{ia}), there is an excellent correlation as presented in Fig. 4. Even with such a good correlation no definite conclusions can be drawn until more of the compounds listed in Tables 1–4 are tested. That the receptor molecule would be a protein containing SH-groups is also acceptable for irritant chemicals of the first and third groups noted previously, since compounds in these groups have been reported to combine either reversibly or irreversibly with SH-groups in proteins (Dixon and Needham, 1946; Dixon, 1948; Mackworth, 1948), and on this basis Dixon (1948) had postulated that these chemicals would exert their activity by combining

with SH-groups. Henkin and Bradley (1969) have suggested that conformational shift in a thiol containing protein could lead to taste stimulation by complexing with a tastant. Since taste, being a specialized chemical sense, has evolved from the more primitive common chemical sense it is possible that both share a primary characteristic and that SH-containing proteins are involved for both.

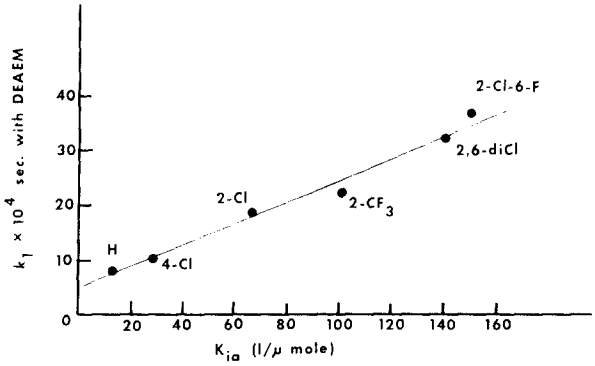


FIG. 4. Chemical reactivity toward DEAEM vs biological activity for benzalmalononitriles. Data for k_1 obtained from Tarantino and Sass (personal communication, 1969).

Some Possible Applications

It is of interest to note that 2-nitro-2 butene, which has the following formula: CH_3—$CH{=}CNO_2CH_3$, and would be part of the second group of sensory irritants, is closely related to the most potent irritant we have tested. This compound has been reported by Lampe *et al.* (1958) to produce eye irritation in humans. Also, Murphy *et al.* (1963) reported a decrease in respiratory rate in guinea pigs exposed to 0.37 to 1.14 ppm similar to the decrease in respiratory rate we have observed in mice with our series of compounds. Deichman *et al.* (1958) and Heimann *et al.* (1958) have pointed out that nitro-olefins may be of great importance in producing sensory irritation in humans since they could be formed as reaction products from nitrogen oxides and olefinic hydrocarbons in exhaust gases of automobiles or from other sources of air pollution. Comparing the data of Murphy *et al.* (1963) with the data of Amdur and Mead (1955) we may estimate that the nitro-olefins are about 40–100 times more potent as irritants than sulfur dioxide. This emphasizes the importance of recognizing the potential sensory irritation of various chemicals in polluted atmosphere, particularly when unsaturated hydrocarbons are present and strong dienophiles are formed.

ACKNOWLEDGMENTS

In conducting the research described in this report, the investigators adhered to the "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

This work was performed under contract No. DAAA13-67-C-0316, Department of the Army, Edgewood Arsenal, W. E. Sultan Contract Project Officer and continued under Research Grant No. 1-R01 OH-00367 from National Institute for Occupational Safety and Health and under Special Fellowship No. 5 FO3 ES 46,198 from National Institute of Environmental Health Sciences.

I acknowledge the technical assistance of Mr. J. Facchina, Mr. C. Tibbets and Mrs. S. Oka. The analytical model was extended by Mr. C. Tibbets, who wrote the computer programs for

calculations and statistical analysis. I am grateful to the personnel of the Chemical Research Laboratory, Edgewood Arsenal for discussion and encouragement and for the data on chemical reactivity supplied for Fig. 4.

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