DOE/ER/60617-7

IONIZATION IN LIQUIDS

PROGRESS REPORT

November 1, 1990 - October 31, 1993

RECEIVED
FEB 01 1995
OSTI

Principal Investigator: George Bakale, Ph. D.

Case Western Reserve University
Department of Radiology
Cleveland, Ohio 44106

April 30, 1993

Prepared for

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

Grant No. DE-FG02-88ER60617

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, makes 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.

MASTER

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

DT

DISCLAIMER

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

TABLE OF CONTENTS

PERSONNEL and DIVISION OF SUPPORT	1
PRESENT STATE OF KNOWLEDGE	2
SUMMARY	4
PROGRESS REPORT: 1990 - 1993	
I. <u>Introduction</u>	7
II. <u>Electron and Ion Transport and Reactions in Model Liquids</u>	
A. Electron Transport and Reaction in Mixtures	7
B. Mobility and Radii of Fullerene Ions	8
C. Radiation-Induced Reactions of Fullerene Ions	11
III. <u>Electrons and Ions in Biomimetic Systems</u>	11
IV. <u>Electrons in Biological Systems: k_es of Chemical Carcinogens</u>	13
V. <u>Radon</u>	19
VI. <u>References</u>	19
APPENDIX CONTENTS	
I. Publications, Abstracts and Manuscripts in Preparation	i
II. Activities of P.I. Pertinent to "Ionization in Liquids" Project	iii

PRESENT STATE OF KNOWLEDGE

The 1990-1993 report period can be viewed as a time of transition in which a new dimension was brought into chemistry by buckminster fullerene, the 60-carbon atom icosahedron that resembles a soccer ball or the geodesic domes of Buckminster Fuller. This "new dimension", the third, will be exploited in the nanoscale technology that is emerging in which the superconducting properties of fullerene's doped analogs will play a key role. Our studies of electron transport and reactions in model liquids has been applied to probing several physico-chemical properties of C_{60} . The unexpectedly high chemical reactivity of fullerene and its expected high physical stability combine to yield unique building blocks with which novel products can be synthesized that range from molecular electronic devices to diamond-like materials. The charge-accepting properties of fullerene combined with fullerene's being regarded as a "radical sponge" present to radiation physicists, chemists and biologists a new tool that can be applied in their respective fields. We have gained some familiarity with this tool and look forward to seeing how we can apply this experience in the forthcoming renewal period.

Another "tool" with which we are familiar and which has been available for decades is the reversed micelle which offers the opportunity to encapsulate polar components in a biomimetic membrane that is solubilized in a nonpolar solvent. The compartmentalization possibilities that this tool presents to assemble pseudo-biological systems that serve as biological models has been under-utilized in radiation physics, chemistry and biology. We also have experience with this tool and plan to use it more extensively in the next renewal period.

The pendulum of scientific change has recently swung back to the area on which we have focused our efforts for more than a decade. The development of a carcinogen-screening test that is based on the only physico-chemical property that is common to most carcinogens, electrophilicity, has been our major contribution to the areas of environmental health science and genetic toxicology. The test that we have developed is based on k_e , the rate constant of electron attachment, which we measure in a nonpolar solvent using a pulse-conductivity technique. Our publications and presentations in this area have stimulated others to invite us to participate in inter-laboratory

blind screenings of chemicals, and the results that were obtained in one of these studies will soon be presented at an international workshop on identifying rodent carcinogens which is sponsored by the National Institute of Environmental Health Science. In a presentation at the most recent national meeting of the American Chemical Society, we demonstrated how we have scaled-down to counter-top dimensions the pulse-conductivity system that we use to screen carcinogens. Further, we demonstrated how this portable carcinogen-screening device can be applied to monitoring complex mixtures of carcinogens such as cigarette smoke. Thus, we have provided a tool to the field of environmental health science which can be used to identify chemical carcinogens as well as to screen potentially hazardous mixtures. Through these studies, we have contributed the carcinogen-screening "litmus paper" sought by Bridges [Bridges, 1975] and have established an "electron-based toxicity index" that Christophorou suggested [Christophorou, 1980], both of which are goals that DOE's Office of Environmental Health Research has also pursued. The challenge that is now apparent is to establish more firmly the theoretical basis of the k_e -carcinogenicity relationship and determine if that basis can be applied to understanding the mechanism of action of the most thoroughly characterized human carcinogen, ionizing radiation. Through our continued interdisciplinary efforts we expect to make significant contributions to that goal.

SUMMARY

Significant progress was made toward reaching the major objectives of the **"1990-1993 Proposed Research Program"** in all three phases of our studies. In the area of Model Liquids, studies of the influence of solvent structure on electron transport that had been proposed in 1990 were completed for two model systems. In the high mobility neopentane-tetramethylsilane (TMS) system, pulse-conductivity measurements of the dependence the electron mobility μ_e on solvent composition revealed a sub-linear dependence of $\log \mu_e$ on solvent composition [Bakale and Schmidt, 1990] rather than the linear dependence that generally had been assumed to be applicable [e.g. Minday et al., 1972]. Extension of the study of the μ_e -composition dependence to include mixtures of the lower μ_e solvents cyclohexane and isooctane with TMS also revealed striking departures from a logarithmic dependence of μ_e on composition [Bakale, Lacmann, Schmidt, 1992b]. The dependence of μ_e on solvent structure that was observed in the cyclohexane/TMS model systems spans a five-hundredfold range of μ_e from 0.2 to 100 cm²/Vs that reflects a change in the electron-transport mechanism from trap-to-trap hopping at low mobility to delocalized electron transport controlled by scattering on the spherical TMS solvent molecules in the high mobility transport regime. This wide range of μ_e should encompass the mobilities of electrons that are produced by ionizing radiation and which reach thermal energies in most condensed phases in less than a picosecond prior to the occurrence of solvation or other secondary processes in polar media. These results, therefore, provide insight pertinent to electron transport in a variety of other systems.

Another study conducted in model liquids focused on one of the most intensively studied molecules thus far in the '90's, viz. the newly discovered third allotropic form of carbon, buckminsterfullerene or simply, fullerene. This unique molecule which is comprised of a truncated icosahedron of 60 carbon atoms and thus resembles the geodesic domes of Buckminster Fuller, is also known as "buckyball" or C₆₀. These unique and well characterized spheres serve as ideal "sinks" for both negative and positive charge and thus provide a novel opportunity to investigate several factors pertinent to electron and ion transport and reactions in liquids in which the unique physico-chemical properties of these spheres can be exploited. Among the results obtained in our studies are the Stokes radii of the C₆₀ ion as well as that of a radiolytic product that is assumed to be a dimer, i.e. C₁₂₀. A preliminary account of these studies will be presented in July at the 8th International

Conference on Conduction and Breakdown in Dielectric Liquids, and another manuscript is in preparation [Bakale, Lacmann, Schmidt, 1993a,b; see **APPENDIX**].

Progress in the second major area of our studies, electron-related processes in biomimetic systems, is described in detail in our appended paper from the ***Journal of Physical Chemistry*** on electron attachment to reversed micelles of Aerosol OT (AOT) that encapsulated varying quantities of water in the solvents isooctane and TMS [Bakale, Beck, Thomas, 1992a]. AOT or sodium bisdiethylhexylsulfosuccinate is a classical amphiphilic molecule which forms inverted micelles in nonpolar solvents in which its nonpolar ethylhexyl tails extend while the charged polar heads of the AOT cluster around available water. This polar pool of water is coated with a nonpolar hydrocarbon sheath that is similar in structure to a membrane and evokes usage of the term "biomimetic" to describe these surfactants that efficiently solubilize biomolecules in their water pools. Attempts to exploit this unique property and to extend our study of electronic processes occurring in the picosecond time regime to ionic processes that dominate at millisecond times were only partially successful which we ascribe to the need for instrumentation that had been requested in 1990 but could not be obtained with the available funds. Preliminary experiments were conducted in using an alternative ion-current monitor that was developed for another application [Bakale, Mc Creary, Nagel, 1993b] to monitor charge transfer in these biomimetic systems.

In the third area of study, which is electron attachment to chemical carcinogens and other bioactive molecules, the fruition of a decade of studies of electron-chemical interactions was realized. In two papers published in the journal ***Carcinogenesis***, our studies of electron attachment to genotoxic and nongenotoxic carcinogens and noncarcinogens were reported [Bakale and McCreary, 1990b; 1992b]. A rigorous statistical analysis of these results was also reported in ***Carcinogenesis*** which demonstrated that our electron attachment screening test complements *in vitro* short-term tests in identifying carcinogenic chemicals [Ennever and Bakale, 1992]. The structure-activity relationship between the electron attachment rate constant k_e and the carcinogenicity of a chemical which we have found prompted our being invited to participate in an inter-laboratory blind screening of chemicals that was organized by Dr. E. Zeiger of the National Institute of Environmental Health Sciences (NIEHS). A preliminary account of this study was presented [Zeiger et al., 1991], and we then participated in an international prospective screening of chemicals that were currently being tested in rodent bioassays conducted under the aegis of the National Toxicology Program (NTP).

Our k_e -screening results were reported in **Mutagenesis** [Bakale and McCreary, 1992a] and will be compared with the predictions of others who participated in this study at an international workshop entitled "Predicting Chemical Carcinogenesis in Rodents" which was organized by the NIEHS and will be held there May 24-25. Our k_e -screening studies of carcinogens have prompted another group to develop a computer-based method of calculating the k_e s of chemicals (Benigni et al, 1989; 1992; Benigni, 1991], and a third group has confirmed that the k_e carcinogen-screening test complements other screening tests and therefore should be included in a battery of short-term tests in order to optimize the predictivity of the battery [Kitchin et al., 1993].

In addition to these studies, the P.I. has contributed toward designing and fabricating a radon generator [Bakale, Rao et al., 1993d] that was used in studies of the biological effects of radon and its daughters [e.g. Jostes et al., 1991; Schwartz et al., 1992]. Although the fabrication of the radon generator was not part of the "Ionization in Liquids" project, the availability of the radon generator is noted in this Report because use of the generator to study the interactions of radon and its progeny with cellular-mimetic spheres is proposed in the "**1993-1996 Proposed Research Program**". Similarly, the ion-current monitor noted earlier was developed without the use of DOE funds but was used in several studies that are reported as well as for studies that are proposed for 1993-1996.

In concluding this Introduction to the **Progress Report**, it is noted that the most salient feature of our program should not be regarded to be any single facet of the three-pronged study but rather the interdisciplinary nature of the overall program. This spanning and overlapping of the disciplines is perhaps best exemplified through our publications which range from the physical extremes of **Physical Letters** and **Chemical Physics Letters** to **The Journal of Physical Chemistry** and **Radiation Research** and on to the biological end of the spectrum represented by **Carcinogenesis** and **Mutagenesis**. The multi-disciplinary aspects of the "Ionization in Liquids" project and the contributions it has made to making genetic toxicologists more aware of electrons in liquids should be considered when reviewing the Report that follows.

PROGRESS REPORT: 1990-1993

I. Introduction

Progress during the 1990-1993 report period is best demonstrated in the publications and presentations that are included in the **APPENDIX** in which drafts of several manuscripts that are in preparation are also included. Although additional background material is presented on these subjects in the Report that follows, the emphasis will be on those areas of research where work is in progress and has not yet been reported. The reviewer is therefore urged to refer to the **APPENDIX** in order to appreciate fully our progress which is described in the Report that follows.

Studies of electron and ion transport and reactions in nonpolar solvents such as cyclohexane and isooctane may appear to have insignificant relevance to the myriad of charge-transfer processes that occur in biological systems. It has been our contention over the last 15 years, however, that an electron undergoing a redox transfer, for example in respiration, or involved in the formation of a covalent bond between a carcinogenic electrophile and a nucleophilic target biomolecule is governed by the same physico-chemical interactions as an excess electron in a nonpolar liquid. Evidence to support this premise continues to accumulate, an example of which is the recent application of polaron theory to electron states in proteins [Balabev et al., 1991] which is the same mechanism of electron transport as in liquid ethane and propane [Bakale et al., 1975]. Evidence also grows that water in biological systems has significant structure which influences electron transport [e.g. Gerstein and Lynden-Bell, 1993; Fukazaki et al., 1992]. Studies of this nature and of cellular structure [e.g. Hoffman, 1993] should also be assimilated in order to assess the work that follows.

II. Electron and Ion Transport and Reactions in Model Liquids

A. Electron Transport and Reactions in Mixtures

Studies of electron transport were completed in two model systems, descriptions of which are both appended. The high-mobility mixture neopentane-tetramethylsilane (TMS) in which μ_e varies from 70 to 100 cm²/Vs was studied at the Hahn-Meitner Institut (HMI) in Berlin [see Bakale and Schmidt, 1990; **Appendix Items 1.A** and **1.A'**]. Our measurements were confined to this high μ_e system because the 15 MeV Linac that delivers the high dose rate pulses needed for lower μ_e studies was inoperative during the P.I.'s stay at HMI. Even in the limited high- μ_e region that was accessible, deviations from the logarithmic dependence of μ_e on

composition were observed. These results prompted extension of the study to the lower- μ_e solvents cyclohexane and isooctane in mixtures with TMS. These studies were conducted using the Linac during the P.I.'s next visit to HMI and the results have also been reported [Bakale, Lacmann, Schmidt 1992b; **Appendix 1.B**]. Again deviations from a logarithmic dependence of μ_e on composition were observed, but an additional aspect of the study that was attempted but not reported was determining how the high field electron drift velocity, v_d , above the critical field, E_c , is influenced by solvent composition. E_c is the value of the electric field at which the onset of deviation of v_d with the external field E occurs; this deviation can be either super-linear as in liquid ethane and propane in which v_d increases with E^2 [Bakale and Schmidt, 1973] or sub-linear as in TMS in which v_d is proportional to $E^{1/2}$ [Sowada et al., 1977]. These preliminary studies indicate that the $E^{1/2}$ dependence of pure TMS at $E_c = 20$ kV/cm changes to a linear dependence at the highest values of E that can be reached in either pure cyclohexane or isooctane (~ 200 kV/cm for experimental conditions at HMI). Efforts to delineate the dependence of E_c on composition were hindered by the threat of dielectric breakdown at high fields, the occurrence of which would damage the oscilloscope used for the measurements of drift time. Since the $E^{1/2}$ dependence provides important information on heating of the electrons by the external field, these efforts may be resumed if a means of protecting the monitoring oscilloscope were found.

The studies of electron transport that were conducted provide information that was needed to analyze the studies of electron attachment that had been conducted earlier at HMI in collaboration with Dr. G. Beck. Preparation of a manuscript on these electron attachment studies was delayed when the studies that are now described were assigned a higher priority due to the volatile nature of the molecule studied.

B. Mobility and Radii of Fullerene Ions

A study in the area of model liquids that was not proposed in 1990 but which appeared warranted in view of the intriguing structural and charge-accepting properties of the subject of the study was our work on buckminsterfullerene, also known as fullerene or C_{60} . This third allotropic form of carbon was virtually unknown in early 1990, but that changed soon afterward as evidenced by this molecule receiving the "Molecule of the Year" award from *Science* [Koshland, D.E., 1991], a special issue of *Accounts of Chemical Research* that was devoted to fullerene [McLafferty, 1992] and a monograph [Hammond and Kuck, 1992].

Our studies of C_{60} were initiated at HMI in July, 1992 with two objectives in mind, which were to measure the k_e of fullerene and the mobility of the attachment product. These studies were greatly facilitated by using a Hewlett Packard digitizer which underscored the need for updating our data acquisition method at CWRU. Despite this excellent data-collection equipment, samples of fullerene dissolved in cyclohexane or n-pentane could not be prepared without also adding impurities that obscured the evaluation of k_e . An upper limit of $1.3 \times 10^{12} \text{ M}^{-1}\text{s}^{-1}$ was estimated for the k_e in n-pentane, but the focus of our efforts shifted to the ion mobilities that were measured in two solvents, cyclohexane and n-pentane. From these mobilities the Stokes radius was calculated as is described in the two fullerene manuscripts that are appended [**Appendix Items 1.C** and **1.D**]. These ion mobility measurements stimulated us to initiate analogous studies at CWRU in which an ion-current monitor that had been designed for another purpose (*vide infra*) could be used.

An ion current monitor (ICM) that was designed and developed in a joint effort with Life Systems, Inc. for the purpose of monitoring anions formed when electrons attach to carcinogens was applied to fullerene studies at CWRU. A description of the monitor was recently presented in which the ion current decays of several types of samples were reported [Bakale, McCreary, Nagel, 1993; **Appendix Item 3.J** and **3.J'**]. Discussion of this application of the monitor to screening carcinogens is deferred to Section IV, but in order to demonstrate the response of the ICM to solutions of fullerene, a brief description of it is now presented.

The monitor is used to sample current in the same ion chamber that has been used in all of our pulse-conductivity studies done at CWRU. The commercial components used with the ICM are a Hewlett-Packard 150 kVp x-ray unit (30 ns pulse), a Keithley 300-3300 V power supply, an Interoptics RS232C fiber optic link, and an Altec 386DX personal computer (PC). The ICM is triggered by a pulse of x-rays and samples ion current at 50 μs intervals for 2 ms, which cannot be varied. Collected data is transferred from the ICM to the PC for display and analysis. In a typical measurement of ion current decay, ten ion currents are collected and averaged with the PC with which data analysis is also done. Typical responses of the ICM to pure cyclohexane and a 2.5 μM solution of fullerene in the same solvent are compared in **Figure 1**. Additional examples of applying the ICM to other types of study are noted in the remainder of the report and **Appendix Item 3.J** and **3.J'**.

Figure 1. Change of ion current decay in 2.5 μM . fullerene solution (squares) relative to pure cyclohexane (X's) at $E = 50 \text{ kV/cm}$. Extrapolation of data points yields drift time from which mobility is obtained.

C. Radiation-Induced Reactions of Fullerene Ions

In addition to studies of the k_e and mobility of fullerene ions, an unexpected third area of study of C_{60} arose from discovering that gamma irradiation of fullerene solutions markedly changed the mobility of the dominant ion in fullerene solutions. We assume that the radiolytic product is the dimer, C_{120} , which is based on the Stokes radius that is derived from the mobility of the ion that was observed in the irradiated solutions [see Appendix 1.C and 1.D]. We had preliminary discussions with analytical chemists at HMI concerning identification of the radiolytic product(s), and analysis of the product shall be attempted when the P.I. returns to HMI in June, 1993. Studies of irradiated fullerene solutions using the ICM were also conducted at CWRU and are currently in progress. Examples of ion current decay in fullerene solutions before and after gamma irradiation are illustrated in **Figure 2**. Through the studies at CWRU we have found that the ions that are formed by radiolysis are unstable and undergo various types of reactions that appear to be light-sensitive. In view of the fullerenes being much more chemically active than was initially assumed, such reactions are expected and precautions must be taken to avoid spurious reactions. Additional discussions of fullerene reactivity, including C_{60} being a "radical sponge," are in the appended manuscripts.

III. **Electrons and Ions in Biomimetic Systems**

The section of the **1990-1993 Proposed Research Program** on this topic stated "Highest priority in this area will be completion of the manuscript entitled *Dynamics of Electron Attachment to AOT/H₂O Reversed Micelles*." That manuscript was published [Bakale et al., 1992a] and is Appendix Item 2.B. Several salient points on this work were presented in an invited lecture delivered by our collaborator, Prof. J.K. Thomas of the University of Notre Dame, at the conference entitled "The Solvated Electron: 25 Years After." An abstract of this presentation is also appended, Item 2.A. The seemingly paradoxical results of the observed k_e in isooctane appearing to be **greater** than diffusion-controlled in isooctane whereas k_e in TMS was significantly **less** than diffusion-controlled posed a major obstacle that was a challenge to overcome, but this was done as is described in the appended manuscript. This apparent paradox illustrates the complexity of the results and discussion in the paper which we shall not attempt to simplify with a cursory summary in this REPORT.

Figure 2. Change of ion current decay in solution of 1.5 μ M fullerene in cyclohexane prior to (squares) and after (triangles) gamma irradiation to 50 krad.

The second major effort proposed in this area was to study charge-transfer processes in biomimetic micellar systems in order to determine if processes pertinent to radiation-induced biological damage were accessible. We noted in the proposal "that the proposed study presents formidable technical problems...". We also noted that a "key component that is expected to be needed to conduct the proposed studies is the digital oscilloscope for which funds are requested...". Thus, we were prepared to accept this challenge, but funding cuts precluded our doing so. An attempt was made to use the ICM to conduct the proposed study which was reported in the 1991-1992 PROGRESS REPORT and is now briefly summarized.

The reversed-micellar system that was studied was that which is most thoroughly characterized, viz. AOT/H₂O/isooctane. Ion currents were recorded with the ICM as is illustrated in **Figure 3** for the conditions stated in the figure legend. The limiting external field that could be applied in the micellar system was significantly less than that which we routinely use in our fullerene studies, i.e. 10 rather than 50 kV/cm. An attempt to enhance the structure of the water in the micellar pool in order to reduce the self-conductivity of the system by adding a highly polar solute, p-nitrophenol, to the system had a negligible effect on the ion decay. We similarly attempted to enhance the water structure of the pool by encapsulating a biomolecule, leucine, in the system but this also had a negligible effect on the ion current. These discouraging results combined with the more interesting results obtained in the fullerene studies diverted our focus from this area. However, the newly proposed study of determining how biomimetic systems influence ion currents resulting from attachment to chemical carcinogens will add incentive to return to studies of biomimetic systems.

IV. Electrons in Biological Systems: k_e s of Chemical Carcinogens

The first objective that was proposed in 1990 was "...to publish the results of the remainder of the chemicals that we have screened." From the publications that are appended, it is clear that we have reached this goal. This extensive list of publications and manuscripts warrants a few notes on each appended item to facilitate the reviewer's assimilating the information that is presented. The appended materials are in approximately chronological order of preparation, and the notes are presented in the same order.

Appendix Item 3.A (Bakale and McCreary, 1990a) is a chapter on the book **Mutation and the Environment** which is based on an invited lecture presented at the Fifth International Conference on Environmental Mutagens. This chapter

Effect of 100 μM (X's) and 250 μM (squares) AOT and water at 5:1 mole ratio on ion decay in i-octane at 10 kV/cm relative to pure isooctane (dashed line).

focuses on the relationships among electrophilicity, mutagenicity and carcinogenicity, or the E/C/M relationship, and illustrates that the k_e test can be exploited to identify nonmutagenic chemicals that are carcinogens. This was the most vivid demonstration until that time of how the k_e test complements conventional *in vitro* carcinogen-screening tests, and the presentation appears to have favorably influenced two of the most dominant genetic toxicologists who were in the audience. One was Dr. E. Zeiger of NIEHS who invited us to participate in the blind-screening of chemicals that is described in Appendix Item 3.F, and the other was Dr. J. Ashby of Imperial Chemicals who organized the workshop that is described in Appendix Item 3.H.

Appendix Item 3.B (Bakale and McCreary, 1990a) is a more comprehensive paper on the same topic that was published in the journal **Carcinogenesis**. The most salient feature of the paper is that the k_e test correctly identifies 27 of 46 carcinogens for which the Ames *Salmonella* assay yields negative responses. It should also be noted that all of the chemicals screened were those that were tested by the NTP and reported to yield negative or equivocal *Salmonella* responses [Zeiger, 1987]. The k_e test also was found to yield 8 *negative* responses to 20 putative *noncarcinogens* that are all mutagens.

Appendix Item 3.C (Bakale and McCreary, 1992a) represents achieving the second objective that was proposed in 1990, viz. "...measuring the k_e s of the 44 chemicals now undergoing NTP rodent testing...". Additional discussion of this paper is deferred to Item 3.H.

Appendix Item 3.D (Bakale and McCreary, 1992b) is "the other side of the coin" to Item 3B, i.e., in this study we screened all of the NTP-tested chemicals reported by Zeiger that yield *correct mutagenicity responses*. This paper provided the critical results that were needed to prepare the next paper, which is:

Appendix Item 3.E (Ennever and Bakale, 1992) reports a comprehensive statistical analysis that illustrates that the k_e test complements the Ames *Salmonella* test. The co-author, Dr. F.K. Ennever of Wake Forest University, has published extensively on the optimization of carcinogen-screening strategies, and her endorsement of the k_e test in this paper should be a valuable contribution to more widespread acceptance of the k_e -carcinogenicity structure-activity relationship.

Appendix Item 3.F (Zeiger et al., 1991) is an abstract of Dr. Zeiger's presentation at the Environmental Mutagen Society Annual Meeting at which he reported on the inter-laboratory blind-screening of 100 coded chemicals for which the other participants in the study did not know the mutagenicity of the chemicals

that were screened. A full report on this study is forthcoming and shall be submitted for publication in the journal *Mutagenesis*.

Appendix Item 3.F' (Zeiger et al., in preparation) is the introduction to a draft of the manuscript noted in the preceding item. Completion of the manuscript has been delayed due to a major reorganization at NIEHS (see also **Item 3.H**).

Appendix Item 3.G (Bakale and McCreary, in preparation) describes our contribution to the Zeiger inter-laboratory study. Final revisions to the manuscript are expected to be made following the Workshop described in the next item. It should be noted that ten fewer chemicals were screened with the k_e test than the other methods due to reasons described in the manuscript.

Appendix Item 3.H (Ashby et al., 1993) is the description of the NIEHS Workshop at which all of those who participated in predicting the carcinogenicity of the chemicals being tested by the NTP will provide additional information on the test method that they used. The participants who made predictions and reference to their predictions are: Benigni, R. (1991); Enslein, K., Blake, B.W. and Borgstedt, H.H. (1990); Lewis, D.F.V., Ioannides, C. and Parke, D.V. (1990); Rosenkranz, H.S., and Klopman, G. (1990); Tennant, R.W., Spalding, J., Stasiewicz, S. and Ashby, J. (1990). From the Workshop schedule, it should be noted that our lecture on the k_e test will be presented on the afternoon of the first day and will be followed by the presentation of Dr. Benigni who developed a computer-based method of evaluating k_e s (Benigni et al., 1989, 1992). This work by Benigni's group is particularly significant since it identifies the physico-chemical parameters that combine to yield k_e . *Thus, k_e serves as a link between the physical and chemical properties of a chemical and that chemical's carcinogenic properties.* In view of the relevance of Benigni's work to the k_e test, the abstract of their most pertinent paper follows on the next page.

Appendix Item 3.I (Bakale and McCreary, 1993) summarizes the results of our k_e screening of the NTP chemicals and is the crux of our Workshop presentation.

Appendix Item 3.J (Bakale, McCreary and Nagel, 1993a) is the extended abstract of our presentation at the March national meeting of the American Chemical Society in which we described how the ICM, which is referred to as a portable electrophile detector or PED, can be used to identify chemical carcinogens. A more detailed description of the presentation is provided in the next item.

Appendix Item 3.J' (Bakale, McCreary and Nagel, 1993a) provides an overview of a decade of development of the k_e test and describes how we have