DOE/ER/60617--T2

IONIZATION IN LIQUIDS

FINAL TECHNICAL REPORT

November 1, 1993 - December 31, 1995

Case Western Reserve University
School of Medicine
Department of Radiology
Cleveland, Ohio 44106

March 29, 1996

Prepared for

The U. S. Department of Energy
Office of Health and Environmental Research
Washington, D. C. 20585

Grant No. DE-FG02-88ER60617

George Bakale, Ph. D.
Principal Investigator

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

MASTER

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, make any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

**Portions of this document may be illegible
in electronic image products. Images are
produced from the best available original
document.**

TABLE OF CONTENTS

FINANCIAL STATEMENT of ESTIMATED COSTS	1
PERSONNEL	2
EXECUTIVE SUMMARY	3
1993-1995 FINAL REPORT:	
I. <u>Electrons in Biological Systems</u>	5
A. Excess Electrons as Probes of Carcinogen Electrophilicity	5
B. Cost Effectiveness of k_e as a Carcinogen-Screening Test	7
C. Conversion of k_e to a Carcinogen-Screening Electronic Device	9
II. <u>Electron and Ion Transport and Reactions in Model Liquids</u>	9
A. Radiation-induced Dimerization of Fullerenes	9
B. Radiolytic Synthesis of Fullerene Derivatives	10
III. <u>References</u>	12
APPENDIX	15

Bakale, G. et al., (1994) "Pittcon '94 Meeting Report: Analysis of Environmental Biohazards" *American Environmental Laboratory*, 6, No. 6, pp. 1, 6, 8.

Bakale, G., (1994) "A Carcinogen-Screening Test Based on Electrons" in Linking the Gaseous & Condensed Phases of Matter: The Behavior of Slow Electrons, L.G. Christophorou, W.F. Schmidt and E. Illenberger, eds., pp. 561-566 (Plenum, New York).

Henderson, S.J., Hettich, R.L., Compton, R.N. and Bakale, G. (1996) "Small-Angle X-ray Scattering and Mass Spectrometry Studies of γ -Irradiated C_{60} in Cyclohexane", *J. Phys. Chem.*, in press

Bakale, G., (1996; in editorial review) "Predicting Carcinogenicity: The k_e Test" in Testing, Predicting and Interpreting Carcinogenicity K. Kitchin, ed. (Marcel Dekker, New York).

Bakale, G. et al., (in review) " C_{60} and C_{70} Fullerene Ions in Nonpolar Liquids: Mobility and Radiation Chemical Changes", submitted to *J. Phys. Chem.*

Bakale, G. and McCreary, R.D., (in review) "Comparison of k_e Electrophilicity with Ames *Salmonella* Mutagenicity for 90 Coded Chemicals", submitted to *Mutagenesis*

Zeiger, E., Ashby, J., Bakale, G., Enslein, K., Klopman, G. and Rosenkranz, H.S. (in review). "Prediction of *Salmonella* Mutagenicity", submitted to *Mutagenesis*

PERSONNEL

Principal Investigator

G. Bakale, Ph. D.
75 % effort and salary

Pulse-conductivity measurements
(11/93 - 4/94)

R. D. McCreary
75 % effort; 25 % salary

OTHER SUPPORT of P.I. during Report Period

DOE Grant DE-FG02-88ER60658 (11/93 - 12/94)
"Mutagenicity of Radon and Radon Daughters"
H.H. Evans, P.I.

5 % effort and salary

Hahn-Meitner Institut (7-10/94 and 6-9/95)
W.F. Schmidt, P.I.

15 % effort and salary

NIH Grant CA15378-23 (12/95)
"Radiation-Induced Modifications in Protein Synthesis"
N.L. Oleinick, P.I.

60 % effort and salary

EXECUTIVE SUMMARY

The objective of the studies proposed in 1993 was to provide new information on electron and ion transport and reactions in model liquids and biomimetic systems that is pertinent to the roles of charged species in inducing radiobiological damage and to elucidating the inter-relationship among the carcinogenicity, mutagenicity and electrophilicity of chemicals. The concordance between the electron attachment rate constant, k_e , and the carcinogenic properties of chemicals has been the focus of our studies for more than a decade and is viewed as being a step closer to the initiating step of carcinogenesis than are computer-based studies of structure-activity relationships (SARs) that are an accepted method of predicting the carcinogenicity of chemicals. The values of k_e that we measure using a pulse-conductivity technique provide an indication of electron reactivity that SARs can obtain only indirectly, which is illustrated by the development of a SAR method to predict the k_e s of chemicals which are then used in predicting the carcinogenicity of chemicals [e.g. Benigni et al., 1989, 1992, 1996]. Thus, the k_e -carcinogenicity results that we have measured can be regarded as the first step toward the "electron-based toxicity index" that Christophorou suggested be developed 15 years ago [Christophorou, 1981]. Our k_e database includes more than 300 chemicals and provides a firm basis to determine which structural factors influence both k_e and carcinogenicity. This database can be used to prioritize future k_e studies of other solute/solvent systems in order to test the robustness of the electron-based toxicity index that was developed.

Our 1993-1996 progress in the area of using physico-chemical techniques to identify chemical carcinogens is described in five appended papers, the first of which is a meeting report of a symposium entitled "Analysis of Environmental Biohazards" which the P.I. organized for Pittcon '94, the Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, and which was a feature article in *American Environmental Laboratory*. Another paper entitled "A Carcinogen-Screening Test Based on Electrons" is based on an invited lecture presented at the NATO Advanced Study Institute in Patras, Greece entitled *Linking the Gaseous & Condensed Phases of Matter: The Behavior of Slow Electrons*. A third paper is a review entitled "Predicting Carcinogenicity: The k_e Test" which was prepared at the invitation of Dr. K. Kitchin of the U.S. EPA Health Effects Research Laboratory who is editor of the book *Testing, Predicting and Interpreting Carcinogenicity* in which the review will appear. Two other manuscripts, "Prediction of *Salmonella* Mutagenicity" and "Comparison of k_e Test Electrophilicity with Ames *Salmonella* Mutagenicity for 90 Coded Chemicals" were also prepared in the 1993-1995 report period and are currently in the final stages of peer review.

All of these papers complement our earlier DOE-supported studies pertaining to the identification of biohazardous agents and are consistent with the recommendations made by the Galvin Commission for "...a particular need for long term, basic research in disciplines

related to environmental clean-up... (that)... could lead to both reduced cleanup costs and smaller environmental impacts at existing sites and to the development of a scientific foundation for advances in environmental technologies" [Galvin, 1996]. Application of the k_e test to environmental problems is discussed in this FINAL REPORT as is using k_e to develop an electronic monitoring device that could spawn a new generation of hazard-monitoring instrumentation that could be applied to reducing human exposure to biohazards from a myriad of environmental sources. Using k_e to bridge the physico-chemical gap between analytical and microbiological techniques in identifying and prioritizing the biohazard potential of mixtures in a novel and cost-effective manner is also discussed as is the "foundation" on which our carcinogen-screening studies are based. This foundation has been our >20 years of studies in which elucidating fundamental electron and charge transfer processes in model liquids and in biomimetic systems were the primary objectives.

A common thread in all of our studies of the interactions between electrons and chemical carcinogens is the influence of structure in biomimetic media in which biologically critical electron-transfer reactions occur. In our efforts to elucidate the role that structural effects play in these processes, we proposed in 1993 to continue our studies of charge transfer to the uniquely structured fullerenes C_{60} and C_{70} and of the transport properties of the ions that are thus formed. Our studies of these novel molecules involved collaborations with two groups, each of which was ideally suited to characterize the fullerenes through very different but also complementary techniques; one group is at the Hahn-Meitner Institut (HMI) in Berlin with whom we had extensive earlier collaborations and the other is at Oak Ridge National Laboratory (ORNL). The latter group used small-angle x-ray scattering (SAXS) and mass spectrometry (MS) and is currently using small-angle neutron scattering (SANS) to characterize the products that are formed in gamma-irradiated solutions of the fullerenes in several solvents. Two manuscripts submitted to *The Journal of Physical Chemistry* in which the results of these studies are described are appended.

1993-1995 FINAL REPORT

I. Electrons in Biological Systems

A. Excess Electrons as Probes of Carcinogen Electrophilicity

The studies on electron attachment to chemical carcinogens that were proposed in 1993 are based on one of the fundamental tenets of the somatic mutation theory of carcinogenesis which served as the crux of decades of cancer research conducted by J. and E. Miller who demonstrated that electrophilic chemicals react with electron-rich cellular DNA [Miller and Miller, 1971; 1977]. The Millers proposed that electrophile-DNA interaction was the initiating step in the multi-step process of chemical carcinogenesis and that electrophilicity was the one physico-chemical property that is common to most chemical carcinogens. Thus, identification of chemical carcinogens could be effected if a method to identify electrophilic chemicals were found, which also served as the basis for the most widely used short-term carcinogen-screening test (CST), the *Salmonella*/microsome assay developed by B.N. Ames and hereafter referred to as the Ames test [Ames, 1972]. In that assay, histidine-deficient strains of *Salmonella* are exposed to the test chemical; if the chemical or metabolite of the chemical is sufficiently electrophilic to induce a base-pair or frameshift mutation in the DNA of the auxotrophs, reversion to prototrophy occurs and is easily detected by growth of the revertant colonies. The initial success of this assay prompted Ames to proclaim in 1973 that "carcinogens are mutagens" [Ames, 1973], which appeared to be verified in subsequent validation studies in which the Ames test was found to identify carcinogens with an accuracy of 90% [e.g. McCann et al., 1975]. This strong correlation between mammalian carcinogenicity and bacterial mutagenicity was the basis for Bridges' noting in his 1976 review of short-term tests that the DNA of the Ames tester strains could be viewed as a sensitive probe of a chemical's electrophilicity [Bridges, 1976]. This statement, the work of the Millers and the Ames test strongly influenced our two decades of subsequent research in this area out of which the k_e test was developed in our DOE-supported studies that were augmented by support from the National Institute for Occupational Safety and Health (NIOSH), National Institute of Environmental Health Sciences (NIEHS) and the Edison BioTechnology Center of Northeast Ohio. This FINAL REPORT summarizes these 20 years of developing a CST that can be a valuable tool in solving many of the current problems related to environmental health.

In the decade that followed its introduction, the Ames test was used in more than 3,000 laboratories [Ames, 1982] and more than 200 short-term CSTs were developed to identify chemical carcinogens [Ashby, 1989], most of which were intended to complement the Ames test. Among these CSTs was the k_e test, our physico-chemical test that uses electrons rather than electron-rich DNA as a probe of the test chemical's electrophilicity. This test involves measuring the rate constant of electron attachment, k_e , by a pulse-conductivity technique in

which electrons are generated in a nonpolar liquid by a pulse of ionizing radiation. The technique and application of it to screening a variety of chemicals have been reported in numerous papers [e.g. Bakale et al., 1981, 1982; Bakale and McCreary, 1987, 1990a,b; 1992a,b] and summarized in several reviews [e.g. Bakale, 1988, 1989] in addition to the publications that are appended.

The preceding description of the 1975-1985 decade of development of CSTs suggests that the 90% accuracy of the Ames test as a screening tool to identify chemical carcinogens precludes the need for any other CST. In the mid-80's, however, evidence began to accumulate that a significant fraction, 53/123 or 43%, of the chemical carcinogens in a newly reported database are nongenotoxic, i.e. these chemicals yield "false-negative" Ames test responses, whereas 22 of 73 putative noncarcinogens (30%) were found to yield "false-positive" responses in the Ames test [Zeiger, 1987]. This evidence for the existence of significant numbers of nongenotoxic carcinogens and genotoxic noncarcinogens was obtained in long-term, two species (mouse and rat) bioassays that were conducted under the aegis of the National Toxicology Program [NTP] which should provide the least equivocal comparisons of the genotoxic and carcinogenic properties of chemicals that are available. From such comparisons of the first 73 chemicals that were screened in this data base, Tennant et al. concluded that three other in vitro bioassays performed as poorly as the Ames test in identifying chemical carcinogens [Tennant et al., 1987]. Subsequent analyses of the NTP data base [Ashby and Tennant, 1988; Tennant and Ashby, 1991 and references therein] which now includes >350 chemicals yielded the same conclusion.

The preceding summary illustrates only a few of the more salient points of the complex problem of identifying carcinogens via CSTs; additional aspects of which have been discussed in a special issue of *Mutation Research* [Rosenkranz, 1988], in the "Discussion Forum" section of *Mutagenesis*, and in other reviews [e.g. Ashby, 1989]. The need for a STT that complements the Ames test appears to have been underscored through this last decade of intensive and extensive screening of chemical carcinogens, and it appears that an in vitro CST cannot meet this need. This assertion is based on Ashby and Tennant concluding in their survey of 222 NTP-screened chemicals that there is "... a major need for new assays... with which to predict nongenotoxic carcinogens" [Ashby and Tennant, 1988].

In concluding this summary of two decades of CST research, another general method of screening carcinogens is noted, viz. structure-activity relationships (SARs). The SAR that provides the most facile prediction of the carcinogenicity of a chemical is the Structural Alert (SA) method derived by Ashby which is based on the functional groups of the test chemical [Ashby; 1985]. Several other CSTs based on SARs of 100 coded chemicals have recently been shown to be strongly concordant with the Ames test, but the k_e test which is also

included in this study yielded a significantly lower concordance with mutagenesis than the SAR-based tests [Zeiger et al., 1996; see Appendix]. This again reflects that the measure of electrophilicity that k_e provides is significantly different than DNA reactivity and SA, which was first illustrated in our k_e screening of the NTP-tested carcinogens and noncarcinogens that yield contrary or equivocal Ames test responses [Bakale and McCreary, 1990 a,b]. Our rigorous analysis of the latter results illustrated that the k_e test complements not only the Ames test but also SA [Ennever and Bakale, 1992], and this complementarity was recently shown to extend to other CSTs which is described in the appended manuscript now in review [Bakale and McCreary, 1996].

B. Cost Effectiveness of k_e as a Carcinogen-Screening Test

Although the two appended reviews entitled "A Carcinogen-Screening Test Based on Electrons" and "Predicting Carcinogenicity: The k_e Test" should be read for a full appreciation of how k_e can be used most efficaciously to identify chemical carcinogens, the following table from the former review succinctly illustrates that k_e compares favorably with other methods of identifying carcinogens.

TABLE 1. Comparison of key characteristics of rodent bioassays and the Ames *Salmonella* and k_e short-term carcinogen-screening tests.

<u>Characteristic</u>	<u>Rodents</u>	<u>Ames <i>Salmonella</i></u>	<u>k_e test</u>
Cost (US \$)	> 10 ⁶	~ 1,000	~ 400
Time	> 2 years	> 2 days	~ 1 hour
Basis	rodent carcinogens are human carcinogens	bacterial mutagens are carcinogens	electrophiles are carcinogens

Clearly, the k_e test offers cost and time advantages over rodent bioassays and/or the Ames test, but these apparent advantages are of no value if the k_e test does not identify carcinogens as accurately as the other screening methods. To illustrate that the k_e test compares favorably with the Ames test in identifying carcinogens as well as putative noncarcinogens, the responses of the k_e and Ames tests for 282 chemicals are compared in Table 2 using the three measures of the predictivity of short-term tests that are most frequently used to evaluate CSTs. Sensitivity and specificity are the percentages of carcinogens and noncarcinogens, respectively, that a test correctly identifies, and accuracy is a combination of these two criteria. From these results it is apparent that the k_e test compares favorably with the Ames test for all three measures of predictivity, but of greater significance to the value of k_e as a CST is another conclusion that is not evident from the predictivity criteria, which is now discussed.

Table 2. Comparison of three measures of predictivity for the Ames and k_e tests.

<u>Predictivity Criterion</u>	<u>Ames <i>Salmonella</i></u>	<u>k_e test</u>
Sensitivity ¹	84/156; 56%	100/156; 64%
Specificity ²	94/126; 75%	89/126; 71%
Accuracy ³	178/282; 63%	189/282; 67%

¹Number of positive responses obtained/number of carcinogens tested.

²Number of negative responses obtained/number of noncarcinogens tested.

³Number of correct responses obtained/number of chemicals tested.

The point not illustrated by the results in Table 2 is the advantage of combining the Ames and k_e tests together in a battery which significantly increases the sensitivity relative to that of using either test alone. For the 156 carcinogens included in the database of Table 2, the sensitivity of the Ames- k_e battery is 84% whereas the sensitivity of the Ames test is 56%. Also pertinent to carcinogen-screening strategy is that for the 126 noncarcinogens in the same database the k_e test yields only 22 false-positive responses to the 94 nonmutagenic noncarcinogens that were screened. Thus, the 28% increase of the sensitivity of the Ames- k_e battery relative to the Ames test sensitivity more than offsets the 18% decrease of the specificity of the battery relative to that of the Ames test. The value of the trade-off in the increased sensitivity being offset by the decreased specificity in using the Ames- k_e battery would depend upon the screening application which was discussed in our study of the complementary value of k_e [Ennever and Bakale, 1992]. The appended study of the responses of the k_e test and several structure-based CSTs to 100 coded chemicals for which the *Salmonella* mutagenicity responses were determined provides additional insight on those applications where the k_e test can be used most efficaciously in designing a screening battery [Bakale and McCreary, 1996].

The preceding illustration of the sensitivity of the Ames/ k_e test battery being 84% is similar to the value of 82% reported earlier for the same battery for 107 NTP-screened carcinogens [Ennever and Bakale, 1992]. Of greater pertinence to the cost-effectiveness of the k_e test is a comparison of the sensitivities of the Ames/ k_e battery and the mouse bioassay, i.e. the sensitivity of the mouse bioassay that is obtained from the percentage of rat carcinogens for which a mouse bioassay yields a positive response. For the NTP-screened rat carcinogens considered in this work, that percentage is 69% which can be viewed as the upper limit of sensitivity that could be attained with a CST having a surrogate target such as *Salmonella* DNA in the Ames test or freely drifting electrons in the k_e test rather than mouse tissue in the mouse bioassay. The electrons of the k_e test yield a sensitivity of 64% for the same NTP-screened chemicals, which although satisfactory is of less significance than the

result pertaining to the complementary nature of the k_e and Ames tests. This comparison of the sensitivities of the Ames/ k_e battery and the mouse bioassay illustrates that the Ames/ k_e battery identified more rat carcinogens correctly than did the NTP mouse bioassays that cost >\$200 million, which again vividly illustrates the cost effectiveness of the Ames/ k_e test battery.

C. Conversion of the k_e Test to a Carcinogen-Screening Electronic Device

The physico-chemical k_e screening test that was developed in this project provides the basis for a low cost electrophile detector that could serve as a universal monitor of biohazards. This electrophile-sampling device or ESD with its electron-based endpoint would provide a means to link biohazard potential with electronic sampling techniques and thus would be a candidate to be a cost effective means to determine the biohazard potential of samples taken from a myriad of sources ranging from the air we breath and the food we eat to toxic wastes. Moreover, the numerous bioassay-directed analytical protocols that have been developed to identify biohazardous components of complex mixtures from various sources should be compatible with the ESD, and the ESD will also complement these bioassays. Thus, converting the k_e test to an ESD could bridge the physico-chemical gap between the analytical and microbiological techniques that are now used to identify and prioritize the biohazard potential of mixtures. This novel and cost-effective screening device could spawn a new generation of hazard-monitoring instrumentation that could be applied to reducing human exposure to biohazards from a myriad of environmental sources.

II. Model liquids and solutes

A. Radiation-induced dimerization of fullerenes

The rationale for undertaking a study of the icosahedral fullerenes was that a better understanding of fullerene reactivity and transport would complement the k_e -carcinogenicity studies described in the preceding Section by yielding additional insight on factors that influence the reactions of both positive and negative charges in liquids since fullerene serves as an ideal "sink" for both negative and positive charges. In addition, the fullerenes are excellent radical "sponges" and thus should have an extremely interesting radiation chemistry which is illustrated by the dimer that is formed in the γ radiolysis of fullerene-hydrocarbon solutions. Finally, the well characterized geometry of the fullerenes make them ideal candidates for studies of solvent-solvent interactions via the Stokes-Einstein (SE) equation that relates the diffusion coefficient, D , of a particle of radius R to the viscosity, η , of the solvent via

$$D = k_B T / \chi \pi \eta R \quad (1)$$

where T is temperature, k_B is the Boltzmann constant, and χ denotes a factor that depends on the specific model used for the motion of the ion through the solvent. Equation 1 is valid only for $R \gg R_{\text{solvent}}$ with the liquid considered as a continuum and the ion as a large, heavy particle. The constant $\chi = 6$ denotes the non-slip or sticking condition for which no shear occurs between the surface of the tracer and the adjacent liquid; or in other words, a thin layer of solvent is bound to the solute tracer, whereas $\chi = 4$ describes the slip condition in which shear occurs. Despite these constraints, the SE equation was referred to recently as "...a pillar of insight into the molecular dynamics of liquids" [Tarjus and Kivelson, 1995], which is an attribute that we exploited in our study.

The HMI Linac was used to conduct measurements of the fullerene ion mobilities in neopentane, n-pentane, cyclohexane, tridecane and carbon disulfide via the time-of-flight pulse-conductivity technique that we have used >20 years. One-component ion-current decays were generally observed in the fullerene solutions which indicates that ions of only one size are present in the solutions. These ions are assumed to be fullerene anions and cations formed by charge transfer from the solvents due to the higher electron affinity and lower ionization potential, respectively, of the fullerenes relative to the solvents. The results of these mobility studies are described in the appended manuscript entitled " C_{60} and C_{70} Fullerene Ions in Nonpolar Liquids" which is now in review [Bakale et al., 1996]. A table from that manuscript follows from which it is evident that analysis of the data with the SE equation leads to the conclusion that the degree of solvation of the ions in the solvents studied is cyclohexane > neopentane $\approx$ n-pentane > tridecane > carbon disulfide. Other salient conclusions are: (i) fullerene ions serve as model charge-carriers that can be used to probe ion-solvent interactions via the SE equation; (ii) ions of C_{60} and C_{70} solvate hydrocarbons to varying degrees but more so than these ions solvate CS_2 ; (iii) ion formation in the γ -radiolysis of fullerene/hydrocarbon solutions is strongly mediated by the solvent; and (iv) ions formed by γ -radiolysis are a radical-adduct product with a radius that is approximately twofold greater than that of the parent fullerene ion.

B. Radiolytic synthesis of fullerene derivatives

The preceding studies of fullerene ion mobility and especially our discovery of an apparent radiolytic dimerization product triggered efforts to identify that product. Through collaboration with ORNL, SAXS/MS characterization studies of the fullerene radiolytic products were initiated and the results of this collaboration, which are in press, are appended [Henderson et al. 1996]. The fact that radiolytic products isolated at CWRU could be sent to ORNL for characterization suggests that the radical-substituted products formed by radiolysis are sufficiently stable to be used as building blocks in the emerging field of nanotechnology.

TABLE I. Summary of temperature dependences of viscosity, η , and mobility, μ , in $[10^{-4}\text{cm}^2/\text{Vs}]$ and Stokes radii, r , in $\text{\AA}$, measured in fullerene solutions in neo-pentane, n-pentane, c-hexane and tridecane before and after irradiation to 25 krad.

			C₆₀				C₇₀			
			without		with γ -irr.		without		with γ -irr.	
Solvent	T/°C	η /cP	μ	r	μ	r	μ	r	μ	r
neo-pentane	0	0.33	3.6	7.2	2.3	11.3	4.2	6.2	2.6	10.0
	26	0.20	5.0	8.5	3.3	12.9	6.3	6.7	3.8	11.2
<i>Radius($\text{\AA}$)^a</i>				7.8		12.1		6.5		10.6
n-pentane	-78	0.72	1.8	6.6	0.84	14	1.4	8.4	1.0	11.8
	-32	0.37	3.4	6.8	1.9	12.4	3.1	7.4	2.0	11.5
	24	0.21	5.5	7.4	3.4	11.9	5.2	7.8	3.8	10.6
<i>Radius($\text{\AA}$)^a</i>		--		6.9		12.8		7.9		11.3
c-hexane	7	1.3	--		0.52	12.6	0.76	8.6	0.51	12.8
	22	0.93	1.1	8.3	0.67	13.6	1.3	7.0	0.79	11.6
	58	0.54	1.8	8.7	--		1.8	8.7	1.4	11.2
	63	0.51	--		1.22	13.6	--		--	
<i>Radius($\text{\AA}$)^a</i>				8.5		13.3		8.1		11.9
tridecane	0	2.9	0.39	7.5	0.19	15.4	0.50	5.9	0.21	13.9
	23	1.8	0.76	6.2	0.32	14.7	0.84	5.6	0.39	12.1
	58	0.98	1.5	5.8	0.60	14.4	1.3	6.7	0.76	11.4
<i>Radius($\text{\AA}$)^a</i>				6.5		14.8		6.1		12.5
CS₂^b	-78	0.85	2.2	4.5			1.6	6.2		
	0	0.436	4.5	4.3			3.8	5.1		
	26	0.353	5.5	4.4	5.1	4.7	4.8	5.0	4.2	5.7
<i>Radius($\text{\AA}$)^a</i>				4.3		4.7		5.4		5.7

a) Average Stokes radii from all values of mobility measured in each solvent.

b) C₆₀: 7.7 μM ; C₇₀: 7.1 μM

III. References

- Ames, B.N. (1972) "A Bacterial System for Detecting Mutagens and Carcinogens" in *Mutagenic Effects of Environmental Contaminants*, E. Sutton and M. Harris, eds., pp. 57-66, (Academic Press, New York).
- Ames, B.N. (1982) "Mutagens, Carcinogens, and Anti-carcinogens" in *Genetic Toxicology: An Agricultural Perspective*, R. A. Fleck and A. Hollaender, eds., pp. 499-508, (Plenum Press, New York).
- Ames, B.N., Durston, W.E., Yamasaki, E., Lee, F.D. (1973) "Carcinogens Are Mutagens: A Simple Test System Combining Liver Homogenates for Activation and Bacteria for Detection", *Proc. Natl. Acad. Sci. USA*, **70**, 2281-2285.
- Ashby, J. (1985) "Fundamental structural alerts to potential carcinogenicity or noncarcinogenicity", *Environ. Mol. Mutagen.* **7**, 919-921
- Ashby, J. (1989) "Origins of common uncertainties in carcinogen/mutagen screening", *Environ. Mol. Mutagen.* **14**, Suppl. 16, 51-59.
- Ashby, J. and Tennant, R.W. (1991) "Definitive relationships among chemical structure, carcinogenicity and mutagenicity for 301 chemicals tested by the U.S. NTP", *Mutat. Res.*, **257**, 229-306.
- Bakale, G., (1988) "Theoretical Implications of the k_e Carcinogen-Screening Test", in *Chemical Carcinogens*, P. Politzer and L. Roberts, eds., pp. 320-344, (Elsevier, Amsterdam).
- Bakale, G., (1989) "Detection of Mutagens and Carcinogens by Physical Chemical Techniques," in *Hazard Assessment of Chemicals: Current Developments*, J. Saxena, ed., pp. 85-124, (Hemisphere Publishing, Washington, D.C.).
- Bakale, G., (1994) "A Carcinogen-Screening Test Based on Electrons" in *Linking the Gaseous & Condensed Phases of Matter: The Behavior of Slow Electrons*, L.G. Christophorou, W.F. Schmidt and E. Illenberger, eds., pp. 561-566 (Plenum, New York). (See APPENDIX).
- Bakale, G., (1996; in editorial review) "Predicting Carcinogenicity: The k_e Test" in *Testing, Predicting and Interpreting Carcinogenicity* K. Kitchin, ed. (Marcel Dekker, New York). (see APPENDIX)
- Bakale, G., Gundel, L., Hawthorne, S., Lewtas, J. and Markell, C. (1994) "Pittcon '94 Meeting Report: Analysis of Environmental Biohazards" *American Environmental Laboratory*, **6**, No. 6, pp. 1, 6, 8 (see APPENDIX).
- Bakale, G., Lacmann, K. and Schmidt, W.F. (1996, in review) " C_{60} and C_{70} Fullerene Ions in Nonpolar Liquids: Mobility and Radiation Chemical Changes", submitted *J. Phys. Chem.* (see APPENDIX)

- Bakale, G. and McCreary, R.D., (1987) "A Physico-Chemical Screening Test for Chemical Carcinogens: The k_e Test," *Carcinogenesis* **8**, 253-264.
- Bakale, G., and McCreary, R.D., (1990a) "Response of the k_e Test to NCI/NTP-Screened Chemicals. I. Nongenotoxic Carcinogens and Genotoxic Noncarcinogens", *Carcinogenesis* **11**, 1811-1818.
- Bakale, G., and McCreary, R.D., (1990b), "Electrophilicity of Nongenotoxic Carcinogens and Genotoxic Carcinogens as Measured by the k_e Test", in *Mutation and the Environment*, M.L. Mendelsohn and Albertini, R.J., eds., pp. 355-365, (Wiley-Liss, New York).
- Bakale, G. and McCreary, R.D., (1992a) "Response of the k_e Test to NCI/NTP-Screened Chemicals. II. Genotoxic Carcinogens and Nongenotoxic Noncarcinogens", *Carcinogenesis* **13**, 1437-1445.
- Bakale, G. and McCreary, R.D., (1992b) "Prospective k_e Screening of Potential Carcinogens Being Tested in Rodent Bioassays by the NTP", *Mutagenesis*, 7, 91-94.
- Bakale, G. and McCreary, R.D., (1996, in review) "Comparison of k_e Electrophilicity with Ames *Salmonella* Mutagenicity for 90 Coded Chemicals", *Mutagenesis*, (see APPENDIX).
- Bakale, G., McCreary, R.D. and Gregg, E.C. (1981) "Quasifree Electron Attachment to Carcinogens in Liquid Cyclohexane", *Cancer Biochem. Biophys.* **5**, 103-109.
- Bakale, G., McCreary, R.D. and Gregg, E.C. (1982) "Quasifree-Electron Attachment to Carcinogens", *Int. J. Quantum Chem.: Quantum Biol. Symp.* **9**, 15-25.
- Bridges, B.A., (1976) "Short-term screening tests for carcinogens", *Nature* **261**, 195-200.
- Benigni, R., Andreoli, C. and Giuliani, A., (1989) "Structure-Activity Studies of Chemical Carcinogens: Use of an Electrophilic Reactivity Parameter in a New QSAR Model", *Carcinogenesis* **10**, 55-61.
- Benigni, R., Cotta-Romusino, M., Andreoli, C. and Giuliani, A., (1992) "Electrophilicity As Measured by k_e : Molecular Determinants, Relationship with Other Physical Chemical and Quantum Mechanical Parameters, and Ability to Predict Rodent Carcinogenicity", *Carcinogenesis*, **13**, 547-553.
- Benigni, R. and Andreolli, C. (1996, in review) "Prediction of rodent carcinogenicity of further 30 chemicals bioassayed by the US National Toxicology Program", *Mutagenesis*.
- Christophorou, L.G. (1980), "Negative Ions of Polyatomic Molecules", *Environ. Health Persp.* **36**, 3-32.
- Ennever, F.K. and Bakale, G. (1992) "Response of the k_e test to NCI/NTP-Screened Chemicals. III. Complementary Value of k_e in Screening Carcinogens", *Carcinogenesis* **13**, 2059-22065.
- Galvin, (1996), from "Alternative Futures for the Energy National Laboratories" cited in *Federal Register* **61**, No. 28, 4975-4978.

- Henderson, S.J., Hettich, R.L., Compton, R.N. and Bakale, G. (1996) "Small-Angle X-Ray Scattering and Mass Spectrometry Studies of Gamma-Irradiated C₆₀ in Cyclohexane", *J. Phys. Chem.* (in press; see APPENDIX).
- McCann, J., Yamasaki, E. and Ames, B.N., (1975) "Detection of Carcinogens as Mutagens in the *Salmonella*/microsome Test: Assay of 300 Chemicals", *Proc. Natl. Acad. Sci. USA*, **72**, 5135-5139.
- Miller, E.C. and Miller, J.A. (1971) "The Mutagenicity of Chemical Carcinogens: Correlations, Problems, and Interpretations" in *Chemical Mutagens--Principles and Methods for Their Detection*, A. Hollaender, ed. pp. 83-119 (Plenum, New York).
- Miller, J.A. and Miller, E.C. (1977) "Ultimate Chemical Carcinogens as Reactive Mutagenic Electrophiles" in *Origins of Human Cancer, Book B*, H.H., Hiatt, J.D. Watson and J.A. Winsten, eds., pp. 605-628 (Cold Spring Harbor Lab).
- Tarjus, G.; Kivelson, D. (1995) "Breakdown of the Stokes-Einstein Relation in Supercooled Liquids", *J. Chem. Phys.*, **103**, 3071-3073).
- Tennant, R.W. and Ashby, J. (1991) "Classification according to Chemical Structure, Mutagenicity to *Salmonella* and Level of Carcinogenicity of a further 39 Chemicals Tested by the U.S. NTP", *Mutat. Res.*, **257**, 209-228.
- Zeiger, E., Ashby, J., Bakale, G., Enslein, K., Klopman, G. and Rosenkranz, H.S. (1996, in review). "Prediction of *Salmonella* Mutagenicity", submitted to *Mutagenesis*, (see APPENDIX).

APPENDIX

Bakale, G., (1994) "A Carcinogen-Screening Test Based on Electrons" in Linking the Gaseous & Condensed Phases of Matter: The Behavior of Slow Electrons, L.G. Christophorou, W.F. Schmidt and E. Illenberger, eds., pp. 561-566 (Plenum, New York).

Bakale, G., Gundel, L., Hawthorne, S., Lewtas, J. and Markell, C. (1994) "Pittcon '94 Meeting Report: Analysis of Environmental Biohazards" *American Environmental Laboratory*, 6, No. 6, pp. 1, 6, 8.

Henderson, S.J., Hettich, R.L., Compton, R.N. and Bakale, G. (1996) "Small-Angle X-Ray Scattering and Mass Spectrometry Studies of Gamma-Irradiated C₆₀ in Cyclohexane", *J. Phys. Chem.* (in press).

Bakale, G., (1996; in editorial review) "Predicting Carcinogenicity: The k_e Test" in Testing, Predicting and Interpreting Carcinogenicity K. Kitchin, ed. (Marcel Dekker, New York).

Bakale, G., Lacmann, K. and Schmidt, W.F. (1996, in review) "C₆₀ and C₇₀ Fullerene Ions in Nonpolar Liquids: Mobility and Radiation Chemical Changes", submitted to *J. Phys. Chem.*

Bakale, G. and McCreary, R.D., (1996, in review) "Comparison of k_e Electrophilicity with Ames *Salmonella* Mutagenicity for 90 Coded Chemicals", submitted to *Mutagenesis*.

Zeiger, E., Ashby, J., Bakale, G., Enslein, K., Klopman, G. and Rosenkranz, H.S. (1996, in review). "Prediction of *Salmonella* Mutagenicity", submitted to *Mutagenesis*.

See below

all removed for separate processing.