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Results of Screening NCI/NTP Nongenotoxic Carcinogens and Genotoxic Noncarcinogens with the k$_e$ Test

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DISCLAIMER

ELECTROPHILICITY OF NONGENOTOXIC CARCINOGENS AND GENOTOXIC NONCARCINOGENS AS MEASURED BY THE k_e TEST

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ELECTROPHILICITY/MUTAGENICITY/CARCINOGENICITY RELATIONSHIPS

The interdependence of the electrophilic and carcinogenic properties of chemicals that was demonstrated by the Millers two decades ago (Miller and Miller, 1971) rekindled interest in the somatic mutation theory of carcinogenesis. Interest in this theory grew with the development of a reverse-mutation bacterial assay in the laboratory of B.N. Ames that permitted the mutagenic properties of the chemicals to be determined quickly and yielded results which indicated that "carcinogens are mutagens" (Ames, 1972). Subsequent validation studies of this bioassay, the Salmonella typhimurium/microsome or "Ames test", by Ames' group (McCann et al., 1975) and others provided additional support for the correlation between mutagenicity and carcinogenicity which led to the worldwide deployment of the Ames test in thousands of laboratories (Ames, 1984) and to the development of more than 100 other short-term tests that continue to be used to identify potential carcinogens via various end-points of genotoxicity.

This decade of growth for short-term carcinogen-screening tests was shaken by the release of results of the carcinogenic properties of chemicals which had been obtained via long-term animal studies that were conducted under the aegis of the National Cancer Institute (NCI) and the National Toxicology Program (NTP). The NCI/NTP results and the then recently completed Gene-Tox Carcinogen Data Base (Nesnow et al., 1986) were the focus of attention of an EPA-sponsored workshop entitled "Relationship between Short-Term Test Information and Carcinogenicity" (Auletta

and Ashby, 1988; Kier, 1988) which was soon followed by Zeiger's report that "only 54% of the 149 carcinogens or equivocal carcinogens were (Ames test) mutagens" in the 224 chemicals that had been tested in the NCI/NTP study (Zeiger, 1987). This malaise in the short-term screening of carcinogens intensified a few months later when Tennant et al. reported that three other short-term bioassays performed as poorly as the Ames test in identifying carcinogens among the 73 NCI/NTP-screened chemicals that were considered (Tennant et al., 1987). A special issue of Mutation Research "was conceived as there is general agreement that the problem of battery selection is now at a cross-road" [Rosenkranz, 1988] in which various views on the problem were expressed (e.g., Ashby and Purchase; Bridges; Ramel;Waters et al., all 1988).

While this issue of Mutation Research was in preparation, Ashby and Tennant reported their comprehensive analysis of the results of 222 NCI/NTP-tested chemicals and found a strong association among the structure, Ames test mutagenicity and rodent carcinogenicity of those chemicals (Ashby and Tennant, 1988). Thus, the Ashby/Tennant paper provided justification for the continued use of in vitro tests to identify a large class of genotoxic carcingens as well as support for the use of "structural alerts" of electrophilicity to reach the same objective; however, their analysis of the NCI/NTP data base also indicated that a signficant number of carcinogens are nongenotoxic and a group of putative noncarcinogens is genotoxic. These two classes of chemicals have received considerable attention within the last year (e.g. Ashby; Douglas et al.; Ennever and Rosenkranz; Green; and Trosko; all 1988) and were the chemicals screened in this study. Ashby and Tennant also drew attention to electrophilicity as an indicator of carcinogenicity, which returns us to the work of the Millers that was initially cited and to the k_e test which we regard as the prime measure of electrophilicity.

USING ELECTRONS TO MEASURE ELECTROPHILICITY

Studies of the correlation between the electrophilic and carcinogenic properties of chemicals that precedes and includes the work of the Millers have recently been reviewed (Bakale, 1989). The same review also outlines the intense interest that the unique transport and reaction

properties of excess electrons have generated since their being "discovered" about twenty years ago, and this area has also been recently reviewed (Holroyd, 1987). One of the characteristic features of the reactions of excess electrons that was stressed in these reviews is the strong dependence of the electron attachment rate constant, k_e, on the solvent in which reaction occurs. This is perhaps best exemplified by the four orders of magnitude difference in the k_e's of the electron scavenger SF_6 in two solvents that appear similar, CH_4 and C_2H_6 (Bakale et al., 1975).

The strong dependence of k_e on the reaction medium suggests that if k_e is to provide a meaningful measure of the electrophilicty of a carcinogen, k_e must be measured in a solvent that simulates (to the electron) the microenvironment in which a carcinogen interacts with the biological target. Although cyclohexane may appear (to us) to have little similarity to the carcinogen-target bioenvironment, an electron may view this differently if the electron-transport properties in the transiently structured water that separates the carcinogen and target influences their physical interaction. DNA is an example of a biomolecule that has associated with it a sheath of water that facilitates electron transport (van Lith et al., 1986), and additional evidence of this structured microenvironment has been presented (Bakale and McCreary, 1987; Bakale, 1988, 1989).

Although an electron is certainly the perfect probe of the electrophilicity or "electron-loving" capacity of a chemical, the degree to which electrophilicity is reflected in k_e is arbitrary. In our earliest screening study of the k_e's of carcinogens (Bakale et al., 1981), we assumed that a diffusion-controlled k_e indicated that the electron acceptor was an electrophile since such a k_e also indicates an absence of any barrier to the electron attachment process. We have used the same diffusion-controlled k_e as the boundary between positive and negative responses in our subsequent carcinogen-screening studies (Bakale et al., 1982; Bakale and McCreary, 1987; Bakale, 1988 and 1989). Additional details on this boundary which is $3.0 \times 10^{12} M^{-1} s^{-1}$ in cyclohexane at $21^{\circ}C$ are also presented in these studies in which values of k_e that exceed this boundary due to enhancement of the electron-solvent encounter radius by physical interactions are discussed.

EXPERIMENTAL

The same pulse-conductivity system, solvent purification technique and sample-preparation procedures that were described in detail in an earlier study (Bakale and McCreary, 1987) were again used in this work. Details related to obtaining k_e from the observed electron half-life have also been presented (Bakale and McCreary, 1987; Bakale, 1989). All measurements of k_e were made in cyclohexane at $21 \pm 2^{o}C$.

RESULTS AND DISCUSSION

Those chemicals included in the 1987 Zeiger survey of NCI/NTP-screened chemicals which were reported to be rodent carcinogens and to yield negative or equivocal Ames test mutagenicity responses are listed in Table 1 if k_e is positive or in Table 2 if k_e is negative. Also included in these tables are the presence or absence of "structural alerts" (SA's) of carcinogenicity/electrophilicity which were either reported in the 1988 survey by Ashby and Tennant or obtained from the information that they provided on S/A (see Figure 1 in Ashby and Tennant, 1988). Additional information from the Ashby/Tennant or Zeiger surveys which pertains to the level of effect of the carcinogens or the tumor sites is also included in Tables 1 and 2.

Chemicals for which no evidence of carcinogenicity was found in the NCT/NTP long-term rodent bioassays but which were reported by Zeiger to yield positive or equivocal Ames test responses are listed in Table 3 if k_e is negative and in Table 4 if k_e is positive. Also included in these tables are the SA's reported by Ashby and Tennant.

Omitted from Tables 1-4 are 7 carcinogens and 4 non-carcinogens that could not be screened with the k_e test but which were reported by Zeiger to yield "incorrect" or equivocal Ames test responses. These chemicals include nine that are insoluble in solvents that are miscible with cyclohexane, one (calcium cyanamide) that cannot be dissolved without decomposition (Stecher, et al, 1968) and another (2,3,7,8-tetrachlorodibenzo-p-dioxin) that requires special handling/containment facilities that were unavailable.

The results presented in Tables 1 and 3 illustrate that

Table 1. Rodent carcinogens[a] that yield positive k_e responses and negative or equivocal responses[b] in the Ames Salmonella assay; also listed are the presence (+) or absence (-) of a "carcinogenic structural alert", SA, the level of effect of the carcinogen, LE, and the tumor site.

Chemical	SA[c]	LE[d]	Site[e]
Allyl isothiocyanate	+	D	UB
Benzyl acetate	(-)	(B)	(LO)
Chlordane	-	C	L
Chlordecone	(-)	(A)	(L)
Chlorobenzilate	-	C	L
Chlorodibromomethane	-	D	L*
Chlorothalonil	-	C	K
Dichlorodiphenylethylene	-	C	L
Decabromodiphenyl oxide	-	C	L**
Dicofol	-	D	L
Di(2-ethylhexyl)phthalate	-	A	L,L*
Heptachlor	-	C	L
Hexabromobiphenyl	-	(A)	(L)
Hexachloroethane	-	C	L
Isophorone	-	D	K
Monuron	(+)	(B)	(L,O)
N-Nitrosodiphenylamine	-	C	UB
Pentachloroethane	-	C	L,L**
Phenesterin	+	A	CS,HS,LU,MG
Reserpine	-	A	AG,MG,SV
1,1,1,2-Tetrachloroethane	-	C	L,L**
1,1,2,2-Tetrachloroethane	-	C	L
Tetrachloroethylene	(-)	(A)	(L,O)
Tetrachlorvinphos	(+)	(A)	(L,O)
1,1,2-Trichloroethane	-	B	AG,L
Trichloroethylene	-	C	HS,L*
2,4,6-Trichlorophenol	-	A	HS,L*
Zearalenone	-	B	L**,PTG

Key to Table 1 on following page.

Table 1 (continued)

a. Tested in NCI/NTP long-term bioassays in mice and rats of both sexes (Zeiger, 1987).

b. Negative mutagenicity responses in the Ames Salmonella assay reported by Zeiger except allyl isothiocyanate which was reported to yield an equivocal mutagenic response (Zeiger, 1987).

c. Structural alerts for carcinogenic activity reported or, in parentheses, inferred from Ashby and Tennant, 1988.

d. Level of carcinogenic effect reported by Ashby and Tennant, 1988: A- both species; B- 1 species at 2 or more sites; C- 1 species and 1 site; D- 1 sex of 1 species and 1 site. LE's in parentheses not included in analyses by Ashby and Tennant but same criteria applied to results reported by Zeiger, 1987.

e. Tumor sites reported by Ashby and Tennant, 1988: AG- adrenal gland, CS- circulatory system; HS- hematopoietic system; K- kidney; L- liver carcinoma; L*- liver carcinoma and adenoma; L**- liver adenoma; LU- lung; MG-mammary gland; PTG- pituitary gland; SV- seminal vesicle; UB- urinary bladder. General tumor sites reported by Zeiger, 1987, in parentheses: L- liver; O-more than one site; LO- liver and other site(s).

the k_e test yields a correct response for a significant fraction of the NCI/NTP-screened chemicals that are, respectively, "nongenotoxic carcinogens" or "genotoxic noncarcinogens". For the former class of chemicals, 28 of the 50 carcinogens listed in Tables 1 and 2 yield a positive k_e response of which only 4 have a positive SA. Of the 22 carcinogens listed in Table 2 that yield a negative k_e response, 12 have a positive SA. This low concordance between k_e and SA indicates that these two measures of electrophilicity are significantly different which is also apparent from the results presented in Tables 3 and 4 for the genotoxic noncarcinogens. Of the 7 chemicals in this class that yield a negative k_e response (Table 3), 6 have a positive SA; of the 12 noncarcinogens that yield a positive

Table 2. Rodent carcinogens that yield negative responses in the k_e test and negative or equivocal responses in the Ames Salmonella assay; also listed are SA's, LE's and tumor sites which are defined in Table 1.[a]

Chemical	SA	LE	Site[c]
Allyl isovalerate	-	A	HS,LS
Aniline	+	B	CS,MS,SP
Benzene	-	A	12 sites[d]
Chlorendic acid	(-)	(A)	(LO)
3-Chloro-2-methyl propene[b]	(+)	(A)	(U)
5-Chloro-o-toluidine	+	B	CS,L
Cinnamyl anthranilate	-	A	K,L*,P
m-Cresidine	+	C	UB
Dapsone	+	B	IS,SP
Di(2-ethylhexyl)adipate	-	A	L,L*
N,N'-Diethylthiourea	-	C	TG
Dimethyl morpholino-phosphoramidate	+	C	HS
1,4-Dioxane	-	A	L,L*,L**,N
Ethyl acrylate	(+)	(A)	(U)
4,4'-Methylene-bis (N,N'-dimethylaniline)	+	A	L*,TG
Phenazopyridine·HCl[b]	+	A	I/C,L,L*
Piperonyl sulfoxide	-	D	L
Procarbazine·HCl	(+)	(A)	(O)
Succinic acid-2,2-dimethyl-hydrazide	+	D	U
Tris (2-ethylhexyl) phosphate	-	D	L
4-Vinylcyclohexene	(-)	(D)	(U)
2,6-Xylidine[b]	(+)	(B)	(O)

a. Same sources and notations apply as in Table 1 footnotes with following additions.

b. Equivocal Ames Salmonella response reported by Zeiger, 1987.

c. Tumor sites reported by Ashby and Tennant, 1988, and not defined in Table 1 are: I/C- intestine/colon, IS- integumentary system; LS- lymphatic system; MS- multiple organ sites; N- nose; P- pancreas, SP-spleen; TG- thyroid gland; U- uterus. (U) denotes non-liver tumor in single organ reported by Zieger, 1987.

d. Tumor sites reported by Ashby and Tennant, 1988, for benzene are: AG, HS, L*, LU, MG and Harderian gland, ovary, oral cavity, preputial gland, stomach, skin and Zymbal's gland.

Table 3. Chemicals that yield negative responses in the k_e test and positive or equivocal responses in the Ames Salmonella assay; also listed are SA's.[a]

Chemical	SA
L-Ascorbic acid[b]	-
2-Chloroethanol	+
2,4-Dimethyloxyaline·HCl	+
Fenaminosulf	+
HC Blue 2	+
N-(1-Naphthyl)-ethylenediamine·2HCl	+
p-Phenylenediamine·2HCl	+

a. Same sources and notations as in Table 1.

b. Equivocal Ames Salmonella response reported by Zeiger, 1987.

k_e response (Table 4), 4 have a negative SA. This nonconcordance between k_e and SA could be exploited in designing a more predictive carcinogen-screening battery of short-term tests.

The level of effect (LE) and the tumor sites of the carcinogens are included in Tables 1 and 2 to illustrate, respectively, that although k_e correctly identifies a significant number of nongenotoxic carcinogens that induce

Table 4. Chemicals that yield positive responses in the k_e test and positive or equivocal responses in the Ames Salmonella assay; also listed are SA's.[a]

Chemical	SA
Benzoin[b]	-
4'-(chloroacetyl)acetanilide	+
2-(chloromethyl)pyridine•HCl	+
Dimethoate	+
Dioxathion	+
8-Hydroxyquinoline	+
Iodoform	-
Methyl parathion	+
1-Nitronaphthalene	+
4-Nitro-o-phenylenediamine	+
Photodieldrin	-
Tolazamide	-

a. Same sources and notations as Table 1.

b. Equivocal Ames Salmonella response reported by Zeiger, 1987.

tumors in more than one species and/or at more than one site, k_e also fails to identify many trans-species, multiple-site carcinogens. No correlation between the k_e response and LE or tumor site is evident from the information provided in these tables; however, work now in progress should clarify if the same lack of correlation extends to NCI/NTP-screened genotoxic carcinogens as well as establish if the rationale that has been proposed for k_e yielding positive responses to unactivated procarcinogens is valid (Bakale and McCreary, 1987; Bakale, 1988, 1989).

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