**STUDIES ON EYE IRRITATION CAUSED
BY CHEMICALS IN RABBITS
—1. A QUANTITATIVE STRUCTURE-ACTIVITY
RELATIONSHIPS APPROACH TO PRIMARY EYE
IRRITATION OF CHEMICALS IN RABBITS—**

Syoichiroh SUGAI, Kyoji MURATA, Tadaharu KITAGAKI
and Isao TOMITA*

Life Science Institute, Kumiai Chemical Co. Ltd.,
3360 Kamo, Kikugawa-cho, Ogasa-gun, Shizuoka 439, Japan

*Pharmacology Department, University of Shizuoka,
395 Yada, Shizuoka 422, Japan

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Abstract.....A quantitative structure-activity relationship (QSAR) approach has been applied to the analysis of the relation between the structural features of chemicals and the primary eye irritation in rabbits. One hundred thirty one heterogeneous chemicals including medicals, pesticides, detergents and organic solvents were used in this study. The eye irritation ratings were made in three classes on the basis of the recovery time of corneal and conjunctival damages. Thirty six descriptors were used to describe the molecules. To correlate eye irritation ratings with the descriptors, a QSAR model was formulated by the adaptive least-squares method. A three-class discrimination was made as follows ; class I included 23 chemicals which induced the damages recovering within 24 hr, class II included 64 chemicals which induced the damages persisting for more than 24 hr but recovering within 21 days, class III included 44 chemicals which induced the damages not recovering within 21 days. The discriminant function included 18 descriptors. The accuracy in classifying the chemicals was 86.3% in the recognition and 74.0% in the leave-one-out prediction. These results suggest that QSAR analysis is valuable to predict the primary eye irritation of chemicals.

Key words : Eye irritation, quantitative structure-activity relationship, adaptive least-squares method, rabbits.

INTRODUCTION

The Draize eye irritation test has been used as an attempt to predict the human ocular irritancy of chemicals. However, this test may be considered to be associated with unnecessary use of animals, irreproducibility and high cost. The inadequacies of this test have led to efforts developing approaches without *in vivo* testing, such as alternative methods (Parish, 1985 ; Reinhardt et al., 1985 ; Kelly, 1989).

In toxicity study of environmental substances, as one of these approaches, an attempt to estimate chemical toxicity by quantitative structure-activity relationships (QSAR) analysis has been performed using the data from *in vivo* or *in vitro* toxicity study, the physicochemical properties and/or structural features of chemicals (Tinker, 1981 ; Gombar, 1987 ; Enslein et al., 1987). Furthermore, QSAR analysis in toxicity study offers the significance in drug design of a chemical with low toxicity and in mode-of-action study.

In the eye irritation study, it is known that the intensity of eye irritation induced by chemicals depends on chemical physicochemical properties (Friedenwald et al., 1946 ; Hughes, 1946 a, b ; Shopsis et al., 1985 ; Answell and Fowler, 1988). This indicates that physico-chemical properties of chemicals must be available for the prediction of eye irritation of environmental chemicals.

In general, physicochemical parameters, such as acid dissociation constant (pka), and octanol/water partition coefficient (logP), are useful to predict the toxic potential of homologous chemicals (Rainsford, 1978 ; Kubota et al., 1979 ; Hansch et al., 1980), but not for heterogeneous chemicals. One approach for predicting the eye irritation potential of heterogeneous chemicals would be to study the commonality of structural features of those for which information about eye irritation is available. In QSAR analysis, fragment, substructural or topological descriptors have been used for describing the structural properties of heterogeneous molecules (Kowalski and Bender, 1974 ; Stuper and Jurs, 1975 ; Komatsu, et al., 1985).

In present study, the primary eye irritation of 131 heterogeneous chemicals in rabbits was studied and the eye irritation discriminant function was derived using QSAR analysis for predicting of the eye irritation potential of chemicals.

MATERIALS AND METHODS

1) *Chemicals* :

One hundred thirty one heterogeneous chemicals were used (Table 1). These chemicals were purchased from Wako Pure Chemical Industry (Osaka, Japan), Sigma Chem. Co. (St. Louis, USA), Aldrich Chemical Company (Wisconsin, USA), Dojindo Laboratories (Kumamoto, Japan), Tokyo Kasei Kogyo Co. (Tokyo, Japan) for reagent grade.

2) *Animals* :

Female Japanese white rabbits (2.0–2.2 kg) were purchased from Takeo breeder

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(Shizuoka, Japan), housed in metal cages (39×40×44 cm), fed the RC4 diet (Oriental Yeast Co., Tokyo, Japan) and given water ad libitum. The animals were maintained on a constant 12 hr light : dark cycle with temperature and humidity kept at $25 \pm 1^\circ\text{C}$ and $55 \pm 5\%$, respectively.

3) *Primary eye irritation test :*

Three healthy rabbits were used per one chemical. The chemical (solid : 100 mg, liquid : 0.1 ml) was placed into conjunctival sac of the left eye. The right eye was served as a blank. The eyes were examined and the grade of ocular reaction was recorded 1, 4, 24, 48, 72, 96hr, 7, 14 and 21 days after the treatment of chemicals. Corneal opacity, erythema, chemosis, secreta and iritis were classified according to the Draize score (Draize et al., 1944). The eye irritation score and recovery time of corneal and conjunctival damages were used as the grade of eye irritation potential of each chemical for QSAR analysis.

4) *QSAR analysis :*

Descriptors indicating the substructural features were used as independent variables (Kowalski and Bender, 1974). Thirty six substructural features were investigated to describe the molecules. They included 1 physicochemical descriptor, 34 numeric descriptors and 1 indicator variable for structural fragments and moieties. Numeric descriptors indicated the number of times a descriptor appeared in a molecule. A binary descriptor was simply an indication of the presence of a certain structural parameter. To correlate eye irritation with structural features of chemicals, a QSAR model was developed by the ALS method (Moriguchi and Komatsu, 1977 ; Moriguchi et al., 1980). The ALS method does not assume any particular distribution of the data and can be considered to be a nonparametric pattern classifier. The present version of ALS is ALS81, and the source program was obtained from Kitazato University (Tokyo, Japan). The analysis was performed using a MV-6000 mini-computer (Data General Corporation, USA). Eye irritation potential of chemicals were classified on the basis of their magnitude. Each of the classes was given a starting score (Moriguchi et al., 1980) and the scores were used as a dependent variable. The discriminant score (L) was determined for each chemical by the following equation.

$$L = \Sigma(\text{coefficient} \times \text{descriptor}) + \text{constant}$$

The chemicals were classified into calculated classes on the basis of the value of discriminant score. Cutting points for the classification were determined on the basis of the size of classes used for the derivation of the discriminant function (Moriguchi et al., 1980). A certain number of descriptors were preliminarily chosen by backward stepwise elimination, and several subsets of descriptors were selected using every-possible-combination technique so that the number of misclassified chemicals could be minimized. The final subset of descriptors was determined as the discriminant function on the basis of the results of leave-one-out prediction (Stuper and Jurs, 1975).

Table 1. Chemicals used for eye irritation study in rabbits.

	Chemical Name
1. Acetazolamide	66. Ethionine
2. Acetic anhydride	67. 5-Ethoxycarbonyl-2-(1-isopropylimino)-propyl-cyclohexane-1,3-dione
3. Acetylacetone	68. 5-Ethoxycarbonyl-2-propionyl-cyclohexane-1,3-dione
4. N-Acetylimidazole	69. Ethyl 4-(1-ethoxycarbonylmethylimino)-propyl-3,5-dioxocyclohexanecarboxylate
5. 4-Acetylphenyl chloromethanesulfonate	70. Ethyl-4-(1-(hydroxyethylimino)-propyl)-3,5-dioxocyclohexanecarboxylate
6. Adenine	71. N-Ethylmaleimide
7. Adenosine	72. Ethyl 4-(1-morpholinoiminopropyl)-3,5-dioxocyclohexanecarboxylate
8. 5-Amino-1-(2',6-dichloro-4-trifluoro-methylphenyl)-3-(1,1-dimethylethyl)-1H-1,2,4-triazole	73. Ethyl 4-(1-(phenylimino)propyl)-3,5-dioxocyclohexanecarboxylate
9. 5-Amino-1-(2,6-dichloro-4-trifluoro-methylphenyl)3-pentafluoroethyl-1H-1,2,4-triazole	74. Glycine
10. p-Aminodiphenylether	75. Guanosine
11. 4-Aminosalicylic acid	76. L-Histidine
12. 5-Aminosalicylic acid	77. Hydroquinone
13. 3-Amino-1,2,4triazole	78. 2-Hydroxy-5-nitrobenzylbromide
14. 2-Anilino-4-methyl-6-(1-propynyl)-pyrimidine	79. (4-Hydroxyphenyl) chloromethane-sulfonate
15. Benzene	80. p-Hydroxyphenylglyoxal
16. 2-Benzimidazolethiole	81. 5-Hydroxysalicylic acid
17. Benzoic acid	82. Hypoxanthine
18. 4-Benzoyloxyphenyl chloromethane-sulfonate	83. 2-Imidazolethion
19. 2-Benzylsulfonyl-6-methyl-5H-1,3,4-thiadiazolo [3,2-a] pyrimidin-5-one	84. Inosine
20. Bisdiethanolamine salt of 4-propionyl-3,5-dioxo-cyclohexane-carboxylic acid	85. Isobutyl 2-chloro-6-(4',6'-dimethoxy-pyrimidine-2'-yloxy)benzoate
21. 2,6-Bis-(4',6'-dimethoxypyrimidin-2'-yl)oxybenzoic acid	86. 4-Isopropylphenyl chloromethane-sulfonate
22. N-Bromosuccinimide	87. Isopropyliden amino 3-(4,6-dimethoxy-pyrimidine-2-yl)oxy picorinate
23. 2,3-Butanedione	88. Mercaptoethanol
24. Butyl alcohol	89. 2-Mercaptopyrimidine
25. Butyl 2-(4',6'-dimethoxypyrimidine-2'-yl)oxy benzoate	90. 4-Methansulfonyloxyphenyl chloro-methanesulfonate
26. 1-[p-Chlorobenzoyl]-5-methoxy-2-methyl-indole-3-acetic acid (Indomethacin)	91. Methoxybenzene
27. S-(4-chlorobenzyl)-N,N-diethylthiol-carbamate (Benthiocarb)	92. Methoxybenzoic acid
28. 3'-Chlorobenzyl 3-(4,6-dimethoxy pyrimidine 2-yloxy)picolinate	93. 2-Methoxypyrimidine
29. 2-Chloro-6-((4,6-dimethoxypyrimidin-2-yl)thio)benzoic acid	94. 5-Methoxysalicylic acid
30. 2-Chloro-6-(4,6-dimethoxy-2-pyrimidyloxy)benzoic acid	95. Methyl alcohol
31. 3-(4-Chloro-2-fluoro-5-methoxy-carbonylmethylthiophenylimino)-5,6,7,8-tetrahydro-1H-pyridazino [1,2-c][1,3,4]thiadiazol-1-one	96. 2-(4-Methylbenzylsulfonyl)-5H-1,3,4-thiadiazolo [3,2-a] pyrimidin-5-one
32. 9-(4-Chloro-2-fluoro-5-(1-methoxy-ethoxycarbonyl)ethoxyphenylimino)8-thio-1,6-diazobicyclononan-7-one	97. Methyl 3-(4',6'-dimethoxy 2'-pyrimidinyl)pyridine 2-carboxylate
	98. Methyl 2-(4',6'-dimethoxypyrimidine-2'-yl)oxy benzoate

33. Chloroform	99. 1-Methyl-2-(3-pyridyl)pyrrolidine (Nicotine)
34. 6-Chloro-2-hexylsulfonyl-5H-1,3,4-thiadiazolo[3,2-a]pyrimidin-5-one	100. 4-Methylsalicylic acid
35. Chloromethylsulfonylchloride	101. 5-Methylsalicylic acid
36. 6-Chloro-5H-1,3,4-thiadiazolo[3,2-a]pyrimidin-5-one	102. 6-Methyl-5H-1,3,4-thiadiazolo[3,2-a]pyrimidin-5-one
37. Citraconic anhydride	103. Naphtalene
38. Cyclohexandione	104. Nicotineamide
39. Cyclohexane	105. p-Nitroaniline
40. Cystein	106. 5-Nitrosalicylic acid
41. 6,7-Dichloro-2-pentylsulfonyl-5H-1,3,4-thiadiazolo[3,2-a]pyrimidin-5-one	107. Octahydro-2H-benzimidazole-2-thione
42. 1-(2,6-Dichloro-4-trifluoromethyl-phenyl)-5-ethylamino-3-pentafluoroethyl 1H-1,2,4-triazole	108. Octyl alcohol
43. Diethanolamine salt of methyl 4-propionyl-3,5-dioxo-cyclohexane carboxylate	109. Phenylglyoxal
44. Diethanolamine salt of 4-propionyl-3,5-dioxo-cyclohexane carboxylic acid	110. Phenylsulfide
45. Diethylamine salt of 2-propionyl-5-ethoxycarbonyl-cyclohexane-1,3-dione	111. Phenylthiomethyl 2-chloro-6-(4,6-dimethoxypyrimidine-2-yloxy) benzoate
46. ω -Di-ethylamino-2:6-dimethylacet-anilide (Lidocaine)	112. Picric acid
47. Diethylether	113. 2-Pipecoline
48. N,N-di(1-hydroxyethyl)ammonium salt of ethyl 3,5-dioxo-4-propionylcyclohexane-carboxylate	114. Piperidine
49. 0,0'-Diisopropyl 0-(p-nitrophenyl)thio-phosphate	115. Polyethylene glycol mono-p-octyl-phenyl ether (Triton X-100)
50. 2-((4',6'-Dimethoxy)pyrimidine-2'-yl)-oxy-6-ethoxy-benzoic acid	116. 4-(1-Propanyl)phenyl chloromethane-sulfonate
51. Dimethylaminoantipyrine	117. Pyridine
52. Dimethylformamide	118. Pyrimidine
53. 0,0'-Dimethyl 0-(4-nitro-m-tolyl)phosphorothioate (Sumithion)	119. Salicylic acid
54. 0,0'-Dimethyl(phthalimido)methyl phosphorodithioate (Phosmet)	120. Sodiumdodecyl sulfate
55. Dimethylsulfoxide	121. Sodium salicylate
56. 2,4-Dinitrochlorobenzene	122. Sodium salt of 2-propionyl-5-carboxycyclohexane-1,3-dione
57. 2,4-Dinitrofluorobenzene	123. Sodium salt of 2-propionyl-5-ethoxycarbonylcyclohexane-1,3-dione
58. 3,5-Dinitrosalicylic acid	124. Succinic anhydride
59. 3,5-Dinitrosalicylic acid Na salt	125. 5-Sulfosalicylic acid Na salt
60. Diphenylamine	126. Thioacetamide
61. Diphenylether	127. Toluene
62. Disodium salt of 2-propionyl-5-carboxycyclohexane-1,3-dione	128. 1H-1,2,4triazole
63. 5,5'-Dithio-bis(2-nitrobenzoic acid)	129. 3,4,5-Trimethoxybenzoyl methyl reserpate (Reserpine)
64. 1,2-Epoxyethylbenzene	130. 1,3,7-Trimethylxanthine (Caffein)
65. Ethansulfonic acid	131. L-Tryptophane

The results of clinical observation of the eyes are shown in Fig. 1. A significant correlation was found between the recovery days and the score. However, the irritation ratings could not be classified clearly when the scores were used as the basis of classification. In this study, chemicals were therefore classified into three classes on the basis of the recovery day. Class I (mild) included chemicals inducing no or mild damage recovering within 24 hr, class II (moderate to severe) included chemicals inducing severe irritation which persist for more than 24 hr but clears within 21 days and class III (corrosive) included chemicals inducing corrosive effect not recovering within 21 days. These cutting points were determined by modifying the EPA toxicity categories (1981). The chemicals classified by the above criteria are shown in Table 2. The number of chemicals classified into class I was 23 and these scores distributed from 0 up to 7.0. The number of chemicals classified into class II was 64

Table 2. Criteria based on days of recovery for classification of eye irritation for 131 heterogeneous chemicals, the number of chemicals and chemical No. classified into each class.

Class	Criteria	Number	Chemical No.
I : Non-or mild irritant	Minimal irritation recovering within 24 hr	23	6, 7, 8, 9, 10, 13, 17, 21, 26, 31, 58, 59, 63, 71, 72, 73, 80, 83, 97, 107, 111, 112, 119
II : Moderate or severe irritant	Corneal involvement or irritation that persists for more than 24 hr but recovers within 21 days after treatment	64	1, 3, 5, 11, 12, 14, 15, 16, 19, 22, 25, 27, 28, 30, 32, 33, 36, 39, 42, 43, 44, 45, 46, 47, 48, 49, 51, 52, 56, 57, 61, 64, 66, 67, 69, 70, 74, 81, 82, 84, 85, 86, 87, 89, 90, 91, 93, 95, 98, 99, 101, 102, 104, 105, 108, 110, 116, 122, 124, 126, 127, 128, 129, 131
III : Corrosive	Corneal involvement, irritation or eye damage that persists for more than 21 days after treatment	44	2, 4, 18, 20, 23, 24, 29, 34, 35, 37, 38, 40, 41, 50, 53, 54, 55, 60, 62, 65, 68, 75, 76, 77, 78, 79, 88, 92, 94, 96, 100, 103, 106, 109, 113, 114, 115, 117, 118, 120, 121, 123, 125, 130

and these scores distributed from 1.0 up to 42.8. The number of chemicals classified into class III was 44 and these scores distributed 16.9 up to 96.8.

2. The correlation between chemical structural features and primary eye irritation :

Descriptors used in analysis and Spearman rank correlation coefficients calculated by ALS program for each descriptor are shown in Table 3. The number of descriptors with a positive coefficient was 16, while 20 descriptors had a negative coefficient. Descriptor No. 12, 14, 17, 19 and 36 ($-\text{OH}$ on benzene ring, $-\text{COOH}$ on benzene ring, $-\text{SO}_2-$, $-\text{NO}_2$ and $\text{C}=\text{O}$ bonds/number of bonds) had a positive coefficient with a relatively large value, indicating that these substructural features tend to be in the molecule with which chemicals cause severe or corrosive irritation. Descriptor No. 3, 8, 10, 20 and 30 (N atoms, $\text{R}-\text{O}-\text{R}'$, $-\text{NH}_2$ and N atoms/number of atoms) had a negative coefficient with a relatively large value, indicating that these substructural features tend to be in the molecules with which chemicals cause no or mild irritation.

3. Derivation of the eye irritation discriminant function :

Descriptor No. 3, 10, 14, 17, 20 and 36 (N atoms, $\text{R}-\text{O}-\text{R}'$, $-\text{COOH}$ on benzene ring, $-\text{SO}_2-$, $-\text{NH}_2$, $\text{C}=\text{O}$ bonds / number of bonds) were fixed, because each of them had a positive or negative coefficient with relatively large value. Other descriptors were used as candidate variables, and then the several subsets of descriptors were selected using every-possible-combination technique.

Table 3. Descriptors used for the derivation of eye irritation discriminant function in rabbits, for 131 heterogeneous chemicals and the correlation between the descriptor and starting score.

No.	Descriptor	Correlation ^{a)} coefficient
1	Molecular weight	-0.224
2	Cl atoms (N) ^{b)}	-0.059
3	N atoms (N)	-0.341
4	F atoms (N)	-0.155
5	Br atoms (N)	0.150
6	Non- ring -CH ₂ - (N)	0.100
7	Ring -CH ₂ - (N)	0.001
8	R-O-R' (R,R': alkyl, methylene) (N)	-0.265
9	R-O-R' (R,R': phenyl) (N)	-0.175
10	R-O-R' (R,R': phenyl, alkyl, methylene, P atom) (N)	-0.288
11	Aliphatic -OH (N)	-0.172
12	-OH on benzene ring (N)	0.273
13	Aliphatic -COOH (N)	0.138
14	-COOH on benzene ring (N)	0.253
15	Aliphatic -SH (N)	0.150
16	Ring -SH (N)	-0.118
17	-SO ₂ - ^{b)} (N)	0.241
18	-SO ₃ - (N)	0.029
19	-NO ₂ (N)	0.203
20	-NH ₂ (N)	-0.317
21	C-CH ₃ (N)	-0.188
22	N-CH ₃ (N)	0.068
23	Benzene ring (N)	-0.046
24	-COONa, -SONa, -ONa (N)	-0.051
25	Ammonium, ethanolamine, ethylamine salt (N)	-0.121
26	Ring with N atoms (N=1) (N)	0.133
27	Ring with N atoms (N=2) (N)	-0.063
28	Ring with N atoms (N=3) (N)	-0.217
29	Amino acid (B) ^{c)}	-0.103
30	N atoms / number of atoms (N)	-0.250
31	F atoms / number of atoms (N)	-0.152
32	C atoms / number of atoms (N)	0.040
33	H atoms / number of atoms (N)	-0.049
34	C=C bonds / number of bonds (N)	0.050
35	C-C bonds / number of bonds (N)	0.119
36	C=O bonds / number of bonds (N)	0.230

a) Spearman rank correlation coefficient.

b) Numeric descriptor which indicates the number of times a descriptor appears in a molecule.

c) Binary descriptor which is simply an indication of the presence of a certain structural parameters.

d) -SO₂- in -SO₃- was not included. -SO₂- in -SO₃H was included.

The discriminant function is shown in Table 4. The discriminant function consisted of 18 descriptors in the subset in which 12 descriptors with positive and 6 descriptors with negative coefficients were included. Contribution indexes showed high values at descriptor No. 27, 17, 19, 26, 14 (ring with N atom ($N=2$), $-\text{SO}_2-$, $-\text{NO}_2$, ring with N atom ($N=1$), $-\text{COOH}$ on benzene ring) with positive and No. 10, 3, 20, 24 ($\text{R}-\text{O}-\text{R}'$, N atom, $-\text{NH}_2$, $-\text{COONa}$, $-\text{ONa}$, $-\text{SO}_3\text{Na}$) with negative coefficients. The result of discrimination of the irritation classes for the chemicals is shown in Table 5. The accuracy of classification into three classes was 86.3% in the recognition, and 74.0% in the leave-one-out prediction.

4. The prediction of eye irritation potential for chemicals :

The prediction of irritation potential of 28 heterogeneous chemicals including acids, bases, alcohols etc., was made to confirm the reliability of the discriminant function (Table 6). The results are shown in Table 7. The accuracy of classification

Table 4. Eye irritation discriminant function in rabbits, derived from the data of 131 heterogeneous chemicals.

No.	Descriptor	Coefficient	Contribution ^{a)} index
27	Ring with N atoms ($N=2$)	0.966	0.367
17	$-\text{SO}_2-$	1.209	0.305
19	$-\text{NO}_2$	0.478	0.235
26	Ring with N atoms ($N=1$)	0.680	0.220
14	$-\text{COOH}$ on benzene ring	0.607	0.217
36	$\text{C}=\text{O}$ bonds / number of bonds	2.720	0.216
15	Aliphatic $-\text{SH}$	1.438	0.176
12	$-\text{OH}$ on benzene ring	0.221	0.081
4	F atoms	0.092	0.094
2	Cl atoms	0.095	0.052
5	Br atoms	0.379	0.046
6	Non-ring $-\text{CH}_2-$	0.021	0.049
10	$\text{R}-\text{O}-\text{R}'$ (R, R' : phenyl, alkyl, methylene, P atom)	-0.380	0.437
3	N atoms	-0.311	0.416
20	$-\text{NH}_2$	-0.847	0.285
24	$-\text{COONa}$, $-\text{SO}_3\text{Na}$, $-\text{ONa}$	-0.697	0.214
16	Ring $-\text{SH}$	-0.862	0.106
25	Ammonium, ethanolamine, ethylamine salt	-0.269	0.080
	Contant	0.333	

Discriminant score (L) = $\Sigma(\text{coefficient} \times \text{descriptor}) + \text{constant}$; class I, $L < -0.667$; class II, $-0.667 \leq L < 0.667$; and class III, $L \geq 0.667$.

These cutting points are determined on the basis of the size of classes used for the derivation of the discriminant function (Moriguchi, 1977, 1980)

a) A measure of the contribution of the descriptor to discriminant score (L) Contribution index = $|\text{coefficient}| \times \text{S.D. of descriptor}$. (Moriguchi, 1977, 1980).

Table 5. Result of eye irritation discrimination in rabbit, obtained from the discriminant function derived from the data of 131 heterogeneous chemicals.

Recognition					Leave-one-out prediction				
Nmis ^{a)} =18 (0), Rs ^{b)} =0.869					Nmis=34 (0), Rs=0.736				
Accuracy=86.3%					Accuracy=74.0%				
		Calculated					Calculated		
		I	II	III			I	II	III
Observed	I	14 ^{c)}	9	0	Observed	I	11	12	0
	II	3	58	3		II	8	51	5
	III	0	3	41		III	0	9	35

a) Number of misclassified chemicals. The figure in parentheses indicates the number of misclassified by two grades.

b) Spearman rank correlation coefficient.

c) Number of chemicals classified into each class.

Table 6. Chemicals used for the prediction of eye irritation potential in rabbits.

Chemicals	
1. Acetic acid	15. Ethyl alcohol
2. Acetylsalicylic acid	16. 7-Ethynyl-2-hexylsulfonyl-5H-1,3,4-thiadiazole[3,4-a]pyridin-5-one
3. 2-Aminopyrimidine	17. 2-Heptylsulfonyl-7-methoxy-6-nitro-5H-1,3,4-thiadiazolo[3,2-a]pyrimidin-5-one
4. Aniline	18. o-Hydroxydiphenyl
5. Antipyrine	19. 0,0-Diisopropyl S-benzyl phosphorothioate
6. Benzyl alcohol	20. Imidazole
7. S-[Bis(ethoxycarbonyl)ethyl]0,0'-dimethyl phosphorodithioate (Malathion)	21. Maleic anhydride
8. 2,6-Bis(2-fluorophenyl)-3-methyl-1-(3,5-dimethoxyphenyl)-4(1H)-pyridinone	22. Methansulfonic acid
9. Carbontetrachloride	23. Methyl-6-chloro-2-(4',6'-dimethoxy-pyrimidine-2'-yloxy)benzoate
10. 7-Chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one (Diazepam)	24. N-Methyl-2-pyrrolidone
11. 4,4'-Diaminodiphenylether	25. S-4-Phenoxybutyl dimethylthiocarbamate (Panocon)
12. 2,4-Dichloro-5-(1-cyano)cyclopropyltrifluoromethane	26. Purpurine
13. Diethylpyrocarbonate	27. Sulfanilamide
14. Ethanolamine salt of 4-(1-(2-hydroxyethylimino)propyl)-3,5-dioxo-cyclohexane-carboxylic acid	28. 5-Sulfosalicylic acid

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Table 7. Result of the prediction of eye irritation potency in rabbits, for 28 chemicals, obtained from the discriminant function derived from the data of 131 heterogeneous chemicals.

N=28, Nmis ^{a)} = 8(0), Accuracy=72.4%			
Class (Observed)	Class (Calculated)		
	I	II	III
I	3 ^{b)}	2	0
II	1	8	3
III	0	2	9

a) Number of misclassified chemicals.

The figure in parentheses indicates the number of misclassified by two grades.

b) Number of chemicals classified into each class.

into three classes was 72.4% in the recognition. All misclassified chemicals were assigned to the neighboring classes.

DISCUSSION

1. QSAR analysis :

A QSAR analysis was made with the eye irritation data in rabbits and substructural descriptors for 131 heterogeneous chemicals. There was a significant correlation between the chemical structure and the irritation potential. The study showed that a ring with N atoms (N=1 and 2), $-\text{SO}_2-$, $-\text{NO}_2$, C=O bonds / number of bonds, $-\text{COOH}$ and $-\text{OH}$ on benzene ring and halogens were ranked to important descriptors in a positive and $-\text{NH}_2$, R-O-R' were ranked to important descriptors in a negative way for eye irritation discrimination.

2. Prediction of eye irritation for heterogeneous chemicals :

The result of prediction of irritation potential for 28 heterogeneous chemicals was satisfactory. These results suggested that the data set used here for derivation of the function represents a fairly diverse set of chemicals. The irritation potential of chemicals was considered to be predicted by only the topological descriptors explaining certain substructure in the molecule without physicochemical properties.

3. Descriptors with a positive coefficient :

It has been known that structural features contributing to the induction of damage on eye existed. Friedenwald et al. (1946) showed that buffers of picric acid, tannic acid, sulfosalicylic acid and other organic acid which contain common substructure such as aromatic $-\text{COOH}$ and $-\text{OH}$, induced serious damage on cornea. Grant (1947, a, b) reported that SO_2 in high concentration in the form of

liquefied gas was a occasional cause of serious ocular injury. Chemicals with corrosive effects on mucous membrane, such as mustard gas, lewsite and lacrimators, caused severe eye irritation when a high dose was administered and these chemicals contained halogens in their molecule structures (Potts and Gonasum, 1975). In detergents, cationic detergents indicated more potent eye irritation than anionic or non-ionic detergents (Shopsis et al., 1985 ; North-Root et al., 1982), and the molecule of cationic detergents often contained halogens and rings with N atoms such as pyridinium compounds. Substructure $-\text{CH}_2-$ was contained in the molecule of all kinds of detergents with high incidence.

In this study, most of the descriptors with positive coefficient and high contribution index in the discriminant function indicated the substructural features correlating closely with those reported, previously, as the substructures related to irritation.

4. Relation between the substructural features and the mechanism of eye irritation :

One of the mechanism of irritation caused by chemicals were considered to be the affinity of chemicals to protein and lipid which exist in corneal and conjunctival tissues. Descriptors with a positive coefficient mentioned above were considered to indicate the substructural features which contribute to the enhancement of the affinity of chemicals for proteins or lipids in ocular tissue, and the effect of the substructures in a molecule was considered to mainly result from their electronic effects. For explanation of the electronic effects in a molecule, pka was a suitable physicochemical parameter (Kubota et al., 1979). The relationship between the results of irritation in acids and bases, and published pka data (Schanker et al., 1957 ; Schanker, 1959 ; Rainsford, 1978) are shown in Table 8 and then there was a tendency to correlation between pka values and irritation. The results obtained from reagents with chemical modification activity on protein (Means and Freeny, 1971) seemed to confirm the view that the electronic effects contributed to the enhancement of an affinity of chemicals for protein in ocular tissue. All these reagents caused severe eye irritation and they include electronegative substructures in the molecule, such as $\text{C}=\text{O}$ bond, $-\text{NO}_2$ and halogen (Table 9). Friedenwald et al. (1946) reported that the severity of the corneal lesion produced by organic acids were directly related to its acidity and the affinity of the anion for the protein. It has been known that several phenolic acids had affinity for protein and protein-precipitating ability (Van Buren et al., 1969 ; Sabir, et al., 1974). Grant (1947, a, b) concluded that the production of severe ocular injury by liquid SO_2 was the result from its ready penetration to the corneal epithelium in high concentrations and its denaturing action on corneal proteins. Cations such as N-alkylpyridinium and N-alkylpiperidine were considered to be attracted to electron-rich site in the membrane (Hansch and Glave, 1971).

Another factor related to irritation was the hydrophobicity of chemicals and length of methylene chain ($-\text{CH}_2-$) appeared to reflect the effect of chemicals. In alcohols, a significant relationship between the number of $-\text{CH}_2-$ in the molecule

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Table 8. A compariron of previously published pka data with observed rabbits' eye irritation score and the days of recovery in acids and bases.

Chemical	pka	Observed eye irritation	
		eye irritation score	days of recovery
Acids			
Benzoic acid	4.20 ^{a)}	38.9	>21
5-Aminosalicylic acid	4.00 ^{b)}	2.5	2
Acetylsalicylic acid	3.50 ^{b)}	36.7	21
5-Methylsalicylic acid	3.40 ^{b)}	46.7	>21
5-Hydroxysalicylic acid	3.20 ^{b)}	88.0	>21
4-Methylsalicylic acid	3.15 ^{b)}	39.6	21
5-Methoxysalicylic acid	3.10 ^{b)}	82.2	>21
Salicylic acid	2.95 ^{b)}	64.4	>21
5-Nitrosalicylic acid	2.30 ^{a)}	75.8	>21
5-Sulfosalicylic acid	Strong ^{a)}	87.5	>21
Bases			
Piperidine	11.12 ^{c)}	86.1	>21
Quinine	8.40 ^{a)}	47.6	>21
Pyridine	5.22 ^{c)}	61.6	>21
Aminopyrine	5.00 ^{a)}	29.3	>21
Aniline	4.60 ^{a)}	6.8	4
Antipyrine	1.40 ^{a)}	25.6	>21
p-Nitroaniline	1.00 ^{a)}	1.6	2

a) Schanker et al., 1957, 1959; b) Rainsford, 1978; c) Suzuki, 1979

and the score was obtained (Table 10). Detergents and organic solvents caused eye irritation by solving the lipid of cell membrane (Potts and Gonasum, 1975). It has been found that each $-\text{CH}_2-$ in a homologous series increases log P by about 0.5 (Fujita et al., 1964), and that there was a correlation between log P and the effects on lipid membrane in alcohols, benzene derivatives, and anionic and cationic detergents (Ponder, 1939; Kondo and Tomizawa, 1966; Hansch and Glave, 1971). Binding activity of chemical to protein also correlated with log P in many kinds of chemicals (Hansch et al., 1965; Kiehs et al., 1966; Helmer et al., 1968). $-\text{CH}_2-$ may contribute to the membrane-disruption effect and the protein-binding activity of chemicals by increasing the lipophilicity of molecules.

It was suggested that the existence of the substructure relating to the induction of electronic effect in a molecule was one of important factor for chemicals causing irritation, and that this electronic effects offered a good condition for chemicals to interact with the constituents in ocular tissue.

Table 9. A comparison of chemical structures with observed rabbits' eye irritation class, days of recovery of damage and eye irritation potency calculated from obtained discriminant function for 14 reagents with chemical modification activity on protein.

Chemical	Structure	Eye irritation potential	
		Observed ^{a)}	Calculated ^{b)}
5,5'-Dithio-bis-2-nitrobenzoic acid		III (>21)	III
2,3-Butanedione		III (>21)	III
p-Hydroxyphenylglyoxal		III (>21)	III
2-Hydroxy-5-nitrobenzylbromide		III (>21)	III
Phenylglyoxal		III (>21)	III
Cyclohexanedione		III (>21)	III
N-Ethylmaleimide		III (>21)	III
Acetic anhydride		III (>21)	III
N-Acetylimidazole		III (>21)	III
Maleic anhydride		III (>21)	III
Dinitrofluorobenzene		III (>21)	III
Succinic anhydride		III (>21)	III
Citraconic anhydride		III (>21)	III
N-Bromosuccinimide		III (>21)	III

a) Data from eye irritation test in rabbits. The figure in parenthese indicates the days of recovery. b) Data from the discriminant function.

Table 10. A comparison of number of $-\text{CH}_2-$ bond in the molecule with log P value and observed rabbits' eye irritation score and the day of recovery in alcohols.

Alcohol	Number of $-\text{CH}_2-$	log P ^{a)}	Observed eye irritation	
			eye irritation score	days of recovery
Methyl alcohol	0	-0.68	1.3	4
Ethyl alcohol	1	-0.32	10.9	7
Butyl alcohol	3	0.88	19.5	21
Octyl alcohol	7	3.15	32.3	21

a) Hansch and Leo, (1979)

5. Descriptors with a negative coefficient :

Substructure $-\text{NH}_2$, $\text{R}-\text{O}-\text{R}'$ such as phenoxy and alkoxy group, and salts were in the descriptor group with negative coefficient, and it was considered that these substructure contribute to the reduction of irritation. In Table 8, $-\text{NH}_2$ substituted chemicals such as aniline derivatives had weak irritation potency. The contribution of these substructures to the reduction of irritation potential in the molecule may be explained by characteristics of the organic chemicals as follows. Aniline derivatives were weak bases compared with piperidine and pyridine, not stabilizing the conjugate acid of aniline, i. e. anilinium ion, because of the absence of resonance. Concerning $\text{R}-\text{O}-\text{R}'$, the base dissociation constants of ether bond were very low compared with the amines. Friedenwald et al. (1946) showed that sulfosalicylic acid caused no effect on eye over pH7.0. $-\text{COONa}$ and other salt seemed to contribute to the reduction of irritation by decreasing hydrogen ion concentration in the acid solution.

6. Conclusion :

The current analysis was the one of methods of pattern recognition, and the selected substructures were characterized by generously. Substructures which ranked, as descriptors, high in their importance for the discrimination were considered to affect the observed irritation. However, many other factors such as steric effects and lipophilicity seemed to play a role in the relation of structure to irritation. In order to explain the contribution of these factors, it is considered to be necessary that more detailed information from QSAR analysis is needed in each homologous chemicals.

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