III. PROGRESS REPORT 1991-92 AND PROPOSED RESEARCH PROGRAM 1992-93

A. Model Liquids

1. Electron Transport and Reactions

Studies of the influence of solvent composition on electron mobility, μ_e, which we reported for mixtures of neopentane (NP) and tetramethysilane (TMS) [Bakale and Schmidt, 1990] were extended to mixtures of TMS with isooctane (i-octane) or cyclohexane (c-hexane). Whereas our initial TMS/NP study focused on an electron transport regime in which μ_e varied only from 67 cm^2/Vs in NP to 100 cm^2/Vs in TMS, the more recent studies extended to values of μ_e of 7.5 and 0.22 cm^2/Vs in i-octane and c-hexane, respectively. Both of these studies were conducted in collaboration with Dr. W. F. Schmidt at theHahn-Meitner Institut (HMI) in Berlin, and we were joined in the more recent study by Dr. Lacmann who is also at HMI.

Whereas a linear dependence of $\log \mu_e$ on solvent composition had been found in earlier studies of electron transport in mixtures [e.g. Minday et al., 1972], a negative deviation from this dependence was found in TMS/NP mixtures [Bakale and Schmidt, 1990]. In our more recent studies, this negative dependence was found to be enhanced in TMS/i-octane mixtures which is illustrated in Figure 1a. In contrast, a *positive* deviation from linearity was observed in TMS/c-hexane mixtures which is illustrated in Figure 1b. Despite the markedly different dependences of μ_e on solvent composition for these mixtures, the observed dependences are consistent with the percolation model of electron transport that Schiller has developed [e.g. Schiller et al., 1982]. The application of Schiller's model of electron transport via percolation to these mixtures which is illustrated in Figure 1 is discussed in a manuscript that has been submitted to ***Physical Review Letters*** [Bakale et al., 1992b] and is appended.

In mixtures of TMS with both c-hexane and i-octane, deviations from Schiller's model were noted at the highest concentrations of hydrocarbons in TMS that were studied (see Fig.1). On the P.I.'s return to HMI in July and August, 1992, a more detailed study of the dependence of μ_e on composition in the low-μ_e regions of both mixtures will be conducted in order to determine if the observed deviation is artifactual or is a manifestation of a breakdown of the percolation model in media in which electrons are highly localized. When this point is clarified, a more comprehensive manuscript will be prepared and submitted to the ***Journal of Chemical Physics.***

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FIGURE 1a. Dependence of electron mobility in i-octane/TMS mixtures (squares) on composition. Solid and dashed lines are fit to percolation model with n = 1.5 and 1.0, respectively.

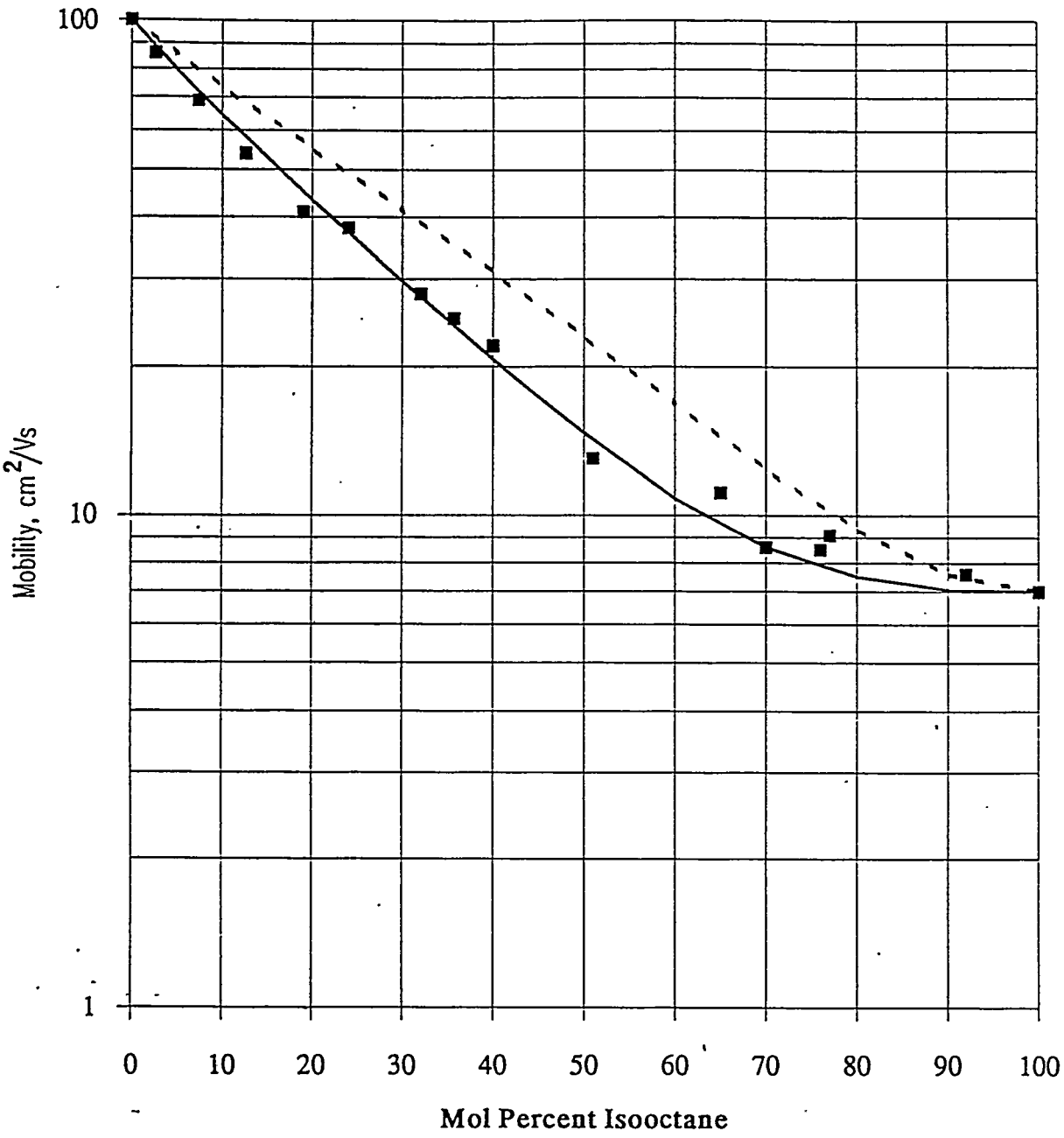

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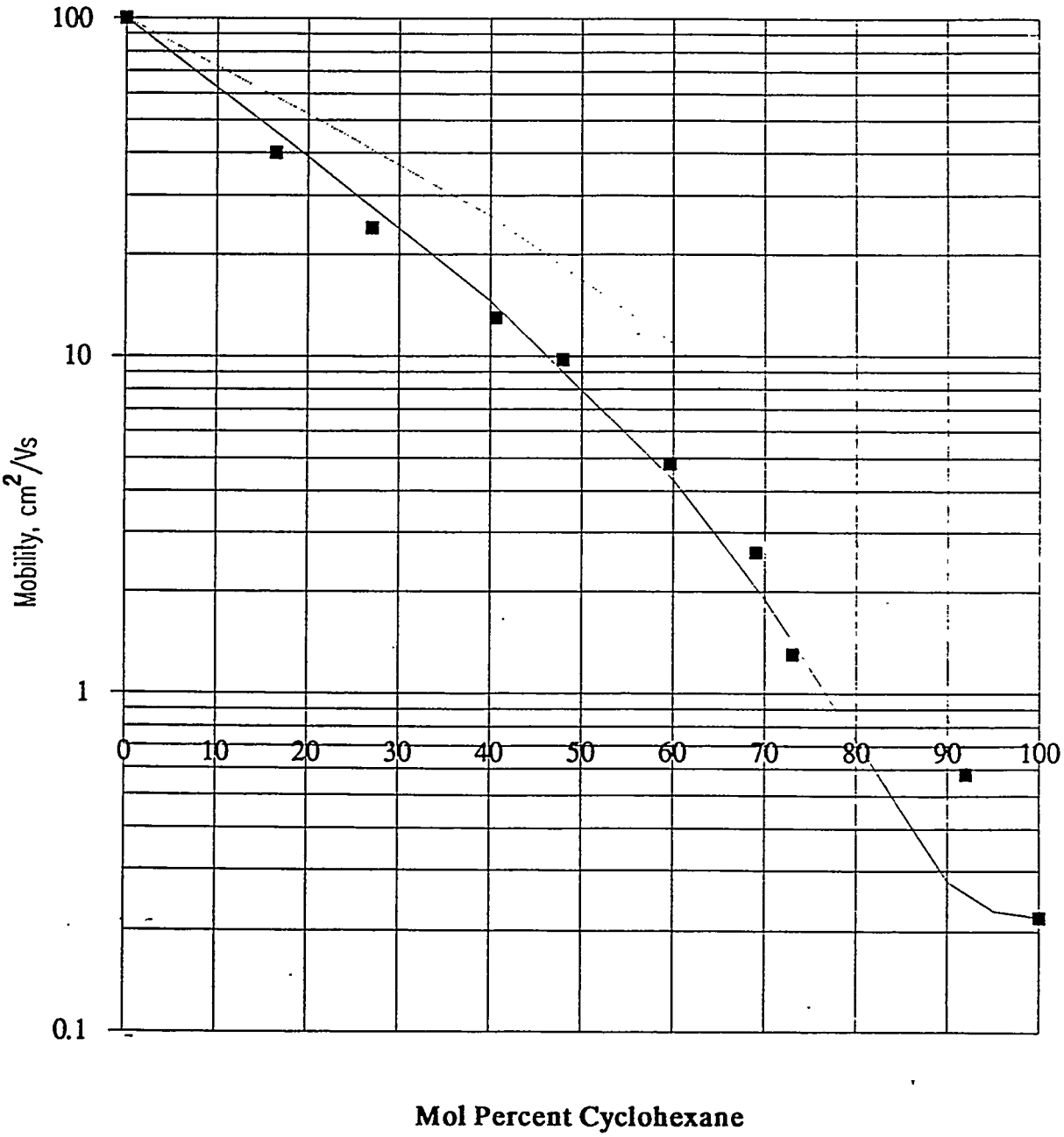
FIGURE 1b. Dependence of electron mobility in c-hexane/TMS mixtures (squares) on composition. Solid and dashed lines are fit to percolation model with n = 1.5 and 1.0, respectively.



Establishing the dependence of μ_e on composition in these mixtures will also permit us to analyze the dependence of the electron attachment rate constant, k_e, on composition in the same mixtures. Values of the k_es of ethyl bromide and carbon tetrachloride in these TMS mixtures have already been measured over the entire composition range by the P.I. in an earlier collaboration with Dr. G. Beck of the HMI. Analysis and publication of these electron-scavenging results, however, had to be deferred until the dependence of μ_e on composition was determined. It was, in fact, the scavenging results that suggested a nonlinear dependence of $\log \mu_e$ on composition in these mixtures which prompted the P.I. to propose that the μ_e dependence on composition be studied [Bakale, 1990].

Another study that is planned for the P.I.'s 1992 visit to HMI is measurement of the k_e and mobility of one of the most intensively studied molecules, buckminsterfullerene or C_{60} [Koshland, 1991]. This molecule is readily soluble in nonpolar solvents, and the k_es of C_{60} will be measured in several solvents including c-hexane, i-octane and TMS using C_{60} that Dr. Schmidt has already obtained. Measurements of the mobility of the C_{60} attachment product, presumably C_{60}^-, will also be attempted.

2. Ion Conductivity

The digitized ion-current monitoring system (ICMS) that was described in our 1990-91 Progress Report (pp.9-10) was used extensively to determine the operating characteristics of the system in order to use the ICMS to characterize charge transport in biomimetic systems *(vide infra)*. Data collection was improved by the routine averaging of multiple (3-9) ion current decays in the 0.05-2.0 ms time window that the ICMS samples. This averaging as well as integration of the areas under the ion decays and extrapolation of the decays to 5-10 ms was done using features of the Microsoft Excel software that is used for data collection in conjunction with an IBM PS/2-70.

An example of the improved signal/noise ratio obtained by averaging is illustrated in Figure 2 for ion current decay in pure octane after the ICMS recovers from the electronic "spike" from excess electrons in the sub-microsecond time regime. The electron spike as well as the sensitivity of the ion current to common impurities is illustrated in Figure 3 in which the effects of air and carbon dioxide on the ion current decay are shown. Each of the four decays in this figure is an average of six pulses. The enhanced ion current in the air-saturated solution is a manifestation of the conversion of electrons to O_2^- and the product anion's contributing to the current observed in the millisecond time regime. The dependence of this enhancement of the ion current on the

Figure 2. Comparison of scatter of six ion decays with average (solid line) for isooctane at 10 kV/cm

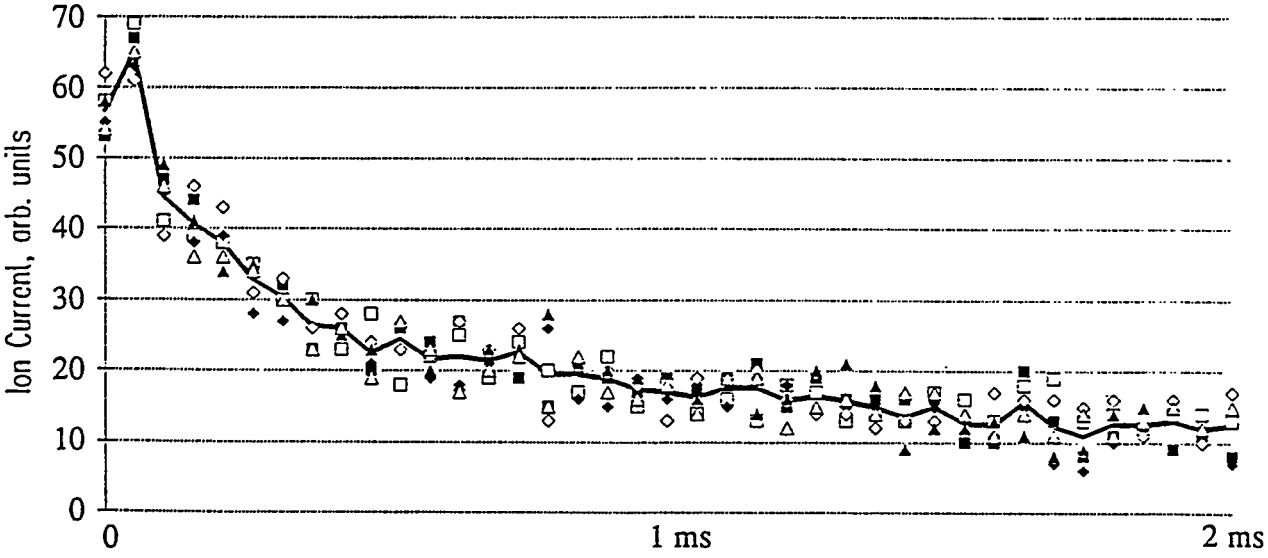
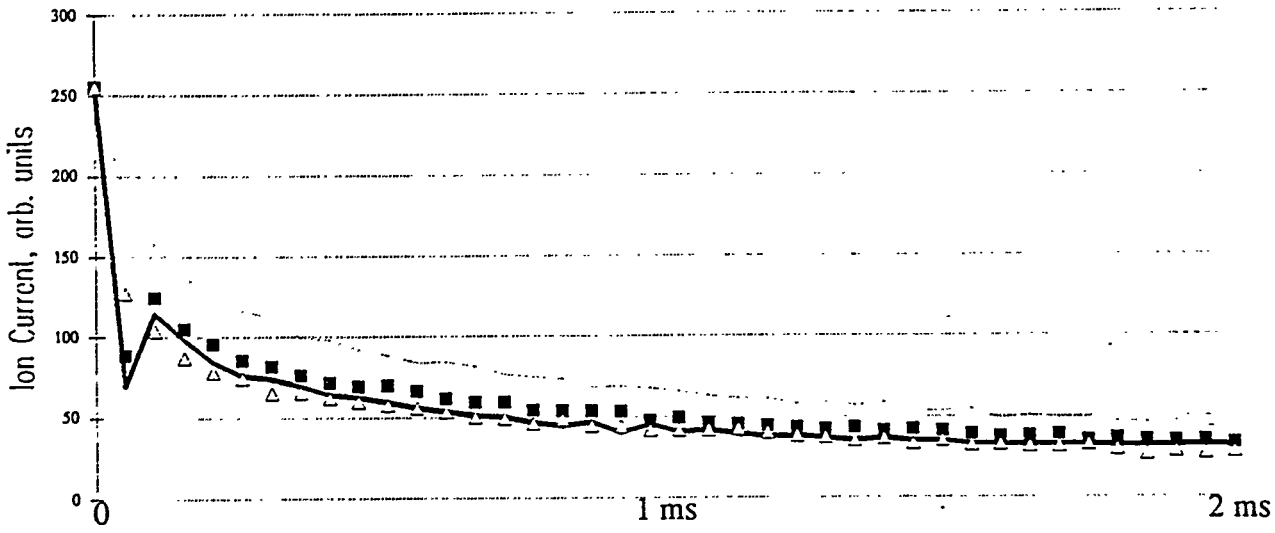


Figure 3. Comparison of ion decays in isooctane freshly degassed (solid line), air-saturated (dashed line), redagassed (squares) and carbon dioxide-saturated (triangles) at 30 kV/cm



concentration of electron scavenger is shown in Figure 4 for biphenyl. The area increase that is plotted is obtained by integration of the ion decay and thus can be measured in Coulombs. The final data manipulation that is demonstrated is the extrapolation of the ion decay in i-octane scavenged with SF_6 to obtain the drift time of the ions, which for the example illustrated in Figure 5 is poorly resolved. Through modification of the software of the ICMS, extension of the data collection window to the 5 to 10 ms is feasible. This modification would greatly improve the quality of the data collected in this wider time window, and funds are requested to effect this modification. The application of the data collection techniques demonstrated in Figures 2-5 is now extended to biomimetic systems.

B. Biomimetic Systems

The manuscript entitled "Dynamics of Electron Attachment to AOT/H_2O Reversed Micelles" was published in the ***Journal of Physical Chemistry*** [Bakale et al. 1992a] and a reprint is appended. Several of the observations and novel concepts that were presented in this paper are expected to elicit comment from kineticists and micellar chemists. Examples are the observed values of k_e appearing to exceed the diffusion-controlled attachment limit (Figure 3, p. 2331 of reprint) and the proposed alignment of the ellipsoidal micelles by the external electric field contributing to this observation (Fig. 4, p. 2332 of reprint). Additional information on the dynamics and kinetics of electron attachment to large spherical particles having a diameter >25Å should be obtained from the proposed study of electron attachment to fullarenes which was alluded to at the conclusion of section III. A.1.

Application of the ICMS described in III.A.2 to studying ion transport in biomimetic systems was initiated in the reversed micellar system that we best understood, *viz.*, AOT/H_2O/isooctane. The presence of water in the system limits the electric field that can be applied to the slightly polar medium. As illustrated in Figure 6, however, a sufficient field can be applied to the AOT/H_2O/i-octane system to induce ion currents that are about half the value of ion currents observed in pure i-octane at significantly greater fields. An attempt was made to enhance the binding of the water in the pool by adding a polar solute to the system that would induce additional structuring of the water in the pool and thereby decrease the ion conductivity. However, as illustrated in Figure 7, p-nitrophenol appeared to have a negligible effect on the ion current in AOT/H_2O/i-octane. Additional studies of this nature in which known "structure-makers" are added to the micellar system will be conducted, and the contribution of hydrated electrons serving as "structure-breakers"[Han and Bartels, 1991] will be considered.

Figure 4. Dependence of Percent Area Increase on Biphenyl Concentration

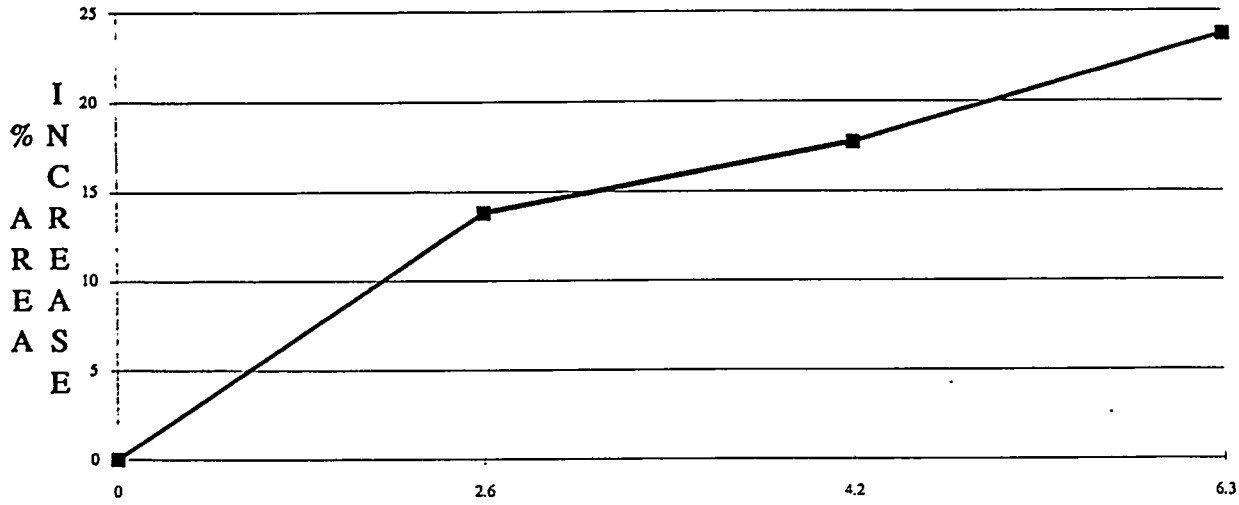


Figure 5. Comparison of decays of ion currents in pure (squares) and SF -saturated (x's) c-hexane and extrapolations of each to 5 ms

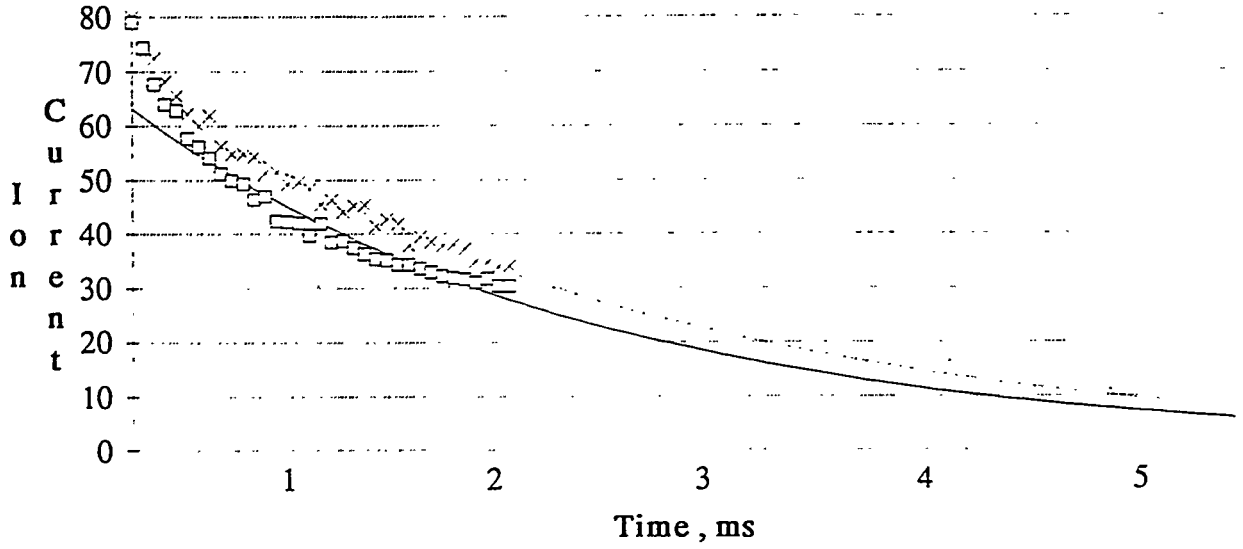


Figure 6. Dependence of 0.1 mM AOT (x's) and 0.25 mM AOT/water (squares) on the ion current decay in i-octane (line) at 10 kV/cm

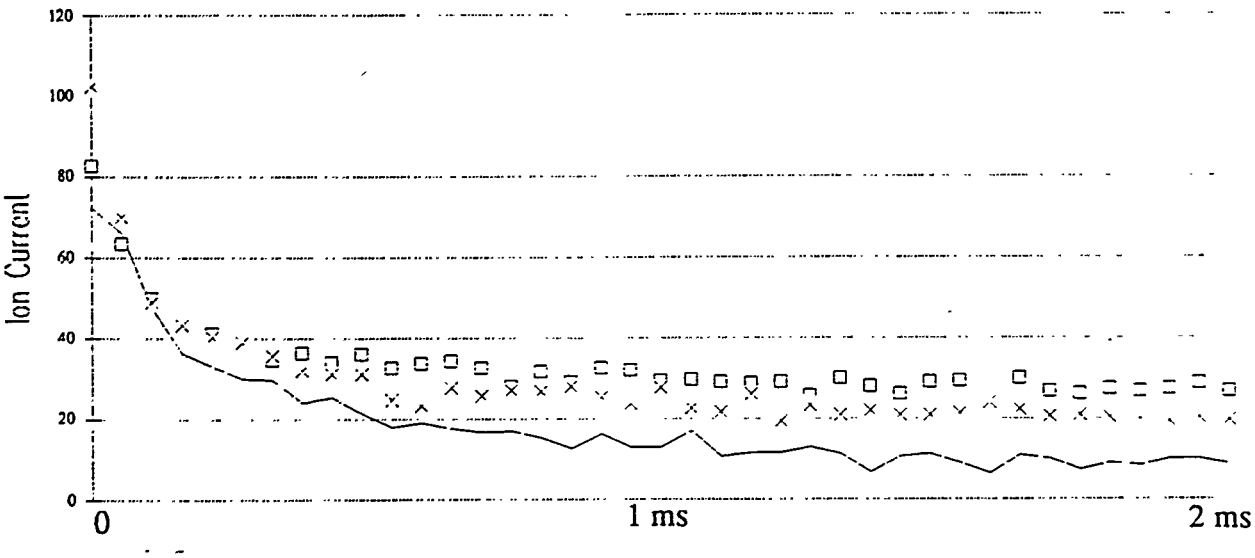
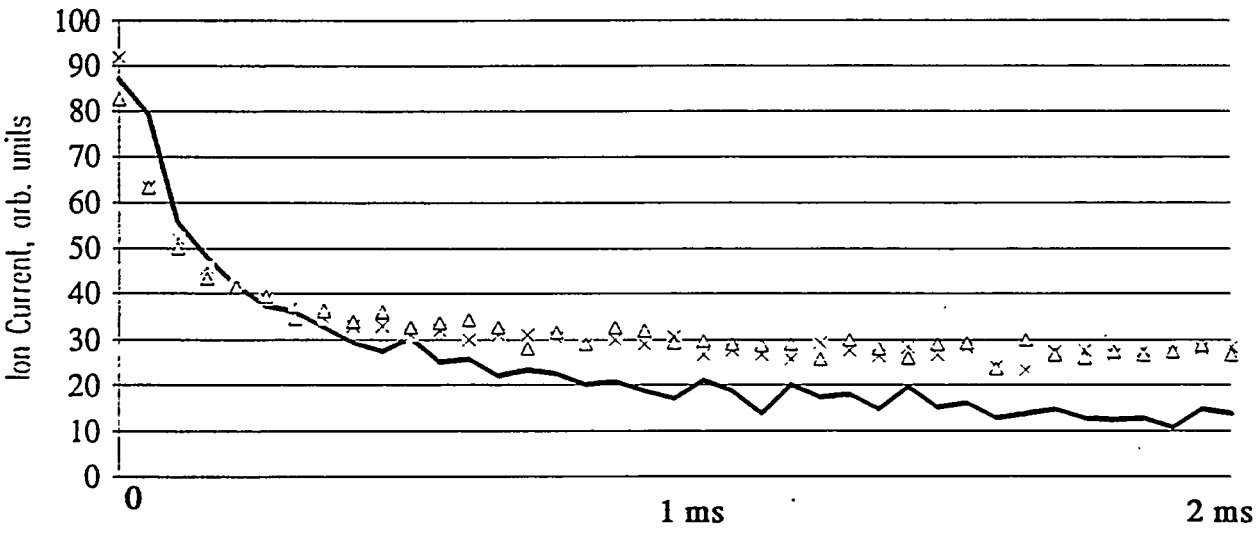


Figure 7. Effect of AOT/water with (triangles) and without p-nitrophenol (x's) on ion current decay in i-octane at 10 kV/cm (solid line)



Application of the AOT/H_2O/i-octane system to encapsulating a biomolecule was done using a method that was recently reported [Leodidis and Hatton, 1990]. With this technique, Leodidis and Hatton rigorously characterized a series of encapsulated amino acids of which leucine was chosen for our initial studies. The ion current decay in the leucine/AOT/H_2O reversed micelles in i-octane is illustrated in Figure 8 from where it is evident that leucine has a negligible effect on the ion current decay relative to that observed in AOT/H_2O reversed micelles (cf. Fig. 6). Also, extrapolation of the ion decay to zero to evaluate t_d yields a mobility of approximately 10^{-3} cm^2/Vs which seems a reasonable value for an ion having a molecular weight of 131. Although these cursory studies suggest that monitoring ionic conductivity in biomimetic systems with the ICMS is feasible, it is also evident that much additional work must be done to interpret the results that are obtained. Our efforts will be focused in this direction in the third year of DOE funding of this research as the effort directed toward carcinogens further declines.

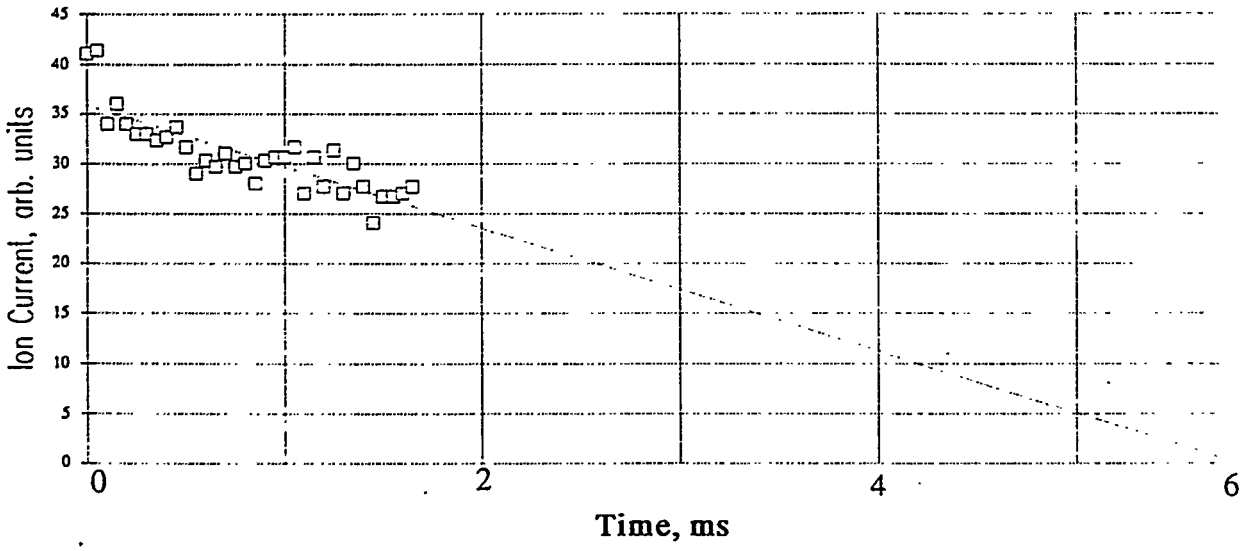
C. Biological Systems

As in 1990-1991, the primary focus in this area was to report the results of our earlier studies in which we measured the k_es of chemicals having carcinogenic or mutagenic properties that have been established or are currently being tested in studies conducted under the aegis of National Toxicology Program (NTP). Consequently, no new studies of the k_es of carcinogens were initiated, and the following studies were reported

1. Published: A reprint in which our prospective k_e screening of 44 chemicals currently being tested in rodent bioassays by the NTP is appended [Bakale and McCreary, 1992 a]. These results are compared with the responses of four other short-term tests (STTs) that were used to screen the same chemicals. The accuracy of the predictions of the k_e and other STTs will be known when the results of the NTP rodent bioassays are released in October, 1992. It is also noted that a fifth STT was used to predict the carcinogenicity of the same 44 chemicals, and the technique that was used for these predictions is based on k_es that were calculated using a quantitative structure-activity relationship (QSAR) [Benigni, 1991]. Additional information on the QSAR technique is cited at the conclusion of this section.

2. In Press: A preprint of another k_e study of carcinogens which complements one of our earlier carcinogen-screening studies [Bakale and McCreary, 1990] is also appended [Bakale and McCreary, 1992b]. In this more recent study which will appear in the June issue of ***Carcinogenesis,*** the k_es of 61 carcinogens and 44 putative noncarcinogens are reported. Diffusion-controlled k_es were measured for 36 or 59 percent of the carcinogens screened, and 29 or 66 % of the noncarcinogens yield k_es that are less than diffusion-controlled. Although these

Figure 8. Extrapolation of ion current decay (squares) in leucine/AOT/water/i-octane solution with E = 10 kV/cm.



values of the k_e test sensitivity and specificity, respectively, may appear to be indicative of a very low accuracy of k_e as a measure of carcinogenicity, we demonstrated that these results when combined with other STTs enhance the predictivity of the screening battery to 80-90%. This aspect of k_e complementing other STTs in the identification of carcinogens is rigorously addressed in another manuscript that is currently under review [Ennever and Bakale, *vide infra*]. More pertinent to our elucidating the interaction of electrons and carcinogens, however, was our finding that electrons in cyclohexane are much less discriminating in attaching to carcinogens than are the electron-rich targets of strains of *Salmonella* that are routinely used as a carcinogen-screening STT. This observation is consistent with the pre-chemical electron-transfer process that was proposed earlier as the initiating step of carcinogenesis. [Bakale and McCreary, 1987; Bakale, 1988].

3. In Review: The manuscript alluded to above in which the value of using k_e in conjunction with other STTs is assessed is also appended [Ennever and Bakale, in review]. This study was done in collaboration with Dr. F.K. Ennever of Wake Forest's Bowman Gray School of Medicine who has published extensively in the area of maximizing the predictivity of batteries of carcinogen-screening tests [e.g. Ennever and Rosenkranz, 1988; Ennever, 1990]. The degree to which k_e enhances the predictivity of the two most widely used STTs is summarized in Table IV of that manuscript (p.25), and the same results are illustrated in Figures 1 and 2 which conclude the manuscript.

In Preparation: Three additional manuscripts pertaining to the k_es of carcinogens or mutagens are at various stages of completion. A brief summary of the current status of each of these follows. It is anticipated that all three of the manuscripts will be submitted for publication in the 1991-1992 grant period.

(a) "Response of the k_e test to NCI/NTP-screened chemicals. IV. Equivocal carcinogens; "G. Bakale R.D. McCreary and F.K.Ennever, to be submitted to ***Carcinogenesis***. As is evident from the title, this is the fourth and concluding paper in our series of k_e studies of NCI/NTP-screened chemicals. The Introduction to the current draft of the manuscript is appended to indicate the scope of this study.

(b) "Prediction of *Salmonella* mutagenicity," E. Zeiger, J. Ashby, G. Bakale, K. Enslein, G. Klopman, and H.S. Rosenkranz, to be submitted to ***Mutagenesis***. The six co-authors of this paper represent six different institutions who collaborated in this inter-laboratory study that was designed by Dr. E. Zeiger of the National Institute of Environmental Health Sciences. The objective of this study was to determine the degree to which the responses of four different

physico-chemical screening tests are correlated with the Ames *Salmonella* test which provides a measure of the bacterial mutagenicity of chemicals. The Introduction of the most recent draft of the manuscript is appended.

(c) "Comparison of k_e test electrophilicity with Ames *Salmonella* mutagenicity for 90 coded chemicals, "G. Bakale and R.D. McCreary, to be submitted to ***Mutagenesis*** with the overview paper by Zeiger et al. and three additional accompanying manuscripts by the developers of the three other carcinogen-screening STTs. As is evident from the appended draft of the manuscript, few revisions to this manuscript are anticipated.

This series of papers combined with the collaboration with Dr. Ennever should persuade many who are skeptical of a role for electron-transfer in the initiating step of carcinogenesis to reassess their opinions. The recent work of Benigni et al. that was alluded to in Section III. C.1 should provide additional incentive for this reassessment. In a recent study, Benigni combined k_es that were calculated using a QSAR technique with another physico-chemical STT to predict the carcinogenicity of the chemicals currently being studied by the NTP [Benigni, 1991]. More recently, Benigni et al. used the same methodology to predict the rodent carcinogenicity of 206 chemicals [Benigni et al., 1992]. These predictions were compared with those of other STTs from which Benigni et al. concluded "... the k_e system performed better than the other systems...a chemical with a k_e higher than 3.0×10^{12} $M^{-1}s^{-1}$ has nearly 80% probability of being a carcinogen." More significantly, the interdependence that Benigni's study found among k_e, the energy of the lowest unoccupied molecular orbital, the absolute electronegativity and the carcinogenicity of chemicals should prompt others to explore this interrelationship, which was one of our objectives in initiating these studies.

D. References

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. (1990) "Ionization in Liquids: Proposed Technical Program 1990-1993," submitted to DOE.

Bakale, G., Beck, G. and Thomas, J.K. (1992a) "Dynamics of Electron Attachment to AOT/H_2O Reversed Micelles", J. Phys. Chem.**96**. 2328-2334. (see Appendix).

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Bakale, G., and McCreary, R.D. (1990) "Response of the k_e Test to NCI/NTP-Screened Chemicals. I. Nongenotoxic Carcinogens and Gentotoxic Noncarcinogens", Carcinogenesis **11**, 1811-1818.

Bakale, G. and McCreary, R.D. (1992a) "Prospective k_e Screening of Potential Carcinogens Being Tested in Rodent Bioassays by the NTP", Mutagenesis **7**,91-94. (see APPENDIX).

Bakale, G. and McCreary, R.D. (1992b) "Response of the k_e Test to NCI/NTP-Screened Chemicals. II. Genotoxic Carcinogens and Nongenotoxic Noncarcinogens" Carcinogenesis (in press; see APPENDIX).

Bakale, G. and Schmidt, W.F. (1990) "Mobility of Excess Electrons in Mixtures of Neopentane and Tetramethylsilane", Chem. Phys. Lett. **175**, 319-321.

Benigni, R. (1991) "QSAR Prediction of Rodent Carcinogenicity for a Set of Chemicals Currently Bioassayed by the NTP" Mutagenesis **6**, 423-425.

Benigni, R., Cotta-Ramusino, M., Andreoli, C. and Guiliani, 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, in press.

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Hann, P. and Bartels (1991) "On the Hydrated Electron as a Structure- Breaking Ion," J. Phys. Chem. **95**, 5367-5370.

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Minday, R.M., Schmidt, L.D. and Davis, H.T. (1972) "Mobility of Excess Electrons in Liquid Hydrocarbon Mixtures." J. Phys. Chem. **76**, 442-446.

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Zeiger, E. (1987) Carcinogenicity of Mutagens: Predictive Capability of the *Salmonella* Mutagenesis Assay for Rodent Carcinogenicity Cancer Res. **47**, 1287-1296.

Zeiger, E., Ashby, J., Bakale, G., Enslein, K., Klopman, G., McCreary, R.D., and Rosenkranz, H.S. (1992) Prediction of *Salmonella* mutagenicity, presented at the Environmental Mutagen Society Annual Meeting, Orlando, FL, April 6-11, 1991 and to be submitted to ***Mutat. Res.*** (see Appendix).

IV. ACTIVITIES PERTINENT TO THIS PROJECT

I. Publications (In addition to those listed in Appendix on next page)

Jostes, R.F., Hui, T.E., James, A.C., Cross, F.T., Schwartz, J.L., Rotmensch, J., Atcher, R.W., Evans, H.H., Mencl, J., Bakale, G. and Rao, P.S. (1991) *In Vitro* Exposure of Mammalian cells to Radon. Radiat. Res. 127, 211-219.

Schwartz, J.L., Rotmensch, J., Atcher, R.W., Jostes, R.F., Cross, F.T., Hui, T.E., Chen, D., Carpenter, S., Evans, H.H., Mencl, J., Bakale, G. and Rao, P.S. (1992) Interlaboratory Comparison of Different /Radon Sources: Cell Survival and RBE. Health Phys. 62, 458-461.

II. Presentations

Bakale, G., Rao, P.S., Mencl, J., Evans, H.H., Nygaard, O.F., "A radon generator/delivery system for radiobiological studies, "presented at the 40th Annual Meeting of the Radiation Research Society, Salt Lake City, UT, March 14-18, 1992.

Bakale, G., "Using excess electrons to identify electrophilic carcinogens,"Procter and Gamble Miami Valley Laboratory, Cincinnati, Ohio, April 1, 1

Table I. Chemicals reported as mutagenic carcinogens or nonmutagenic noncarcinogens by Zeiger (12) which could not be screened with the k_e test.

Chemical (Source)[a]	CASRN[b]	Comment[c]
A. Mutagenic Carcinogens		
Basic Red 9 HCl (Si)	569-61-9	Insoluble
3-Cloro-2-methylpropene (TK)[d]	563-47-3	Volatile
D & C Red 9 (Ra)	5160-02-1	Insoluble
2,4-Diaminoanisole SO_4 (Ra)	39156-41-7	Insoluble
Diglycidyl resorcinol ether	101-90-6	Unavailable
Direct Black (CS)	1937-37-7	Insoluble
Direct Brown 95 (Ra)	16071-86-6	Insoluble
1.2-Propylene oxide	75-56-9	Volatile
Ziram (Ra)	137-30-4	Insoluble
B. Nonmutagenic Noncarcinogens		
EDTA 3Na (Fl)	150-38-9	Insoluble
Pigment Yellow (Ra)	6358-85-6	Insoluble
Sodium diethyldithiocarbonate (CS)	148-18-5	Insoluble
Titanium dioxide (PB)	13463-67-7	Insoluble
Witch Hazel (Ra)	68916-39-2	Complex mixture[e]

Legend:

a. Sources of chemicals for which k_e screening was attempted are: CS, Chem Service; Fl, Fluka; PB, Pfaltz and Bauer; Ra, Radian; Si, Sigma; and TK, Tokyo Kasei.

b. Chemical Abstracts Service registry number

c. Details of solubility, volatility and availability provided in text.

d. Classification of mutagenic response as "equivocal" by Zeiger (12) revised to "positive" by Ashby et al. (15).

e. Complex mixture having unspecified concentrations of active components.

Table II. Electron attachment rate constants, k_es, structural alerts of carcinogenic activity, S/As, and levels of carcinogenic effect, LEs, of NCI/NTP-tested rodent carcinogens reported by Zeiger (12) to yield positive responses in the Ames *Salmonella* test.

Chemical (Source[a])	CASRN[b]	k_e[c]	S/A[d]	LE[d,e]
$k_e > 3.0 \times 10^{12} M^{-1}s^{-1}$				
2-Aminoanthraquinone (TK)	117-79-3	3.9	+	A
2-Aminobiphenyl (Al)	90-41-5	3.8	+	D
1-Amino-2-methylanthraquinone (Ra)	82-28-0	4.2	+	A
4-amino-2-nitrophenol (Al)	119-34-6	4.2	+	D
2-Amino-5-nitrothiazole (Si)	121-66-4	3.3	+	D
Chlorodibromomethane (Ra)[f]	124-48-1	3.2	+[g]	D[g]
Cupferron (Ra)	135-20-6	3.3	+	A
1,2-Dibromo-3-chloropropane (CS)	96-12-8	3.7	+	A
1,2-Dibromoethane (Al)	106-93-4	3.6	+	A
1,2-Dichloroethane (CS)	107-06-2	5.1	+	A
2,6-Dichloro-p-phenylenediamine (Si)	609-20-1	3.4	+	C
1,2-Dichloropropane (CS)	78-87-5	3.5	+[g]	C[g]
1,3-Dichloropropene(CS)	542-75-6	3.4	+	A
Dichlorvos (Ra)[g]	62-73-7	3.3	+	A
Dimethoxybenzidine-4,4'diisocyanate (PB)	91-93-0	4.4	+	B
2,4-Dinitrotoluene (CS)	121-14-2	4.7	+	B
Disperse Yellow 3 (Si)	2832-40-8	3.3	+	A
Lasiocarpine (Ra)	303-34-4	3.1	—[h]	B[i]
2-Methyl-1-nitroanthraquinone (Ra)	129-15-7	4.2	+	A
Michler's Ketone (CS)	90-94-8	4.6	+	A
1,5-Naphthalenediamine (PB)	2243-62-1	3.3	+	A
Nithiazide (Ra)	139-94-6	3.2	+	A
5-Nitroacenaphthene (Al)	602-87-9	5.2	+	A
3-Nitro-p-acetophenetide (Ra)	1777-84-0	3.4	+	D
5-Nitro-o-anisidine (Si)	99-59-2	5.5	+	A
Nitrofen (Ra)	1836-75-5	5.6	+	A
2-Nitro-p-phenylenediamine (CS)	5307-14-2	3.5	+	D

Table II (continued)

Chemical (Source[a])	CASRN[b]	k_e[c]	S/A[d]	LE[d,e]
p-Nitrosodiphenylamine (PB)	156-10-5	5.3	+	A
5-Nitro-o-toluidine (CS)	99-55-8	6.0	+	B
p-Quinone dioxime (PB)	105-11-3	3.1	+	D
Solvent Yellow 14 (Si)	842-07-9	4.1	+[g]	C[g]
Sulfallate (CS)	95-06	5.0	+	A
2,4-Toluene diisocyanate (Si)[j]	584-84-9	5.5	+[g]	A[g]
Toxaphene (CS)	8001-35-2	4.8	+	C
Trifluralin (Ra)	1582-09-8	3.6	+	B
Tris(2,3-dibromopropylphospate) (Al)	126-72-7	4.1	+	A
$k_e < 3.0 \times 10^{12}$				
3-Amino-4-ethoxyacetanilide (Ra)	17026-81-2	0.7	+	D
3-Amino-9-ethylcarbazole (Si)	6109-97-3	2.5	+	A
o-Anisidine (CS)	90-04-0	0.1	+	A
Azobenzene (CS)	103-33-3	1.6	+	B
Bis (2-chloro-1-methylethyl) ether (Ra)	108-60-1	1.0	+	B
3-Chloromethyl pyridine HCl (PB)	6959-48-4	1.9	+	A
4-Chloro-m-phenylenediamine (PB)	5131-60-2	1.0	+	A
4-Chloro-o-phenylenediamine (CS)	95-83-0	0.9	+	A
Cytembena (Ra)	21739-91-3	0.1	—[h]	B[i]
p-Cresidine (PB)	120-71-8	0.1	+	A
2,4-Diaminotoluene (PB)	95-80-7	0.2	+	A
Dimethyl hydrogenphosphite (PB)	868-85-9	0.1	+	B
Direct Blue 6 (PB)	2602-46-2	0.4	+[h]	B[i]
Disperse Blue 1 (Al)	2475-45-8	0.6	+	C
HC Blue 1 (Ra)	2784-94-3	1.9	+	A
Hydrazobenzene (Al)	122-66-7	0.6	+	A
4,4'-Methylenedianiline 2HCl (CS)	13552-44-8	0.1	+	A
6-Nitrobenzimidazole (PB/TK)	94-52-0	2.3	+	C
4,4'-Oxydianiline (Si)	101-80-4	0.2	+	A
Phenoxybenzamine HCl (TK)	63-92-3	1.1	+[h]	A[i]
Pivalolactone (Ra)	1955-45-9	0.2	+	C

Table II (continued)

Chemical (Source[a])	CASRN[b]	k_e[c]	S/A[d]	LE[d,e]
4,4'-Thiodianiline (Al)	139-65-1	0.5	+	A
o-Toluidine (CS)	95-53-4	0.03	+	A
2,4,5-Trimethylaniline (CS)	137-17-7	0.01	+	A
2,6-Xylidine (PB)[k]	87-62-7	0.02	+[h]	B[i]

Legend:

a. As Table I with the addition of Al, Aldrich.

b. As Table I.

c. Units of k_e are $10^{12}M^{-1}s^{-1}$.

d. Ashby and Tennant, 1988 (13) unless denoted otherwise.

e. Levels of carcinogenic effect are: A, Carcinogenic to both rats and mice at one or more sites: B, carcinogenic to one species at two or more sites: C, carcinogenic to both sexes of one species at one site; D, carcinogenic to one sex of one species at one site (13).

f. Mutagenicity or carcinogenicity revised by Ashby et al., 1989 (15).

g. Ashby et al., 1989 (15).

h. Assigned using criteria of Ashby and Tennant, 1991 (14).

i. Assigned using criteria of Ashby and Tennant, 1991 (14) applied to results reported by Zeiger, 1987 (12).

j. Both 2,4- and 2,6- isomers (80 and 20%, respectively) were tested in NCI/NTP bioassays.

k. Classified as mutagenic based on "weak positive" response reported by Zeiger et al., 1988 (17).

Table III. Electron attachment rate constants, k_es, and structural alerts of carcinogenic activity, S/As, of NCI/NTP-tested chemicals classified as noncarcinogens and reported by Zeiger (12) to yield negative mutagenicity responses in the Ames *Salmonella* assay.

Chemical (Source)[a]	CASRN[b]	k_e[c]	S/A[d]
$k_e < 3.0 \times 10^{12}\ M^{-1}s^{-1}$			
Acid Orange 10 (Al)	1936-15-8	0.2	+[e]
Acid Red 14 (Al)	3567-69-9	0.3	—
Aldicarb (CS)	116-06-3	1.5	—
o-Anthranilic acid (CS)	118-92-3	0.9[f]	—
Butylated hydroxytoluene (FS)	128-37-0	0.01[f]	—
Caprolactam (Si)	105-60-2	0.01	—
2-Chloroethyltrimethyl-ammonium chloride (Al)	999-81-5	0.01	—
Chlorpropamide (Ra)	94-20-2	2.5	—
N,N'-Dicyclohexylthiourea (PB)	1212-29-9	1.0	—
Dodecyl alcohol, ethoxylated (Si)[g]	9002-92-0	0.01	—[h]
Ephedrine sulfate (CS)	134-72-5	0.2	—
Ethionamide (Si)	536-33-4	2.6[g]	—
Geranyl acetate (PB)	105-87-3	0.3	—
Lithocholic acid (CS)	434-13-9	0.3	—
Malathion (CS)	121-75-5	2.9[f]	+
D-Mannitol (Si)	69-65-8	0.3	—
d,l-Menthol (Si)	15356-70-4	0.1	—
Pentachloronitrobenzene (CS)	82-68-8	2.6	—
Phenformin HCl (Ra)	834-28-6	0.1	—
Phenol (CS)	108-95-2	0.01	—
N-Phenyl-p-phenylenediamine (CS)	101-54-2	0.1	+
1-Phenyl-2-thiourea (CS)	103-85-5	1.7	—
Phthalamide (PB)	88-96-0	1.9	—
Phthalic anhydride (CS)	85-44-9	1.3	—
Piperonyl butoxide (Si)	51-03-6	0.1[f]	—
Sulfisoxazole (PB)	127-69-5	1.2	+
3-Sulfolene (Si)	77-79-2	2.3	—

Table III (continued)

Chemical (Source)[b]	CASRN[c]	k_e[d]	S/A[e]
Tolbutamide (CS)	64-77-7	2.5	—
Triphenyltin hydroxide (TK)	76-87-9	2.3	—
$k_e \geq 3.0 \times 10^{12} M^{-1}s^{-1}$			
Acetohexamide (Ra)	968-81-0	3.0	—
Anilazine (Si)	101-05-3	4.6	—
Benzoin (CS)	119-53-9	4.5	—
3-Chloro-p-toluidine (CS)	95-74-9	3.7	+
Coumaphos (CS)	56-72-4	4.1	+
Diazinon(CS)	333-41-5	3.5	+
o-Dichlorobenzene (Al)	95-50-1	3.8	—
Endrin (CS)	72-20-8	4.7	—
Lindane (EK)[j]	58-89-9	4.7	—
Malaoxon (Ra)	1634-78-2	3.4	+
Methoxychlor (CS)	72-43-5	4.5	—
1-Phenyl-3-methyl-5-pyrazolone (PB)	89-25-8	3.2	—
2,3,5,6-Tetrachloro-4-nitroanisole (Ra)	2438-88-2	4.3	—
Tetraethylthiurium disulfide (PB)	97-77-8	5.0	—
Vinylidene chloride (CS)	75-35-4	5.3	—

Legend:

a. As Tables I and II with addition of EK, Eastman Kodak and FS, Fisher Scientific

b,c, and d: As Table II

e. Ashby and Tennant, 1991 (14)

f. Bakale and McCreary, 1987 (7)

g. Polymer with average molecular weight of 600.

h. S/A assigned using criteria of Ashby and Tennant, 1991 (14).

i. Assuming molecular weight < 14,700

j. Mixture of conformational isomers of hexachlorocyclohexane.

Table IV. Comparison of overall sensitivity and specificity of k_e and Ames *Salmonella* tests to 171 NCI/NTP-screened chemicals reported by Zeiger (12).

A. Sensitivity to rodent carcinogens

k_e	Mutagens	Nonmutagens	Total
+	36[a]	27[b]	63
−	25[a]	19[b]	44
Total	61	46	107

Sensitivity: k_e (+) = (63/107) x 100% = 59%

Ames (+) = (61/107) x 100% = 57%

B. Specificity to putative noncarcinogens

k_e	Nonmutagens	Mutagens	Total
−	29[a]	8[b]	37
+	15[a]	12[b]	27
Total	44	20	64

Specificity: k_e (−) = (37/64) x 100% = 58%

Ames (−) = (44/64) x 100% = 69%

Legend

a. This work

b. Bakale and McCreary, 1990 (Reference 9).

Figure Legends

Fig. 1 Histograms of the distributions of positive and negative responses of the Ames *Salmonella* and k_e tests for chemicals having the number of positive S/As indicated in each of the following groups: (a) aromatic amino/nitro-type chemicals, (b) natural electrophiles, and (c) miscellaneous groups of structurally-alerting chemicals. The distribution of LEs for each type of response is also indicated and ranges from A-D for rodent carcinogens,which are defined in **footnote e** of Table I, to F which denotes "putative noncarcinogens".

Fig. 2 Histograms analogous to Figure 1 for chemicals having negative S/As and divided into the following groups: (a) having a non-reactive halogen, (b) devoid of an electrophilic center, and (c) with "minor concerns" of electrophilicity. The distribution of LEs is again demonstrated as in Figure 1.

Fig. 3 Histograms of distribution of responses of S/A, *Salmonella* mutagenicity and k_e test to mouse-liver carcinogens classified by Ashby and Tennant as 1) "non-genotoxic" M-L specific, 2) "genotoxic" M-L specific, 3) "non-genotoxic" trans-species/ M-L specific, 4) "genotoxic" trans-species M-L specific, 5) multiple-site because of M-L activity and 6) other carcinogens also M-L active (14).

Fig. 4 Histogram of the distribution of positive k_e responses per number of screened chemicals that are active at the five target sites indicated. The Zymbal's gland and lung are sensitive primarily to genotoxic carcinogens and have positive S/As whereas carcinogenesis at the other three sites occurs primarily via nongenotoxic mechanisms and S/As are negative.

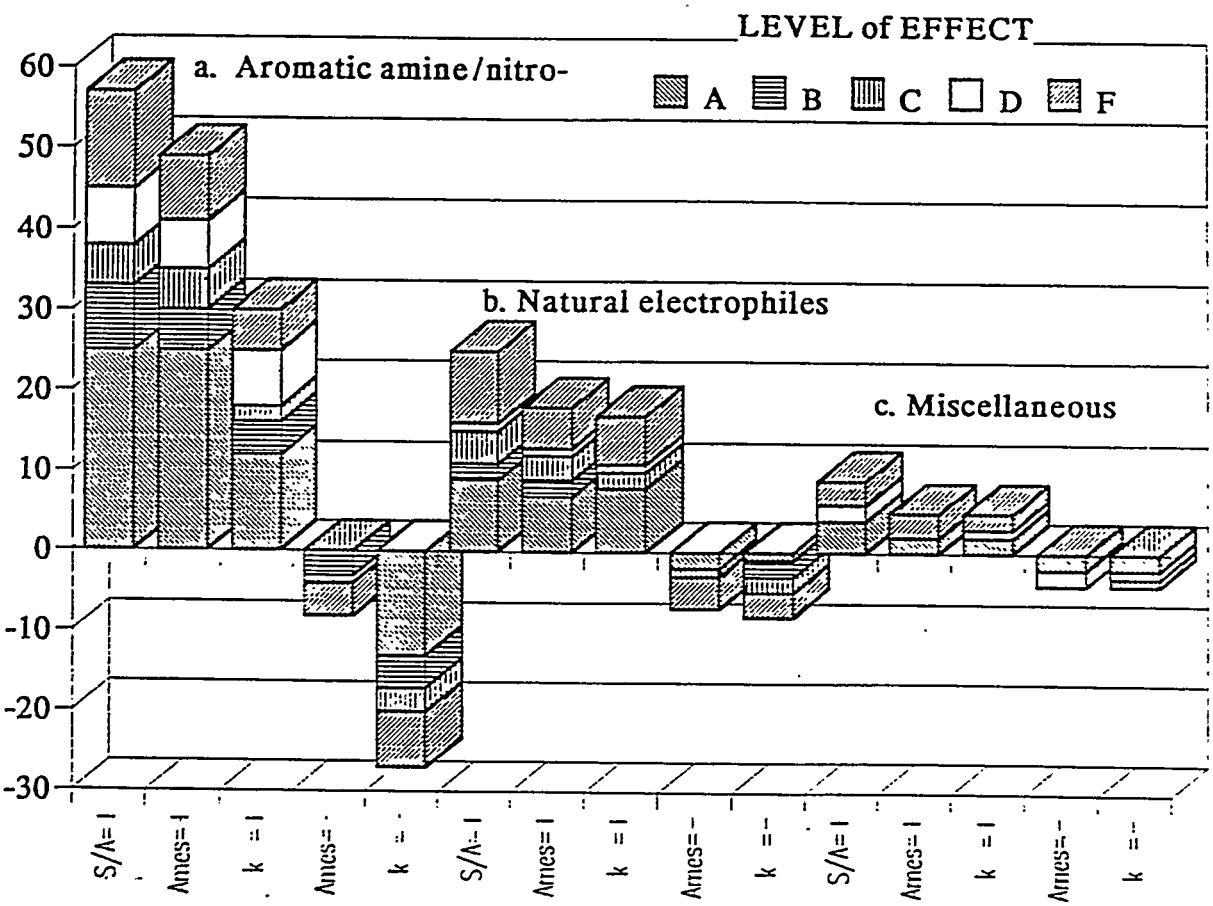
LEVEL of EFFECT
a. Aromatic amine/nitro-
A
B
C
D
F
b. Natural electrophiles
c. Miscellaneous
60
50
40
30
20
10
0
-10
-20
-30

Figure 1.

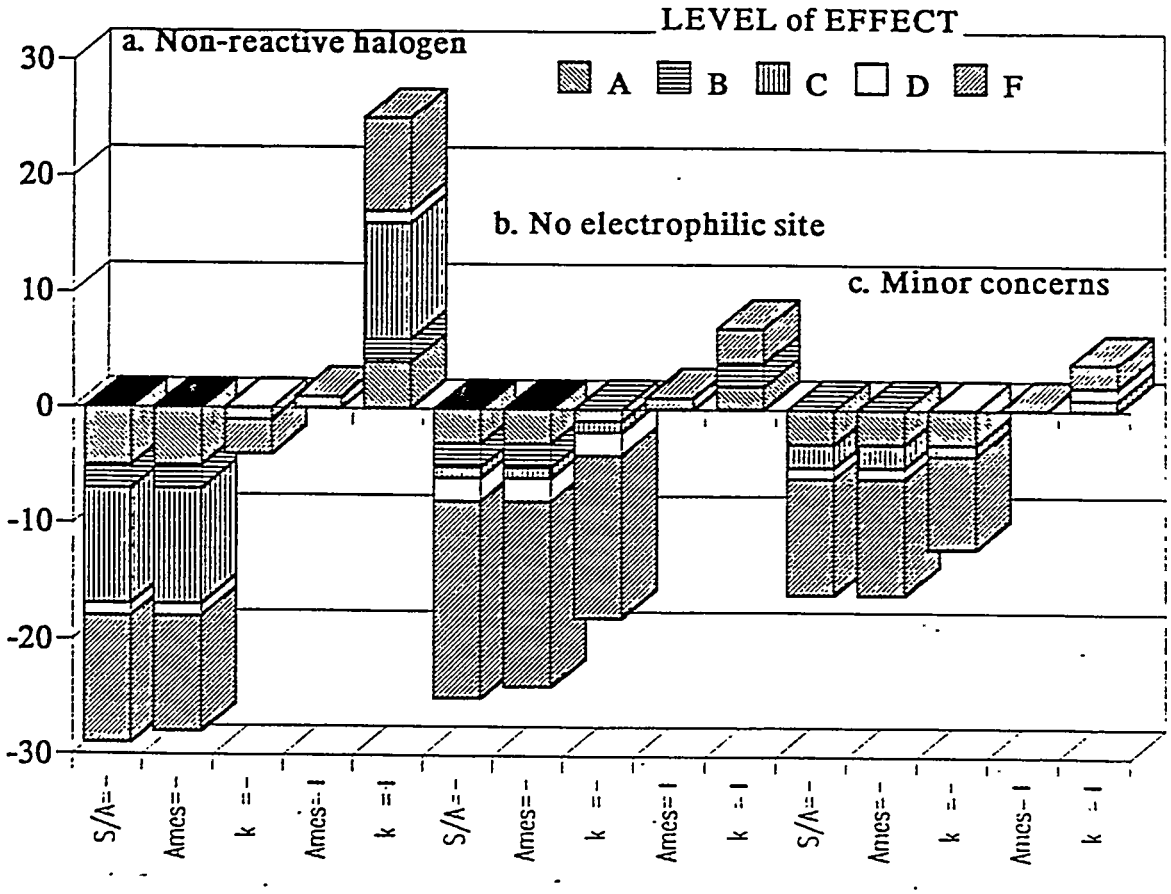
LEVEL of EFFECT
A
B
C
D
F
a. Non-reactive halogen
b. No electrophilic site
c. Minor concerns
30
20
10
0
-10
-20
-30

Figure 2

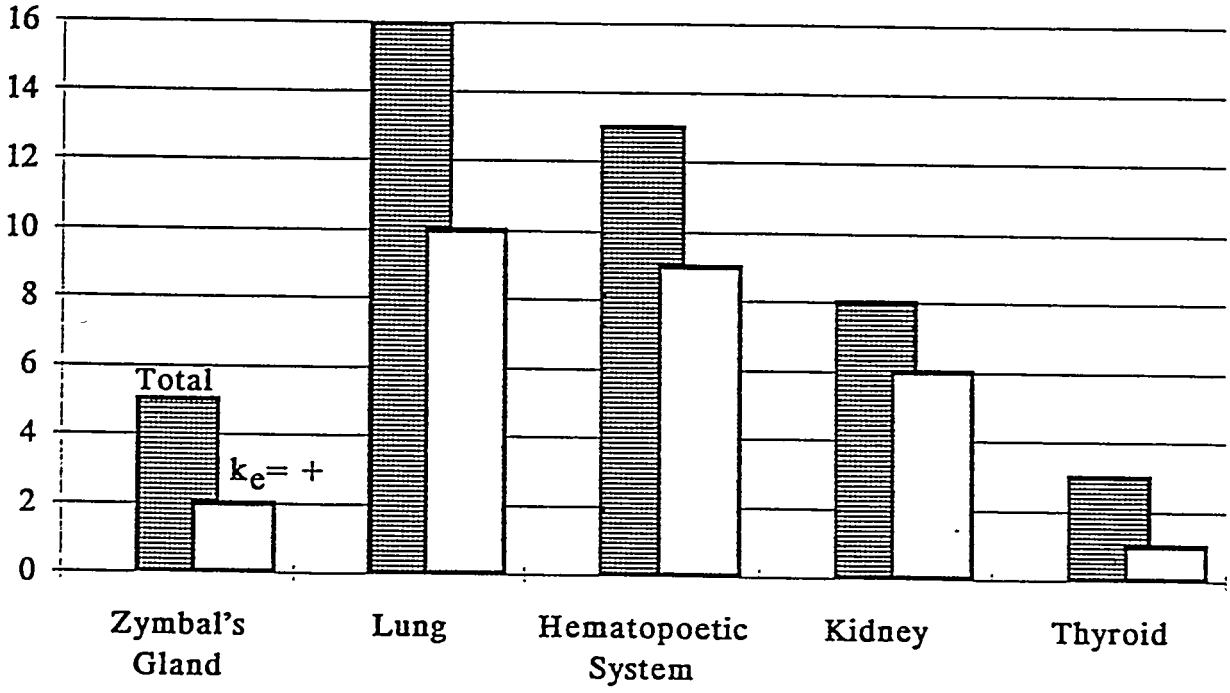
16
14
12
10
8
6
4
2
0
Total
k_e= +
Zymbal's Gland
Lung
Hematopoetic System
Kidney
Thyroid

Figure 4