On the Relationship Between the Chemical Structure and the Carcinogenicity of Polycyclic and Chlorinated Monocyclic Aromatic Compounds as Studied by Means of ^{13}C NMR

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The relationship between chemical structure and carcinogenicity was investigated in thirty-eight polycyclic aromatic compounds and forty-four chlorinated aromatic hydrocarbons by means of ^{13}C NMR. As an "index" serving as a measure of the electronic structure of aromatic compounds, which is closely related to the carcinogenicity, the averaged ^{13}C NMR chemical shift over all the aromatic carbons in the molecule was used. In polycyclic aromatic compounds, the averaged chemical shifts of the carcinogenic compounds are within the range from 127.11 to 127.87 ppm, but those of the noncarcinogenic compounds are not within this range. In chlorinated monocyclic aromatic compounds, the carcinogenic compounds was within the range from 127.76 to 132.64 ppm. Thus, we proposed that the "index" is very useful for the discernment of the carcinogenicity of polycyclic and chlorinated monocyclic aromatic compounds.

It is known that aromatic compounds sometimes have carcinogenicity.[1-3] In order to obtain useful information about the detailed mechanism of their carcinogenicity, several approaches have been employed. One of them is the quantum-chemical approach,[4-10] which has provided insight into the close relationship between the carcinogenicity and the electronic structure and has also made possible an interesting prediction as to the carcinogenicity of aromatic compounds. Nevertheless, this approach is not practical for the discrimination of the carcinogenicity. Another approach is to relate the carcinogenicity to a physical quantity such as the ionization potential.[11-15]

It has been reported that aromatic hydrocarbons with an ionization potential within any specified range show carcinogenic activity and that the carcinogenicity increases with a decrease in the potential energy.

It is known that ^{13}C NMR chemical shifts of the aromatic compounds considered here are closely related to the electronic environments of the individual carbon and to the ionization potential.[16,17] This means that the ^{13}C NMR chemical shift of aromatic compounds provides a measure of whether or not the molecules have carcinogenic activity.

The purpose of this paper is to study the relationship between the carcinogenicity and the averaged ^{13}C chemical shifts over all the carbons in the ring structure of aromatic compounds, and to clarify whether or not the averaged ^{13}C chemical shift is useful for the discernment of the carcinogenicity.

The averaged chemical shifts employed in this work reflect the electronic structure and electronic state of the molecules as follows. The dominant factor governing the ^{13}C chemical shift of a nucleus i is the paramagnetic contribution as given by Eq. 1, with an average excitation energy approximation.

$$\delta_i \propto \frac{1}{\Delta E} \cdot Q_i, \tag{1}$$

where ΔE is the averaged excitation energy of a molecule and Q is a factor containing electron density and bond order. Thus, the averaged chemical shift defined above is expressed by Eq. 2:

$$\begin{aligned} \bar{\delta} &\propto \frac{1}{N} \sum_i^N \frac{1}{\Delta E} \cdot Q_i \\ &= \frac{1}{\Delta E} \cdot \frac{1}{N} \sum_i^N \cdot Q_i \\ &= \frac{1}{\Delta E} \cdot \bar{Q} \end{aligned} \tag{2}$$

in which $\bar{Q} = \frac{1}{N} \sum_i^N Q_i$,

where N is the number of carbon atoms considered here. As may be seen from this equation, the averaged chemical shift involves information on the averaged excitation energy and the averaged value of Q. Thus, we can indirectly get information on the electronic structure and electronic state of the aromatic compounds through the observation of the averaged chemical shift for all the carbons in the ring structure of the molecule.

Experimental

Twelve compounds (**1**)—(**12**) (see Table 1) and forty-four compounds (**1**)—(**44**) (see Table 2) were obtained from the Tokyo Kasei Co. and were used without further purification.

The ^{13}C NMR spectra were recorded with Fourier Transform spectrometers, Hitachi R-900 FT and JEOL GX-400 FT, operating at 22.6 MHz and 100.4 MHz respectively, at 35 °C and at 27 °C, using an external lock system and a deuterated lock system. Deuteriochloroform was used as the solvent. The concentration of the sample solution was 25—62%(w/v). The chemical shift was significantly independent of the concentration of the sample within this concentration range.

The chemical shifts were expressed in ppm relative to tetramethylsilane.

The number of accumulations is about 400—14000: data points, 16K or 32K: spectral width, 1800 Hz: flip angle, 25°,

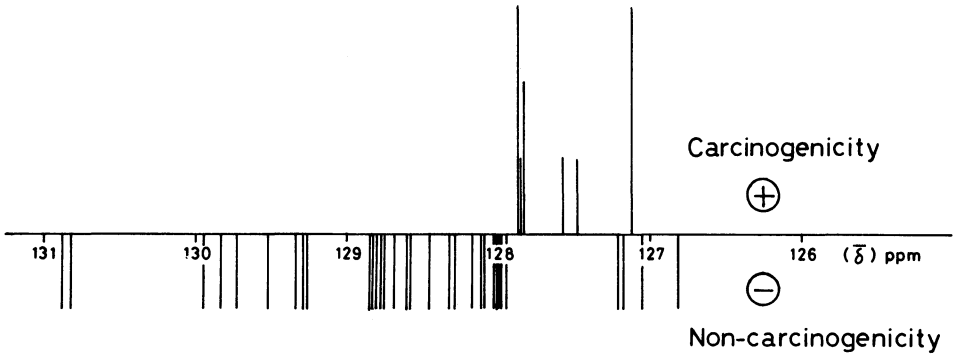

Fig. 1. The relationship between the averaged ^{13}C NMR chemical shifts (δav) of individual polycyclic aromatic compound and their carcinogenicity.

and interval of pulse to pulse, 3 s. (The detailed assignment of the ^{13}C NMR spectra for some samples is carried out by means of T_1 measurement,[18] selective decoupling[19a,b] and an isotope-label shift.[20])

Results and Discussion

Polycyclic Aromatic Hydrocarbons. The averaged ^{13}C NMR chemical shifts (δ av) over all the ring carbons in polycyclic aromatic compounds and their subsituted compounds, together with the ionization potential and the known carcinogenicity, are shown in Table 1, while the relationship between the averaged ^{13}C NMR chemical shifts (δ av) of the individual compounds and their carcinogenicities is shown in Fig. 1.

The averaged ^{13}C NMR chemical shift values for the compounds have already been estimated from the reported data.[23a,b] On the other hand, some compounds (**1**)—(**12**) with carcinogenicity have already been investigated.[4,5,17b,21,22,23b,25] Hawever, in many compounds the carcinogenicity is unknown. Thus, the compounds listed in Table 1 are divided into three groups: carcinogenic, noncarcinogenic, and undetermined groups. It has been reported that benz[*a*]anthracene (**4**), dibenz[*a,h*]anthracene (**8**), benzo[*a*]pyrene (**9**), 7-methylbenz[*a*]anthracene (**11**), and 7,12-dimethylbenz[*a*]anthracene (**12**) are carcinogenic.[4,22,23,25] The values of δ av for the corresponding compounds are within the range from 127.11 to 127.87 ppm. On the other hand, it is found that the averaged ^{13}C chemical shift values for naphthalene (**1**) and anthracene (**2**) are larger than 127.87 ppm, while those for 9,10-dimethylanthracene (**10**) and triphenylene (**6**) are smaller than 127.11 ppm. These compounds are not carcinogenic.[4,17,22,23b] These findings suggest that there is a correlation between the averaged chemical shifts and the carcinogenicity, and that the chemical shift range from 127.11 to 127.87 ppm, the "index range," can be used to discern whether or not compounds being considered are carcinogenic. For phenanthrene (**3**), contrary results on its carcinogenicity have been reported.[4,5] The averaged chemical shift was 128.68 ppm. Thus, according to the above "index" range, phenanthrene (**3**) can be expected to be noncarcinogenic. Next, let us consider pyrene (**5**) and dibenz[*a,c*]anthracene (**7**), which have been reoported to be noncarcinogenic.[4,5,17b,22]

The values of these compounds are slightly higher than that of 7,12-dimethylbenz[*a*]anthracene (**12**), which is carcinogenic: they are the lowest values in the "carcinogenic index." This means that it is not easy to determine clearly, by the use of the carcinogenic index, whether compounds which are near the lower limit of the carcinogenic index are carcinogenic or noncarcinogenic. When methyl groups were substituted into polycyclic aromatic hydrocarbons, the increase in carcinogenicity or the appearance of the carcinogenic activity were recognized in some compounds.[17a] For methyl-substituted benz[*a*]anthracene (**4**), the increase in the carcinogenicity has been well-confirmed in 7-methylbenz[*a*]anthracene (**11**) and 7,12-dimethylbenz[*a*]anthracene (**12**). However, methyl-substituted naphthalene and anthracene are known to be noncarcinogenic. Such experimental findings will be studied through the averaged ^{13}C chemical shifts of methyl-substituted aromatic hydrocarbons.

We predicted the carcinogenicity of the methyl-substituted compounds to be as is shown in Table 1; we did so on the basis of the averaged chemical shifts. The values of δ av for all methyl-substituted compounds, (**14**)—(**16**) and (**19**)—(**28**), appear at a field lower by from 0.17 to 2.54 ppm than that of naphthalene. This suggests that these compounds are noncarcinogenic. Also, the values of the δ av for 1-methylnaphthalene (**13**), 1,4-dimethylnaphthalene (**17**), and 1,5-dimethylnaphthalene (**18**) appear at a field higher than that of naphthalene by ca. from 0.05 to 0.09 ppm; these values are not in the range from 127.11 to 127.87 ppm. Therefore, it may be suggested that these compounds are noncarcinogenic. The values of δ av for di-, tri-, and tetramethyl-substituted anthracene, (**32**)—(**38**), showed a variety of shifts ranging from ca. 0.16 to 1.66 ppm.

None of them is carcinogenic, since all of them showed a field shift lower than the "index." 1-Methylanthracene (**29**) and 2-methylanthracene (**30**) also seem to be noncarcinogenic, judging from their low

Table 1. The Averaged ^{13}C NMR Chemical Shifts over All the Ring Carbons of Polycyclic Aromatic Hydrocarbons, Ionization Potentials, and Their Carcinogenicities

Compound	Averaged Chemical Shift/ppm	Ionization Potential IP/eV	1/IP	Predicted Carcinogenicity[a]	Carcinogenicity[b] Literature
Naphthalene (**1**)	128.28	8.13	0.1230		− (4, 22)
Anthracene (**2**)	128.17	7.43	0.1346		− (4, 22)
Phenanthrene (**3**)	128.68	8.19	0.1221	−	± (4, 5, 21)
Benz[*a*]anthracene (**4**)	127.86	7.54	0.1326		+ (4, 22, 25)
Pyrene (**5**)	127.16	7.50	0.1333		− (4, 5, 17b))
Triphenylene (**6**)	126.82	8.15	0.1227		− (4, 22)
Dibenz[*a*,*c*]anthracene (**7**)	127.21				− (4, 5, 22)
Dibenz[*a*,*h*]anthracene (**8**)	127.83	7.58	0.1319		++ (4, 22)
Benzo[*a*]pyrene (**9**)	127.11	7.23	0.1383		+++ (4, 22, 25)
9,10-Dimethylanthracene (**10**)	127.06	7.14	0.1401		− (17a), 23b))
7-Methylbenz[*a*]anthracene (**11**)	127.47	7.37	0.1357		+ (4, 23b))
7,12-Dimethylbenz[*a*]anthracene (**12**)	127.87	7.22	0.1385		+++ (4, 23b))
1-Methylnaphthalene (**13**)	128.08[c]			−	
2-Methylnaphthalene (**14**)	128.75[c]			−	
1,2-Dimethylnaphthalene (**15**)	128.45[c]			−	
1,3-Dimethylnaphthalene (**16**)	128.82[c]			−	
1,4-Dimethylnaphthalene (**17**)	128.02[c]			−	
1,5-Dimethylnaphthalene (**18**)	128.10[c]			−	
1,6-Dimethylnaphthalene (**19**)	128.76[c]			−	
1,7-Dimmethylnaphthalene (**20**)	128.83[c]			−	
1,8-Dimethylnaphthalene (**21**)	130.87[c]			−	
2,3-Dimethylnaphthalene (**22**)	129.26[c]			−	
2,6-Dimethylnaphthalene (**23**)	129.52[c]			−	
2,7-Dimethylnaphthalene (**24**)	129.73[c]			−	
2,3,5-Trimethylnaphthalene (**25**)	129.32[c]			−	
2,3,6-Trimethylnaphthalene (**26**)	129.96[c]			−	
1,6,7-Trimethylnaphthalene (**27**)	129.26[c]			−	
1,5,8-Trimethylnaphthalene (**28**)	130.82[c]			−	
1-Methylanthracene (**29**)	127.96[d]			−	
2-Methylanthracene (**30**)	128.61[d]			−	
9-Methylanthracene (**31**)	127.57[d]			+	
1,4-Dimethylanthracene (**32**)	127.97[d]			−	
1,8-Dimethylanthracene (**33**)	127.99[d]			−	
1,4,9-Trimethylanthracene (**34**)	128.59[d]			−	
2,7,9-Trimethylanthracene (**35**)	128.33[d]			−	
1,4,5,9-Tetramethylanthracene (**36**)	128.84[d]			−	
1,4,5,8-Tetramethylanthracene (**37**)	127.94[d]			−	
1,4,5,8,9-Pentamethylanthracene (**38**)	129.83[d]			−	

a) − For noncarcinogenic and + for carcinogenic. b) Carcinogenicity reported by reference. This may be due to biochemical evidence or molecular orbital calculation. −: For noncarcinogenic, +: for carcinogenic, ±: for noncarcinogenic or carcinogenic, ++, +++: for strongly carcinogenic. c) From Ref. 23a). d) From Ref. 23b).

chemical shifts. However, 9-methylanthracene (**31**) falls in the range of the "index." The carcinogenicity in the compound has not yet been determined, but the observed value of δ av suggests that the compound is carcinogenic. Most polycyclic aromatic compounds form charge-transfer complexes which consist of an electron-donor part and an acceptor part.

It has been proposed that any specified value of the ionization potential formation for charge-transfer complexes is associated strongly with the ionization potential. This causes steric hindrance effects to the DNA conformation, and so such compounds are carcinogenic. The relationship among the ionization potential, the averaged ^{13}C NMR chemical shift, and the carcinogenicity is shown in Table 1. The ionization potentials and their reciprocals in some compounds are shown in Table 1. It is known that carcinogenic compounds have ionization potentials of from 7.22 to 7.58 eV and that naphthalene (**1**), phenanthrene (**3**), and triphenylene (**6**), with higher ionization potentials than this range, are not carcinogenic. In the carcinogenic compounds, those with lower ionization potentials showed stronger carcinogenicities. However, 9,10-dimethylanthracene (**10**), which has the lowest ionization potential, anthracene (**2**) and pyrene (**5**), all within the range from 7.22 to 7.58 eV, are not carcinogenic. In these cases, the local electronic state in the molecules should be determined in addition to the ionization potential in order to estimate their carcinogenicity.

Chlorinated Aromatic Hydrocarbons. The relationship between the averaged ^{13}C NMR chemical shift

Table 2. The Averaged ^{13}C NMR Chemical Shifts over All the Ring Carbons of Chlorinated Aromatic Hydrocarbons, Ionization Potentials, and Their Carcinogenicities

Compound	Averaged Chemical Shift/ppm	Ionization Potential IP/eV	1/IP	Predicted carcinogenicity	Carcinogenicity[b] Literature
Chlorobenzene (**1**)	129.77[c]	9.42	0.1062		+ (26)
o-Dichlorobenzene (**2**)	131.48[c]	9.06	0.1104		+ (26)
m-Dichlorobenzene (**3**)	130.65	9.80	0.1020		+ (26)
p-Dichlorobenzene (**4**)	131.21	9.67	0.1078		+ (26)
1,2,4-Trichlorobenzene (**5**)	130.24				+ (26)
3,4,5,6-Tetrachlorobenzene (**6**)	131.49				+ (26)
1,2,4,5-Tetrachlorobenzene (**7**)	131.52				+ (26)
1,2,3,4,5-Pentachlorobenzene (**8**)	131.90				+ (26)
p-Chlorobenzyl chloride (**9**)	132.23[c]			+	
Benzyl chloride (**10**)	130.75[c]				+ (27a), b))
Benzylidene dichloride (**11**)	131.23				+ (27a), b))
Benzylidyne trichloride (**12**)	131.80				+ (27a))
Benzoyl chloride (**13**)	132.30				+ (28a), b))
o-Chloroacetophenone (**14**)	129.43			+	
m-Chloroacetophenone (**15**)	131.30			+	
p-Chloroacetophenone (**16**)	131.23			+	
2,6-Dichloroacetophenone (**17**)	131.38			+	
1-Chloro-2,4-dinitrobenzene (**18**)	131.82			+	
2,4-Dichloroacetophenone (**19**)	129.85			+	
2,3-Dimethyl-6-chlorobenzene (**20**)	131.63[c]			+	
o-Chloronitrobenzene (**21**)	129.70			+	
2-Chloro-6-nitrotoluene (**22**)	132.82			−	
4-Chloro-2-nitroanisole (**23**)	131.92			+	
p-Chlorofluorobenzene (**24**)	134.68[d]			−	
p-Chlorobenzonitrile (**25**)	137.10[e]			−	
2,6-Dichlorobenzonitrile (**26**)	133.55				− (29a), b), c))
2,3,4,5,6-Pentachloro-1-nitrobenzene (**27**)	131.72			+	
Ethyl α-(*p*-Chlorophenoxy)isobutyrate (**28**)	132.80				− (29a), b), c)) (30a), b), c))
o-Chlorophenol (**29**)	127.76	9.28	0.1078		+ (29a), b), c)) (31a)—h))
m-Chlorophenol (**30**)	128.82	8.98	0.1114		+ (29a), b), c)) (31a)—h))
p-Chlorophenol (**31**)	131.51	9.07	0.1103		+ (29a), b), c)) (31a)—h))
2,4,5-Trichlorophenol (**32**)	128.94				+ (29a), b), c)) (31a)—h))
2,4,6-Trichlorophenol (**33**)	130.57				+ (29a), b), c)) (31a)—h))
Pentachlorophenol (**34**)	132.64				+ (29a), b), c))
4-Chloro-*m*-cresol (**35**)	129.89			+	
2,4-Dichlorophenoxyacetic acid (**36**)	130.03				+ (29a), b), c))
o-Chloroaniline (**37**)	125.70	8.30	0.1205	−	
m-Chloroaniline (**38**)	126.56	8.60	0.1163	−	
p-Chloroaniline (**39**)	128.41	8.18	0.1223	+	
p-Chloro-*o*-anisidine (**40**)	120.26			−	
1-Chloro-4-nitroaniline (**41**)	128.29			+	
4-Chloro-1,2-phenylenediamine (**42**)	124.60			−	
3,4-Dichloroaniline (**43**)	126.97			−	
(4-Chloro-*N*-methylanilino)cyanide (**44**)	129.25[f]			+	

a) − For noncarcinogenic and + for carcinogenic. b) Carcinogenicity reported by reference. +: For carcinogenic, −: For noncarcinogenic. c) From Ref. 32). d) From Ref. 33). e) From Ref. 34). f) From Ref. 35).

and the carcinogenicity of chlorinated monocyclic aromatic compounds is shown in Table 2 and Fig. 2. These compounds also were divided into three groups: the carcinogenic, noncarcinogenic, and undetermined groups. Chlorobenzene (**1**) and di-, tri-, tetrachlorobenzene (**2**)—(**8**), benzyl chloride and its derivatives (**10**)—(**13**), di-, tri-, and pentachlorophenol (**29**)—(**34**), and 2,4-dichlorophenoxyacetic acid (**36**) have all been reported to be carcinogenic. The averaged chemical shifts in these carcinogenic compounds were within the range from 127.76 to 132.64 ppm. The averaged chemical shift of two non-carcinogenic compounds, 2,6-dichlorobenzonitrile (**26**) and ethyl α-(*p*-chlorophenoxy)isobutyrate (**28**), were larger than those of the carcinogenic compounds. From these "index" values, we predicted the carcinogenicity of the *p*-chlorobenzyl chloride (**9**), (**14**)—(**25**), (**27**), whose carcinogenicities have not yet been studied. *p*-Chlorobenzyl chloride (**9**), chloroacetophenones (**14**)—(**17**), (**19**) chlorobenzene derivatives (**18**), (**20**), (**21**), (**27**), and 4-chloro-2-nitroanisole (**23**) would seem to be carcinogenic, since the averaged chemical shifts of these compounds are within the range of 127.76—132.64 ppm, the index of carcinogenic chlorinated

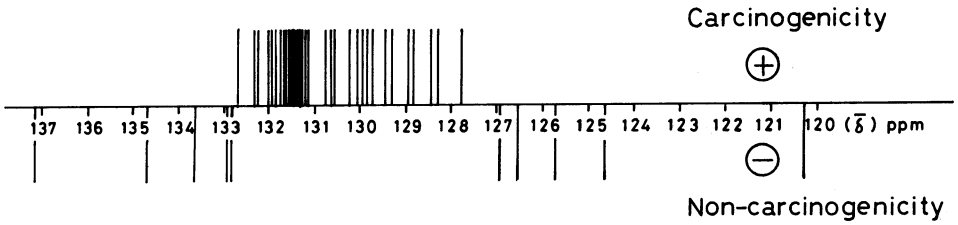

Fig. 2. The relationship between the averaged ^{13}C NMR chemical shifts (δav) of individual chlorinated-monocyclic aromatic compound and their carcinogenicity.

compounds. The averaged chemical shifts of 2-chloro-6-nitrotoluene (**22**), *p*-chlorofluorobenzene (**24**), and *p*-chlorobenzonitrile (**25**) were larger than the "index value" and similar to that of the noncarcinogenic compounds, (**26**) and (**28**). Therefore, we predicted that these compounds would be noncarcinogenic.

In 2-chloro-6-nitrotoluene (**22**), electrons in the ring are attracted by the high electronegativity of the Cl and NO_2 groups, and so the number of electrons in the ring decreased. This moved the averaged chemical shifts downfield. In compound (**24**), the electrons in the ring are attracted to subsituted halogen; this moves its averaged chemical shifts downfield. In *p*-chlorobenzonitrile (**25**) and 2,6-dichlorobenzonitrile (**26**), the electronegativity of the cyano group is so large that the electrons in the ring are attracted by the δ-I effect; therefore, the averaged chemical shifts move downfield as a whole. The larger the δ-I effect, the less the electron density in para positions within the benzene ring.

Next, let us consider the monocyclic chlorine-substituted compounds (**29**)—(**36**). In this table, all of the compounds except 4-chloro-*m*-cresol (**35**) have been reported to be carcinogenic.[29,30,31]

Compound (**35**) also would be carcinogenic judging from the "carcinogenic index" for chlorine-substituted aromatic compounds. The averaged chemical shift value of (4-chloro-*N*-methylanilino)cyanide (**44**) was 129.25 ppm; therefore, the compound seems to have carcinogenicity. The downfield shift may be due to the fact that electrons in the ring are attracted to the Cl group by its large electronegativity, and so the number of electrons in the ring is decreased. In the case of the alkaline compounds, it seems that the downfield shift occurs by means of the solvation in solution. The chlorine atom in the para position may decrease the electrons in the benzene ring rather than that in the meta position, and it seems to cause the averaged chemical shifts downfield as a whole. In general, acid and neutral monocyclic chlorine compounds seem to be carcinogenic, whereas alkaline ones are usually of no carcinogenicity.

As in the case of polycyclic aromatic hydrocarbons, there seems to be a correlation between the carcinogenicity and the ionization potential of the chlorinated aromatic hydrocarbons.

As is shown in Table 2, the ionization potentials of the carcinogenic compounds are within the range from 8.98 to 9.80 eV. We can see the correlation of the averaged ^{13}C NMR chemical shift, the ionization potential, and the carcinogenicity.

Our idea of using the averaged value of the ^{13}C NMR chemical shifts which link the electronic structure and the electronic state was carried out; we found that we could predict the carcinogenicity in aromatic compounds from the chemical-shift value.

The averaged chemical shifts of the known carcinogenic polycyclic aromatics are within the range from 127.11 to 127.87 ppm, while those of the known chlorinated aromatics are within the range from 127.76 to 132.64 ppm: the difference might reflect the slight difference in the mechanism of carcinogenicity between the two types of compounds. There are a few noncarcinogenic compounds that have averaged chemical shifts values within the "carcinogenic index". Therefore, there would be a secondary criterion to judge their carcinogenicity, such as a local electronic state, even though the averaged chemical shift remains the more primary, simple, and useful criterion for carcinogen. Furthermore, a close investigation of carcinogenic compounds and noncarcinogenic compounds with only slightly different chemical-shift values should give insight into the mechanism of carcinogenicity.

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