Carcinogenesis vol.8 no.2 pp.253–264, 1987

A physico-chemical screening test for chemical carcinogens: the k_e test

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A pulse-conductivity technique was used to measure the rate at which excess electrons in liquid cyclohexane attach to carcinogens and non-carcinogens in order to determine if the electron attachment rate constant, k_e, could be used to screen potential carcinogens. The k_es of 114 chemicals are reported; these chemicals are among 182 that had previously been tested in a validation study of several short-term carcinogen-screening bioassays. The remaining 68 chemicals for which k_es were not measured include chemicals that were unavailable, were not sufficiently stable or soluble in cyclohexane, or did not have a well-defined mol. wt. For the 114 chemicals that were tested, 35 are carcinogens, 50 are putative non-carcinogens and 29 have not been adequately tested or yielded equivocal responses in animal-test studies. Diffusion-controlled k_es were measured for 27 of the 35 carcinogens tested whereas the k_es of 45 of the 50 non-carcinogens were less than diffusion controlled. From these results, several measures of the predictive performance of using a diffusion-controlled k_e to indicate a positive response to a carcinogen were calculated and compared with the Ames-test predictiveness in screening the same chemicals. The predictive criteria calculated were sensitivity, specificity, accuracy and predictive value, all of which were greater for the k_e test than for the Ames test. Comparisons of the chemicals that yielded false-negative responses in the k_e and Ames tests indicate a high degree of independence between the two which implies that the tests could be efficaciously used in a battery of short-term tests. Rationales are offered concerning the observed k_e–carcinogenicity correlation and the apparent lack of the need for procarcinogens to be metabolically activated to yield a positive k_e response.

Introduction

The one physico-chemical property that is common to most chemical carcinogens is electrophilicity, which J.A. and E.C.Miller described 15 years ago as the property associated with a molecule having electron-deficient atoms (1). The Millers further noted that electrophiles have the potential to react with electron-rich cellular components such as DNA and that electrophile–DNA interaction may be considered to be the initiating step in the somatic mutation theory of carcinogenesis (1–3). Interest in this theory was revived in the last decade by the success of the Ames *Salmonella*/microsome bioassay, or Ames test (4–6), in identifying chemical carcinogens with an accuracy (*vide infra*) of ~90 per cent in several validation studies (7–11). In his review of the Ames and similar short-term carcinogen-screening tests, Bridges noted that such bioassays are generally based on detecting DNA damage which is 'a very sensitive way of detecting electrophilic reagents' (12). Since we routinely used a pulse-conductivity (PC*) technique to study liquid-phase electron–chemical interactions (13–17), we sought to determine if this technique could be applied to screening potential chemical carcinogens.

The PC technique that we employed in earlier carcinogen-screening studies (18–20) and in this work is described in detail in the Materials and methods section; briefly, the PC technique involves monitoring the decay of excess electrons that are produced in a non-polar solvent by a short pulse of ionizing radiation. In the presence of an electrophilic solute, the high-mobility electrons attach to the electrophile and thereby are converted to low-mobility anions; this change in the mobility of the charge-carriers is readily monitored and an electron-attachment rate constant, k_e, can be extracted from the kinetics of the decay of the conductivity of the solution. A diffusion-controlled k_e (*vide infra*), which indicates the absence of a thermodynamic barrier in the electron–solute interaction, is considered to be a positive indication that the solute is an electrophile and a carcinogen, whereas a less-than diffusion-controlled k_e is regarded as a negative indication of electrophilicity or carcinogenicity. Thus, the electron serves as a probe of test-chemical electrophilicity which is analogous to the role that the DNA of histidine-deficient auxotrophs of *Salmonella typhimurium* plays in the Ames test. The role that mammalian microsomes play in the Ames test, which is to activate procarcinogens to a more electrophilic state, has no counterpart in the k_e test. Discussion of this apparent deficiency of the k_e test is deferred to the concluding section.

Our preliminary reports of using a PC technique to screen chemicals identified as carcinogens or non-carcinogens in animal-test or epidemiological studies have been presented elsewhere (18–20). In these earlier studies, the chemicals that we screened were those that had been tested in five major validation studies of the Ames and other short-term tests (7–11). In comparing the k_e test results with those of the Ames test, we used the two criteria that are generally used to describe the accuracy of a short-term screening test, viz. sensitivity and specificity. 'Sensitivity' is the percentage of positive responses observed for the carcinogens tested and 'specificity' is the percentage of negative responses observed for all non-carcinogens tested (9,21–23); a combined measure of sensitivity and specificity is 'accuracy' which is the percentage of correct positive and negative responses observed for all chemicals tested (24). In our earlier studies, the accuracy of the PC technique as a screen for carcinogens compared favorably with that of the Ames test. For example, for 76 chemicals tested in cyclohexane by the PC technique, the accuracy was 88% (sensitivity and specificity both 88%, ref. 18) whereas the Ames-test accuracy was 83% (sensitivity and specificity 68 and 97%, respectively). In an analogous PC study in which isooctane was used as the solvent, we found for 65 of

*Abbreviations: PC, pulse-conductivity; UHP, ultra-high purity; EtOH, ethanol; BZ, benzene; EtAc, ethyl acetate; DMSO, dimethylsulfoxide; CAS, Chemical Abstracts Services; CPBS, Carcinogenicity Prediction and Battery Selection; PAH, polyaromatic hydrocarbon; CA, chromosome aberrations; SCE, sister chromatid exchange.

the same chemicals a sensitivity of 83% and a specificity of 86% for which the Ames-test sensitivity was 69% and the specificity was 96% (19).

Although these results were encouraging, our studies were subject to the criticism that we had chosen from among the several hundred chemicals screened in the five Ames-test validation studies (7–11) to test only those that we *a priori* expected would yield a correct k_e response. In order to demonstrate that this criticism is invalid, we undertook the present study in which we attempted to measure the k_es of *all* of the chemicals screened in a major validation study. Shortly before we initiated this work, Kawachi *et al.* had reported the results of their six-year study of testing 182 chemicals in eight different short-term tests (25). These tests included Ames *S. typhimurium* strains TA98 and TA100, *Bacillus subtilis* (*rec* assay), hamster lung fibroblast cells (chromosome aberrations, CA, and sister chromatid exchange, SCE), human embryo fibroblasts (CA and SE), bone-marrow cells (CA *in vivo*) and silk worms (mutations). The chemicals in the Kawachi study were comprised of structural analogs of known carcinogens, widely used food additives, drugs frequently administered for long-term illnesses and industrial intermediates produced in large quantities. The significance of the latter three categories of chemicals to human exposure and the chemicals' being subjected to multiple short-term testing made the chemicals selected by Kawachi *et al.* attractive candidates for screening with the k_e test.

Another desirable feature of the Kawachi-tested chemicals was that approximately equal numbers of carcinogens and non-carcinogens (51 and 64, respectively) were screened, which is a factor that becomes significant if 'predictive value' is used as a measure of test performance. Predictive value is the ratio of positive responses observed for the carcinogens tested to the total number of observed positive results and thus depends on 'prevalence', which is the fraction of carcinogens in the chemicals tested (9,21–23). Another validation study that was also reported in 1980 which we considered was that of Bartsch *et al.* (26); however, only seven non-carcinogens were among the 180 chemicals screened in that study, and we therefore chose to screen the Kawachi-tested chemicals.

A final introductory note concerns the terminology used to describe electrons in non-polar media. In our earlier studies, we used the term 'quasi-free' to describe electrons that were neither fully trapped nor free in the solvents cyclohexane and isooctane that were studied. More recently, however, usage of 'quasi-free' has narrowed to describe only highly delocalized electrons for which the electron mobility is at least 100 cm^2/Vs (see, for example, ref. 27–29). Therefore, the more general term 'excess' will be used to describe electrons in the low-mobility liquid cyclohexane that was used in this study.

Preliminary presentations of this work have been reported elsewhere (30,31).

Materials and methods

PC technique

The three major components of a PC system are (i) a pulsed source of ionizing radiation; (ii) an ion chamber to contain the test solution and to provide a volume in which the radiolytic charge-carriers drift and react, and (iii) a method of monitoring the decay of the charge-carriers. A Van de Graaff generator was used to produce single 15-ns pulses of 1-MeV electrons which irradiated the test solution in an aluminum ion chamber. The irradiating electrons entered the chamber through a 0.05 × 9 mm o.d. beryllium window that also served as the cathode. A potential of +1800 V was applied to the 19-mm o.d. aluminum anode that was separated by 0.6 mm from the cathode. This configuration yielded a nearly homogeneous distribution of secondary electrons (13) that thermalized in < 1 ps and drifted with a mobility μ_e of 0.22 cm^2/Vs in the cyclohexane solvent at 21°C (32). Since μ_e is orders of magnitude greater than μ_+, the mobility of the concomitantly generated cations (13,33), the current induced in the circuit external to the ion chamber provided a direct measure of the excess electrons drifting in the 30-kV/cm electric field. This electron current was amplified by a factor of 10 with a Comlinear CLC 100 wideband amplifier, and the current was then monitored on a Hewlett-Packard 1744 storage oscilloscope (100 MHz). After the Van de Graaff electron beam was adjusted to yield an amplified electron current of ~1 mA aross 50 Ω, a trace of the electron-current decay was stored and the oscillogram was photographed for permanent storage and analysis.

Four decay modes were available to excess electrons that escaped geminate recombination and induced the electron current that was monitored in the ~20–600 ns time regime; these were (i) drift to the anode where neutralization occurred, (ii) volume recombination with cations, (iii) attachment to adventitious impurities in the solvent, and (iv) attachment to an electrophilic test solute dissolved in the solvent. Irradiation conditions were chosen to minimize volume recombination (*vide infra*), and with 1800 V applied across 0.06 cm the average electron drift time to the anode was 4.5 μs, which was also a negligible decay mode since the electron half-lifes with respect to attachment were ~600 and 100 ns via decay modes (iii) and (iv), respectively.

A 15-ns pulse of 1-MeV electrons at a beam current of ~0.4 mA produced an inter-electrode dose of ~1.5 rads and a concentration of secondary electrons of $\sim 3.0 \times 10^{11}$ electrons/cm^3 which corresponds to ~0.5 nM. The electron-ion recombination rate constant in c-hexane at 21°C is 1.2×10^{14} M^{-1} s^{-1} (34), which combined with the 0.5 nM concentration of charge-carriers yields an initial electron half-life with respect to recombination of ~17 μs. Thus, electron–ion recombination was negligible in the 50–250 ns time regime where most k_es were measured.

The concentration of test solute, which is denoted by [S], was > 1 μM for all chemicals tested; consequently, pseudo-first-order kinetics applied to the electron attachment process and the electron current decayed exponentially. The electron half-life with respect to attachment, $t_{½}$, was extracted manually from a semi-log plot of the electron-current decay over at least two $t_{½}$s, and k_e was obtained from

$$k_e = \ln 2/t_{½}[S] \quad (1)$$

Additional details related to the derivation of equation (1) and other aspects of applying the PC technique to measure k_es are presented in refs. 13, 14 and 16.

Solvent purification

Cyclohexane (Fisher Scientific, 99 mol %) was freshly purified prior to every k_e measurement. Purification consisted of degassing ~0.5 l of *c*-hexane with ultra-high purity (UHP) argon (Matheson, 99.999 mol % stated purity) before the solvent was passed through a 1.5 m × 2 cm o.d. column of a 50:50 mixture of silica gel (Alltech Assoc. 35/60 mesh) and molecular sieve (Alltech Assoc. 40/60 mesh) that had been freshly activated at ~400°C for >24 h. The first fraction of ~200 ml was collected under an atmosphere of UHP argon in a glass flask that had been baked at ~400°C and was fitted with Teflon stopcocks. The c-hexane was degassed in this flask for an additional 10 min by bubbling with UHP argon and then irradiated with ^{60}Co-γ radiation to a dose of $\sim 10^6$ rads. The c-hexane was then transferred under an argon atmosphere into the ionization chamber where it was subjected to a final 10-min degassing. The electron $t_{½}$ was then measured; if $t_{½}$ > 500 ns, the ion chamber was drained and flushed with UHP argon in preparation for measurement of the k_e of a solution of the test chemical. If the $t_{½}$ of the purified c-hexane were < 500 ns, the ion chamber was drained, flushed with argon and filled with another aliquot of purified c-hexane that was also degassed in the ion chamber. The process was repeated until a $t_{½}$ > 500 ns was observed which generally required not more than two ion-chamber fillings.

Sample preparation

All chemicals were generally used as received except several of the dyes which were dissolved in an appropriate solvent, filtered and recrystallized in order to remove the inert 'filler' upon which the dyes were coated. The stated purity of most chemicals was 95–98%; the purity was checked by h.p.l.c. if the chemical appeared to have decomposed or was known to be unstable. A stock solution of a test chemical was prepared a few hours before k_e was to be measured unless a solubility problem was anticipated; in such cases a stock solution was prepared and stored at ~45°C for ~20 h prior to the k_e measurement.

Preparation of a stock solution typically involved weighing ~15 mg of the test chemical in a 25 ml volumetic flask on a Mettler A30 electronic balance that was accurate to ±0.1 mg. The appropriate solvent was then added to the flask which yielded a solution of the test chemical at a concentration that ranged from 2 to 6 mM for chemicals having mol. wts that ranged from 300 to 100 g/mol, respectively. A 15–50-μl aliquot of the test solution was then added to 25 ml of the purified *c*-hexane, the 1–15-μM solution was degassed in the ion chamber, and $t_{½}$ was measured. A $t_{½}$ in the range of 70–150 ns was sought and usually attained by adding an appropriately larger or smaller volume of the stock solu-

tion; for test chemicals with k_es $< 10^{11}$ or $> 4 \times 10^{12}$ M^{-1} s^{-1}, a more or less concentrated stock solution, respectively, was prepared. Generally, at least two measurements of $t_{½}$ were made in the 70–150 ns range for each of two stock solutions prepared on different days, and the experimental error in the k_es measurement is estimated to be ±5%. Although this protocol was feasible for ~80% of the chemicals tested, problems with test-chemical solubility and reactivity were encountered that required the following modification of the protocol.

The solubility problem was exacerbated by the constraints that the solvent used to dissolve cyclohexane-insoluble test chemicals had to be miscible with cyclohexane and that the quantity of cyclohexane-miscible solvent added had to be limited to avoid markedly changing the μ_e of the solution (35,36). Consequently, stock solutions of cyclohexane-insoluble chemicals were prepared at a sufficiently high [S] in ethanol (EtOH), benzene (BZ) or ethyl acetate (EtAc), so that only 0.1 vol % of such solvents were present in the solutions in which the k_es were measured. Since dimethyl sulfoxide (DMSO) is >10 times more effective in trapping excess electrons in cyclohexane than is EtOH (36), we avoided using DMSO to solubilize cyclohexane-insoluble chemicals.

Problems of test-chemical reactivity were of two general types: (i) chemicals that reacted with the aluminum walls and/or beryllium window of the ion chamber and (ii) chemicals that were radiolytically unstable and therefore were significantly decomposed by the few pulses of radiation that were needed to set the Van de Graaff to the optimum beam current for conducting the k_e measurement. The latter group of chemicals could not be tested and are noted in the Results section; k_es of the former chemicals were measured by minimizing the time that the chemicals were in the ion chamber. This was done by degassing the test solution in a glass bulb connected to the ion chamber and measuring $t_{½}$s at 1, 2 and 3 min after the degassed solution was admitted to the ion chamber. Values of these $t_{½}$s were extrapolated to zero time from a plot of log $t_{½}$ versus t to yield a $t_{½}$ that corresponded to the initial [S].

The measured $t_{½}$s of the test chemicals were corrected by measuring the $t_{½}$ of an appropriate 'blank' of a solution that was of the same solvent composition and was subjected to the same manipulations as the test-chemical solution. A 'worst-case' example is a chemical that reacted with the ion chamber and was soluble only in EtOH. In such a case, k_e was measured using the glass flask for the final degassing of the test chemical dissolved in EtOH/cyclohexane, and $t_{½}$ was determined using the extrapolation procedure described in the preceding paragraph. A blank solution at the same EtOH/cyclohexane composition was then prepared and degassed in the glass flask prior to measuring the $t_{½}$. The reciprocal of the blank $t_{½}$ was then subtracted from the reciprocal of the measured $t_{½}$ of the test-chemical solution to yield a solvent-corrected reciprocal $t_{½}$ that was used in equation (1) to yield k_e.

In concluding this section, we note that several rinsings of the ion chamber with pure cyclohexane interspersed with argon flushings were required to clean adequately the ion chamber of traces of test chemicals. If contamination of the ion chamber were suspected after such rinsings, the $t_{½}$ of pure cyclohexane was checked to ensure that the ion chamber was sufficiently free of test-chemical residue before another chemical was tested.

Results

The 182 chemicals screened by Kawachi *et al.* include several that are so closely related that we have consolidated such compounds into one representative chemical. These chemicals include five caramels that both we and Kawachi *et al.* considered as a single compound. Also, for fluorescent brighteners 24, 225 and 260, we tested fluorescent brightener 28, which was the only fluorescent brightener available, for γ- and β-hexachlorocyclohexane we tested only the γ-isomer, for the iso- and *n*-propyl and butyl esters of *p*-hydroxybenzoic acid we tested only the *n*-isomer of each, for the polychlorinated biphenyls KC 300 and KC 500 we substituted the more readily available Arochlor 1254, and for 'pure' and 'crude' Stevioside, we tested only the former compound. To facilitate comparisons between the chemicals that we tested and those screened by Kawachi *et al.*, we used the same nomenclature as Kawachi and also listed all chemicals alphabetically in the tables that follow. CAS (Chemical Abstracts Services) registry numbers are included for all chemicals for which k_es were measured to ensure that the chemicals are explicitly identified.

The Kawachi-tested chemicals can be conveniently divided into three categories: (i) chemicals that we could not test, (ii) chemicals that were tested and have known carcinogenic properties, and (iii) chemicals that were tested but have unknown carcinogenic properties. Although category (ii) chemicals are most important for determining the sensitivity and specificity of the k_e test (*vide supra*), category (i) and (iii) chemicals are significant in demonstrating that we did not pre-select the chemicals that were tested. Hereafter, specific chemicals will be denoted by the Roman numeral of the table in which each is listed followed by the number in that table.

(i) Chemicals not tested

The first sub-category of chemicals that were not tested are the 23 chemicals listed in Table I, which are 22 that are not commercially available and one that requires hydrolysis for activation. Two of the chemicals I-4 and I-11, are 'controlled substances' that require a Drug Enforcement Agency Class II license for purchase, and I-20 is the chemical that is known to require hydrolysis for activation (37) which is not compatible with the cyclohexane used in the k_e test. The other 20 chemicals are not available from the 21 chemical distributors listed in the legend of Table I.

In Table II are listed six chemicals that are not adequately characterized for k_e to be measured. The acid red screened by Kawachi *et al.* was also denoted by them as Food Red. No. 106, neither designation adequately defines the chemical that they

Table I. Chemicals tested by Kawachi *et al.* that are not commercially available[a], are not accessible[b,c] or require hydrolysis[d]

1. 4-Acetylaminobiphenyl
2. 4-Acetylaminofluorene
3. 4-Aminoquinoline-1-oxide·HCl
4. Barbital[b]
5. *s*-Benzyldiisopropylphosphorothiolate
6. Butylbutanolamine
7. *N*-*n*-Butyl-*N*-nitrosourea
8. *N*-*n*-Butyl-*N*-nitrosourethane
9. *N*-*n*-Butylurethane
10. Chlordiazepoxide
11. Diazepam[b]
12. 4-Dimethylaminostilbene
13. Eulan U-33
14. 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide (Furylfuramide, AF-2)
15. Glycyrrhizic acid trisodium salt
16. Halopericol
17. *N*-Hydroxymethyldimethylphosphonopropionamide
18. Isoascorbic acid sodium salt[c]
19. Lacchaic acid
20. Methylazoxymethanol acetate[d]
21. Mitin FF
22. *N*-Nitrosobutylbutanolamine
23. Thiabendazole

[a]Chemicals are unavailable from the following distributors: Aldrich, Alfa, BDH, Boehringer Mannheim, Chemical Dynamics, Chem Service, Eastman Kodak, EM Science, Fairfield, Fisher Scientific, Fluka, GFS, K&K, Mallinckrodt, MCB, Pfaltz & Bauer, Reidel–de Haën, SCM, Sigma, Strem and U.S. Biochemical.
[b]Drug Enforcement Administration controlled substance.
[c]Isoascorbic acid is readily available; however, the sodium salt tested by Kawachi *et al.* is not available from the distributors listed above.
[d]Hydrolysis is required for activation which is incompatible with the k_e test solvent; see text.

Table II. Chemicals tested by Kawachi *et al.* that are not sufficiently well characterized for k_e to be measured

Chemical	Comment
1. Acid Red (Food Red 106)	More than 100 Acid Red formulations available
2. Annatto, water soluble	Seed extract used as dye
3. Caramel	Structure varies with preparation
4. Cochineal	Dried insect used as dye
5. Dextran	Polysaccharide produced by bacteria; mol. wt varies with substrate
6. Sodium carboxymethyl cellulose	Mol. wt variable; can form reversed micelles in hydrocarbon solvents

Table III. Chemicals tested by Kawachi *et al.* that are not sufficiently soluble in cyclohexane to permit k_e to be measured[a]

Chemical (source[b])	CAS No.[c]	Solvent(s) tested[d]
1. Amaranth (Al)	915-67-3	DMSO/EtOH
2. Aniline·HCl (Si)	142-04-1	EtOH
3. Brilliant Blue FCF (PB)	2650-18-2	DMSO/EtOH
4. Dipyrone (Si)	68-89-3	EtOH
5. D,L-Ethionine (Al)	67-21-0	EtOH
6. Erythrosin (EK)	16423-68-0	DMSO/EtOH
7. Fast Green FCF (PB)	2353-45-9	DMSO/EtOH
8. Fluorescein sodium (CS)	2321-07-5	EtOH
9. Hydralazine·HCl (Si)	304-20-1	EtOH
10. Isoniazid (Si)	54-85-3	EtOH
11. Levodopa (Si)	59-92-7	BZ; DMSO
12. Maneb (CS)	12427-38-2	EtOH; EtAc
13. D,L-Methionine (CS)	59-51-8	DMSO/BZ; EtOH
14. Nitrofurazone (Si)	59-87-0	BZ; EtOH; DMSO/EtOH
15. Potassium bromate (CS)	7758-01-2	DMSO; EtAc; EtOH
16. Potassium metabisulfite (CS)	16731-55-8	DMSO; EtAc; EtOH
17. Saccharin sodium (Si)	128-44-9	EtOH
18. Sodium sulfite (CS)	7757-83-7	DMSO; EtAc; EtOH
19. Stevioside (Si)	77-05-4	DMSO/EtOH
20. Tetrakis(hydroxymethyl)-phosphonium chloride (CS)	124-64-1	DMSO/EtOH
21. Thiourea	62-56-6	EtOH
22. Triamterene (Si)	396-01-0	DMSO/EtOH; EtAc

[a]The limited solubility of <1 μM of the listed chemicals was insufficient to effect a decrease of the electron half-life of 50% which we regard as the minimal change needed to measure k_e accurately. In such cases we attempted to dissolve the chemicals in the solvents or solvent pairs indicated; however, injecting aliquots of these solutions into c-hexane effected dissolution of the solute; see text.
[b]Sources of chemicals tested: Al, Aldrich; CS, Chem Service; EK, Eastman Kodak; PB, Pfaltz & Bauer; Si, Sigma.
[c]Chemical Abstracts Service registry number.
[d]BZ, benzene; DMSO, dimethylsulfoxide; EtAc, ethyl acetate; ETOH, ethanol. Solubility of chemicals was first tested in pure solvent; if not soluble, pairs of solvents (e.g. DMSO/BZ) were tested.

tested which was also classified as having unknown carcinogenic properties (25). The remaining five chemicals listed in Table II have indefinite mol. wts; therefore, the [S] required in equation (1) to obtain k_e cannot be determined.

The third sub-category of chemicals that were not tested are the 22 chemicals listed in Table III which are soluble in neither pure cyclohexane nor in solutions of the indicated co-solvents when such solutions are added to cyclohexane at a concentration of ~0.1% by volume. For example, III-2 is 'insoluble' in pure cyclohexane but dissolves readily in EtOH; however, addition of 25 μl of a 1 mM solution of III-2 in EtOH to 25 ml of cyclohexane effected precipitation of the solute. In a further attempt to solubilize some of the chemicals listed in Table III (e.g. chemicals III-1, 3, 6, 7, 14, 19, 20 and 22), ~2 mg of the chemical was first dissolved in 2 ml DMSO and this solution was diluted to 10 ml with EtOH. Precipitation of these eight solutes occurred, however, when 25 μl of the solute/DMSO/EtOH solution was added to 25 ml of cyclohexane.

Table IV. Chemicals tested by Kawachi *et al.* for which k_e could not be measured accurately due to problem indicated

Chemical (source[a])	CAS No.[a]	Experimental problem[b]
1. Acrylonitrile (CS)	107-13-1	Volatile[c]; facile polymerization
2. Epichlorohydrin (CS)	106-89-8	Immiscible with hydrocarbons; reacts with alcohols[d]
3. Hydrogen peroxide (NA[e])	7722-84-1	Highly unstable; decomposes in organic solvents
4. 3-Hydroxyanthranilic acid (Si)(NA[e])	548-93-6	Unstable; decomposition products found by h.p.l.c. in freshly opened ampoule
5. Maleic anhydride (CS)	108-31-6	Forms esters in alcohol; decomposition product(s) observed in benzene
6. Nitrosomethylurea (CS,Si)	684-93-5	Unstable[f]; samples obtained from sources indicated not 'pale yellow cystals'
7. 1,3-Propane sultone (Si)	1633-83-6	Insoluble in aliphatic solvents; hydrolyzes in polar solvents[g]
8. β-Propiolactone (Si)	57-57-8	Facile polymerization; reacts with alcohols[h]
9. Sodium hypochlorite (NA[e])	7681-52-9	Highly unstable, decomposes in air, anhydrous form is explosive
10. Trichlorfon (CS)	52-68-6	k_es measured using ethanol and benzene bases not reproducible; limited solubility in hexane

[a]See legend to Table III.
[b]Chemical and physical properties obtained from *The Merck Index* (38) except as noted.
[c]Vapor pressure at 24°C is >100 mmHg, ref. (39).
[d]Ref. (40, p. 131).
[e]NA, k_e measurement not attempted.
[f]Stability and appearance data obtained from ref. (41, p. 125). Sample obtained from Sigma was stabilized with ~25% solution of 3% acetic acid which precluded measuring k_e accurately.
[g]Solubility/stability data from ref. (42, p. 253).
[h]Ref. (42, p. 259).

The fourth and final sub-category of chemicals that were 'not tested' are the 10 chemicals listed in Table IV. No attempt was made to measure the k_es of three of the least stable compounds listed; chemicals IV-3 and IV-9 in their anhydrous form are known to react violently with organic solvents and were therefore not tested, and h.p.l.c. analysis of a freshly opened ampoule of IV-4 indicated that the compound had decomposed. Measurements of the k_es of the other seven chemicals were attempted but were not reproducible, which was assumed to be related to the stability problems indicated in Table IV.

(ii) Chemicals tested which are known carcinogens or putative non-carcinogens

The k_e values for the 88 chemicals listed in Table V constitute the basis for determining the sensitivity and specificity of k_e as

an indicator of the carcinogenicity of chemicals. As in our earlier studies of electron attachment to carcinogens (18–22), a diffusion-controlled k_e, which is denoted as k_d, indicates that attachment occurs at every electron-solute encounter. This is regarded as a positive response, i.e. $R = +$, since it indicates that the test chemical is certainly an electrophile and consequently may be a carcinogen. In contrast, a less-than diffusion-controlled k_e, i.e. $k_e < k_d$, is regarded as a negative response, $R = -$, and a negative indication of test-chemical electrophilicity and carcinogenicity.

This boundary between $R = +$ and $-$ is somewhat arbitrary if one considers that the dipole moment of the solute enhances k_e (16) and, therefore, a diffusion-controlled k_e for a polar solute should be significantly greater than that of a non-polar solute. Consequently, the k_e of the non-polar carcinogen CCl_4 which we earlier used to set the $R = +/-$ boundary at 2.9×10^{12} M^{-1} s^{-1} in cyclohexane represents the lower limit of a diffusion-controlled k_e (16,18–20). In addition, factors such as the hard-core radius, polarizability and multi-pole moment of the solute also influence the effective encounter radius of the electron–solute interaction which governs the k_e at which the attachment process is diffusion controlled as defined by the Debye–Smoluchowski equation (16,43–45). Since we do not regard the theory of diffusion-controlled reactions to be adequately developed to permit an accurate value of k_d to be calculated for each of the 88 chemicals listed in Table V, we used our previous approach of setting the $R = +/-$ boundary at 3.0×10^{12} M^{-1} s^{-1}, which is the k_e of the CCl_4 in cyclohexane that we have reported earlier (16) and recently re-measured.

Another note on the k_es listed in Table V concerns those that are $< 10^{12}$ M^{-1} s^{-1} for which only an upper limit of the measured k_e is given. This upper limit of k_e for such non-attaching solutes connotes that the observed $t_{1/2}$ from which k_e was derived may include a significant contribution from an efficient electron-attaching impurity. For example, the presence of 2% of an impurity with a k_e of 3×10^{12} M^{-1} s^{-1} would account for >90% of the reduction of the $t_{1/2}$ effected by chemical V-14. Thus, the k_e of *pure* V-14 is at least an order of magnitude less than the measured value of 5×10^{10} M^{-1} s^{-1}. Conversely, 2% of the same impurity in an efficient electron-attaching solute such as V-3 would effect only a 1.5% overestimation of the measured k_e which is within our estimated experimental error of ±5%. We also note that the k_e of *n*-hexane, V-35, is zero, which is based on the electron-transport properties of *n*-hexane being similar to that of cyclohexane (32).

In addition to the numerical values of k_e listed in Table V, three entries of 'Eq'm' also appear which denote that an electron attachment/detachment equilibrium was observed. Such equilibria have been studied in a variety of solute/solvent systems and are generally characterized by a strong temperature dependence that can be exploited to yield unique thermodynamic information regarding the solute (see, for example, refs. 46–49). In our earlier studies of electron attachment to carcinogens, we arbitrarily classified observations of equilibria as indeterminate or equivocal responses (18–20,30,31). Since none of the chemicals for which an equilibria had been observed are carcinogens, however, we decided to regard an electron attachment/detachment equilibrium as a negative indication of carcinogenicity in this study. Therefore, chemicals V-2, 16 and 65 are assigned an $R = -$ in Table V.

The other short-term test results listed for each of the solutes in Table V are the Ames-test mutagenicity responses, M, which are those reported by Kawachi *et al.* (25). Only tester strains TA98 and TA100 were used by Kawachi's group, and thus the standard Ames-test protocol (5,50) was not rigorously followed. Consequently, the mutagenicity responses listed in Table V should not be considered as being a definitive measure of the accuracy of the Ames test in identifying carcinogens among the chemicals screened. The Ames-test results reported by Kawachi *et al.* are included in Table V to provide an indication of the classes of chemicals where the k_e test and Ames test may respond similarly (*vide infra*).

The remaining information compiled in Table V is the classification of the carcinogenicity of the chemicals tested. The carcinogenicity classifications reported by Kawachi *et al.* are listed under 'C(K)' and are designated '+' and '−' to denote carcinogens and non-carcinogens, respectively, and 'NT' designates those chemicals 'not tested' (25). The criteria used by Kawachi *et al.* in classifying the carcinogenicity of the chemicals were not specified but presumably were based on pre-1978 animal-test and epidemiological studies. More recently, several major surveys of the known carcinogenic properties of chemicals have been reported (52–54) as were a number of well-defined, two-species studies of the carcinogenic properties of many of the Kawachi-screened chemicals (57,59,60). These more recent corroborating or alternative classifications of the chemicals listed in Table V are included under the heading 'C(R)'. As with C(K), C(R)s designated '+' and '−' denote carcinogens and non-carcinogens, respectively, and the references from which the classifications were obtained are also indicated. Additional C(R) notations include '0', which indicates 'no data available' regarding carcinogenicity was reported by Palajda and Rosenkranz in their 1985 survey of ~2200 chemicals (52), and 'ARU' indicates that an 'alternative reference is unavailable', i.e. the chemical was not reported to be either a carcinogen or a non-carcinogen in references (7–11,26,37,51–53,55–64) and also was not reported to be a carcinogen in Vols 1–38 of the IARC Monographs which were recently surveyed by Vainio *et al.* (54). For chemicals having carcinogen or non-carcinogen classifications reported in both screening-test validation studies and either the animal-test studies or the surveys, the latter categories of studies are regarded as superseding the validation-study classifications and thus are the only classifications noted.

The remainder of the 182 chemicals tested by Kawachi *et al.* that are not listed in Table I–V are listed in Table VI. These are the 26 chemicals having 'NT' or 'not tested' carcinogenicity classifications in the Kawachi study which also have not been classified as carcinogens or non-carcinogens in other major Ames-test validation studies (7–11,26,37,51) or in the other studies referenced in Table V (52–64). The column designations and R and M responses are the same as in Table V, and in addition, a column of 'comments' is included in which ancillary information pertaining to several of the chemicals is noted.

The total number of chemicals listed in Tables I–VI is 175, included in which are six 'consolidated' chemicals that represent the 13 chemicals that are described in the first paragraph of this section. Thus, all of the 182 chemicals screened by Kawachi *et al.* (25) have been tested or accounted for in this study, which was one of our objectives.

Another objective of this study was to determine the predictivity of the k_e test in identifying chemical carcinogens, and several measures of this, viz. sensitivity, specificity, accuracy and predictive value, are presented in Table VII. In evaluating these predictive criteria, the carcinogenicity classifications listed under C(R) were used; if only a '0' or 'ARU' entry were available, the classification of carcinogenicity by Kawachi *et al.*

Table V. Comparison of k_e test response R[a] obtained in this work with Ames-test mutagenic response M[b] determined by Kawachi *et al.* for chemicals having carcinogenic properties C; C(K) denotes carcinogenicity classification by Kawachi *et al.*[c] and C(R) indicates a corroborating or an alternative classification of the chemical by the reference(s) noted[d]

Chemical (source[e])	CAS No.[f]	k_e[g]	R[a]	M[b]	C(K)[c]	C(R)[d]
1. Acetanilide (EK)	103-84-4	0.15	–	–	–	–[8]
2. Acetone (FS)	67-64-1	Eq'm	–	–	–	–[7,51]
3. 2-Acetylaminofluorene (Si)	53-96-3	4.0	+	+	+	+[52,53]
4. Acetylsalicylic acid (Si)	50-78-2	1.3	–	–	–	–[8]
5. Allethrin (CS)	584-79-2	2.0	–	+	–	0[52]
6. *p*-Aminoazobenzene (Al)	60-09-3	3.7	+	+	+	+[52,53]
7. *o*-Aminoazotoluene (PB)	97-56-3	3.9	+	+	+	+[52–54]
8. *p*-Aminobenzoic acid ethyl ester (Si)	582-33-2	2.6	–	+	–	ARU
9. *p*-Aminobiphenyl (Al)	92-67-1	4.0	+	+	+	+[52–54]
10. Aminopyrine (Si)	58-15-1	2.5	–	–	–	0[52]
11. Anthracene (Al)	120-12-7	2.9	–	+	–	–[55]
12. Anthranilic acid (CS)	118-92-3	0.9	–	–	NT	–[52,53,56,57]
13. Benzo[a]pyrene (EK)	50-32-8	4.0	+	+	+	+[52–54]
14. Butylated hydroxyanisole (Si)	25013-16-5	<0.05	–	–	–	0[52]
15. Butylated hydroxytoluene (Al)	128-37-0	<0.01	–	–	–	–[56]/+[52,53]
16. *n*-Butylurea (Al)	592-31-4	Eq'm	–	–	NT	–[8]
17. Captan (CS)	133-06-2	4.8	+	+	–	+[52,53,57]
18. Clofibrate (Si)	637-07-0	3.8	+	–	NT	+[58]
19. Chlorpramazine·HCl (Si)	69-09-0	3.2	+	–	–	ARU
20. Cortisone acetate (Si)	50-04-4	2.7	–	–	–	0[52]
21. Dibutylphthalate (CS)	84-74-2	2.6	–	–	–	ARU
22. Dichlorvos (CS)	62-73-7	3.3	+	+	–	–[55]/+[52,53]
23. Di-(2-ethylhexyl)phthalate (Al)	117-81-7	3.0	+	–	–	+[54,59,60]
24. Diethylstilbestrol (Si)	56-53-1	4.9	+	–	+	+[52–54]
25. Dimethoate (CS)	60-51-5	3.5	+	+	+	–[52,53,56]
26. Dimethylamino·HCl (CS)	506-59-2	<0.1	–	–	–	–[8]
27. 7,12-Dimethylbenzanthracene (CS)	57-97-6	1.7	–	+	+	+[52,53]
28. Dimpylate (Diazinon) (CS)	333-41-5	3.5	+	–	NT	–[52,53,56]
29. *N,N'*-Dinitrosopentamethylene tetramine (KK)	101-25-7	2.7	–	–	–	–[52,53]
30. Diphenyl (CS)	92-52-4	3.3	+	–	–	–[9]
31. Ethionamide (Si)	536-33-4	2.6	–	–	–	–[52,56,57]
32. Ethynylestradiol (Si)	57-63-6	<0.2	–	–	+	+[52–54]
33. Fluorene (EK)	86-73-7	3.4	+	–	–	–[7,8]
34. γ-Hexachlorocyclohexane (EK)	58-89-9	4.7	+	–	+	+[52,53]
35. *n*-Hexane (FS)	110-54-3	0	–	–	–	ARU
36. *p*-Hydroxybenzoic acid *n*-propyl ester (Si)	94-13-3	2.0	–	–	NT	–[7,8]
37. Indigo carmine (CS)	860-22-0	<0.1	–	–	–	0[52]
38. Kelthane (Dicofol) (CS)	115-32-2	4.4	+	–	NT	+[52]
39. Malathion (CS)	121-75-5	2.9	–	+	–	–[52,53,56,57,59]
40. 2-Methyl *p*-dimethylaminoazobenzene (PB)	54-88-6	<2.5	–	+	+	+[7,8]
41. 3-Methyl-*p*-dimethylaminoazobenzene (CS)	55-80-1	<2.5	–	+	+	+[7,8,37]
42. 17-Methyltestosterone (Si)	58-18-4	2.6	–	–	–	ARU
43. 1-Naphthol (Si)	90-15-3	2.0	–	–	–	–[8,9]
44. 2-Naphthol (CS)	135-19-3	1.5	–	–	–	–[8,9]
45. 1-Naphthylamine (Si)	134-32-7	3.0	+	+	–	+[52,53]
46. 2-Naphthylamine (Si)	91-59-8	3.0	+	+	+	+[52–54]
47. Nitrofurantoin (Al)	67-20-9	1.9	–	+	–	–[8,51]
48. 4-Nitroquinoline-1-oxide (Al)	56-57-5	3.0	+	+	+	+[52,53]
49. *N*-Nitrosodibutylamine (EK)	924-16-3	5.0	+	+	+	+[52–54]
50. *N*-Nitrosodimethylamine (Si)	62-75-9	4.4	+	+	+	+[52–54]
51. *N*-Nitrosodiphenylamine (EK)	86-30-6	5.6	+	–	–	+[52,53,57]
52. Phenacetin (Al)	62-44-2	<0.1	–	+	+	+[52–54]
53. Phenanthrene (CS)	85-01-8	2.3	–	+	–	–[7,37]

Chemical (source[e])	CAS No.[f]	k_e[g]	R[a]	M[b]	C(K)[c]	C(R)[d]
54. *N*-Phenyl-2-naphthylamine (CS)	135-88-6	3.7	+	−	NT	+[52,53]
55. *o*-Phenylphenol sodium salt (Fl)	132-27-4	3.2	+	−	NT	+[61]
56. Phloxine (CS)	6441-77-6	0.9	−	−	−	−[8]
57. Piperonyl butoxide (CS)	51-03-6	0.1	−	−	−	−[52,56,57]
58. Polychlorinated biphenyls (Arochlor 1254) (CS)	11097-69-1	4.7	+	−	+[h]	+[54,h]
59. Ponceau 4R (PB)	2611-82-7	<0.1	−	−	−	−[8]
60. Potassium sorbate (CS)	590-00-1	<0.1	−	−	−	ARU
61. Progesterone (Al)	57-83-0	4.6	+	−	+	+[52,53]
62. Propyl gallate (CS)	121-79-9	1.7	−	−	−	−[59,60]
63. Propylene glycol (CS)	4254-15-3	<0.01	−	−	−	ARU
64. Pyrene (Al)	129-00-0	2.9	−	+	−	−[8,63]
65. Pyridine (MCB)	110-86-1	Eq'm	−	−	−	0[52]
66. Quinoline (FS)	91-22-5	3.5	+	+	+	+[8]
67. Reserpine (Si)	50-55-5	<2.5	−	−	NT	+[52,53,57,59]
68. Rhodamine B (CS)	81-88-9	4.2	+	+	+	+[52,53]
69. Salicylic acid (CS)	69-72-7	1.5	−	−	−	−[8]
70. Sodium benzoate (CS)	532-31-1	<0.3	−	−	−	0[52]
71. Sodium dehydroacetate (PB)	4418-26-2	<0.3	−	−	−	0[52]
72. Sodium dodecylbenzenesulfonate (Fl)	2211-98-5	<0.2	−	−	−	ARU
73. Sodium lauryl sulfate (CS)	151-21-3	<0.1	−	−	−	ARU
74. Sodium nitrite (CS)	7632-00-0	<0.02	−	+	−	−[7,8]
75. Stilbene (*trans*) (Al)	103-30-0	<1.5	−	−	−	−[8]
76. Styrene (CS)	100-42-5	2.3	−	−	−	+[52,53]/−[59]
77. Succinic anhydride (Si)	108-30-5	<0.3	−	−	+	+[52,53]
78. Sunset yellow FCF (PB)	2783-94-0	<0.9	−	−	−	−[52,53,58]
79. Tartrazine (CS)	1934-21-0	<0.1	−	−	−	0[52]
80. Testosterone propionate (Si)	57-85-2	3.3	+	−	+	+[54]
81. Tetrachloroisophthalonitrile (CS)	1897-45-6	4.1	+	−	NT	+[52]
82. Thioacetamide (Si)	62-55-5	3.6	+	−	+	+[52−54]
83. Tolbutamide (Si)	64-77-7	2.5	−	−	NT	−[52,56,57]
84. Tris (2,3-dibromopropyl)phosphate (Al)	126-72-7	4.1	+	+	+	+[52−54,57]
85. Tween 60 (Si)	9005-67-8	<0.1	−	−	−	ARU
86. Tween 80 (Si)	9005-67-6	<0.1	−	−	−	ARU
87. Urethane (Si)	51-79-6	<0.1	−	−	+	+[52−54]
88. Vitamin E (Si)	59-02-9	<0.1	−	−	−	ARU

[a]R is defined as a positive response (+) for $k_e \geq k_d$, i.e. $\geq 3.0 \times 10^{12}$ M^{-1} s^{-1}; R is negative for $k_e < k_d$ and for chemicals for which an electron attachment/detachment equilibrium was observed (see footnote g).
[b]M denotes the mutagenic response of the Ames *Salmonella* bioassay as reported by Kawachi *et al.*; + and − are positive and negative Ames-test responses, respectively.
[c]C(K), classification of test chemical carcinogenicity as reported by Kawachi *et al.*: +, carcinogen; −, non-carcinogen; NT, not tested.
[d]C(R), corroborating/alternative classification of test chemical carcinogenicity reported in references referred to by superscripts; + and − as indicated in footnote c, ARU denotes 'alternative reference unavailable', 0 indicates 'no data available' in search by ref. (52) or ~2200 chemicals.
[e]Sources of test chemicals same as indicated in legend of Table III with the following additions: Fl, Fluka; FS, Fisher Scientific; KK, K&K Division of ICN and MCB-Matheson, Coleman and Bell.
[f]Chemical Abstracts Services registry number.
[g]Units of k_e are 10^{12} M^{-1} s^{-1}; 'Eq'm' indicates that an electron attachment/detachment equilibrium was observed.
[h]One of two PCBs tested in ref. (25) was the carcinogen Kanechlor 500 for which Arochlor 1254 was substituted in this study; see ref. (62).

listed under C(K) was used. Excluded from the evaluations are the three chemicals for which C(R) is '+/−'; these are chemicals V-15, 22 and 76.

The availability of the Ames-test responses from the study by Kawachi *et al.* invites that a comparison of the predictivity of the two tests be made; therefore, analogous measures of the Ames-test performance in screening the chemicals listed in Table V are also included in Table VII. We again caution, however, that the Ames-test results obtained by Kawachi *et al.* did not adhere to the standard *Salmonella*/microsome protocol prescribed by Ames *et al.* in 1975 (5) and revised in 1983 by Maron and Ames (50); therefore, re-screening the same chemicals with such protocols could significantly change the predictive performance of the Ames test.

Another comparison of the k_e and Ames-test results is made in Table VIII in which the chemicals that yielded false-positive and false-negative responses in each screening test are listed. The purpose of Table VIII is two-fold: (i) to determine if the two tests have a common basis of response which would be manifested by the test yielding similar, albeit incorrect, responses to the same

Table VI. Comparison of k_e test response R obtained in this work with Ames-test mutagenic response M determined by Kawachi *et al.* for chemicals having unknown carcinogenic properties[a]

Chemical (source[c])	CAS No.	k_e	R	M	Comments
1. Acetaminophen (Si)	103-90-2	<0.1	−	−	[b]
2. Buformin·HCl (PB)	1190-53-0	<0.1	−	−	
3. ϵ-Caprolactone (CS)	502-44-3	<0.1	−	−	[b]
4. Captafol (CS)	2425-06-1	5.8	+	+	[b]
5. Chlorpyrifos (CS)	2921-82-2	3.9	+	−	[b]
6. Curcumin (Si)	458-37-7	3.2	+	−	
7. Di-*n*-butylamine (Si)	111-92-2	<0.01	−	−	[b]
8. Ethenzamide (PB)	938-73-8	2.2	−	−	
9. Fenitrothion (CS)	122-14-5	4.7	+	+	
10. Fluorescent brightener 28 (Si)[c]	4404-43-7	<1.0	−	−	
11. Furosemide (Si)	54-31-9	<0.1	−	−	[b]
12. *p*-Hydroxybenzoic acid *n*-butyl ester (Si)	94-26-8	2.4	−	−	
13. *p*-Hydroxybenzoic acid ethyl ester (Si)	120-47-8	2.2	−	−	
14. *p*-Hydroxybenzoic acid methyl ester	99-76-3	2.5	−	−	
15. Imipramine·HCl (Si)	113-52-0	Eq'm	−	−	
16. Indomethacin (Si)	53-86-1	1.4	−	−	
17. Mefenamic acid (Si)	61-68-7	2.1	−	−	
18. *N*-Methylurea (CS)	598-50-5	Eq'm	−	−	[b]
19. Phenylbutazone (Si)	50-33-9	4.4	+	−	[b,d]
20. Rose Bengal (Si)	632-69-9	<0.2	−	−	
21. Sodium propionate (CS)	137-40-6	<0.2	−	−	
22. Theophylline (Si)	58-55-9	<2.1	−	−	
23. Thiram (CS)	137-26-8	4.2	+	+	[b]
24. Tris(2,3-dichloropropyl)phosphate (PB)	78-43-3	3.2	+	+	
25. Vitamin A acetate	127-47-9	1.3	−	+	
26. Xylitol (Si)	87-99-0	<0.1	−	−	[b]

[a]See legends of Tables III and V for explanations of column headings and +, − and Eq'm notations.
[b]Classified by Palajda and Rosenkranz as 'no data available' related to carcinogenic properites; see ref. (52).
[c]Substituted for Fluorescent brighteners 24,225 and 260 that were studied by Kawachi *et al.*(25).
[d]Reported 'not classifiable as to . . . carcinogenicity' by Vanio *et al.* (54).

Table VII. Sensitivity, specificity, accuracy and predictive value of the k_e and Ames tests for 35 carcinogens and 50 non-carcinogens listed in Table V

Predictive criteria: (definition)[a]	k_e test[b]	Ames test
Sensitivity: $\frac{\text{correct '+' responses}}{\text{total carcinogens}} \times 100$	$\frac{27}{35} \times 100 = 77\%$	$\frac{18}{35} \times 100 = 51\%$
Specificity: $\frac{\text{correct '−' responses}}{\text{total non-carcinogens}} \times 100$	$\frac{45}{50} \times 100 = 90\%$	$\frac{41}{50} \times 100 = 82\%$
Accuracy: $\frac{\text{correct '+' and '−' responses}}{\text{total chemicals}} \times 100$	$\frac{72}{85} \times 100 = 85\%$	$\frac{59}{85} \times 100 = 69\%$
Predictive value: $\frac{\text{correct '+' responses}}{\text{total '+' responses}} \times 100$	$\frac{27}{32} \times 100 = 84\%$	$\frac{18}{27} \times 100 = 67\%$

[a]For discussions of the predictive criteria, see text and refs. (21–24).
[b]Excluded from the predictive criteria calculations are chemicals V-15, 22 and 76 which are classified as C(R) = +/− in Table V.

chemicals; and (ii) to determine if the two tests could be used complementarily in or as part of a battery of short-term tests to screen carcinogens. It is evident from Table VIII that of eight false-negative k_e responses to 35 carcinogens, four were among the 17 false-negative Ames-test responses, and of five false-positive k_e responses, one was among the nine false-positive Ames-test responses.

Discussion

The results presented in the preceding section raise a myriad of questions of which each would require extended discussion to be adequately addressed. Such discussions are beyond the scope of this paper which is primarily intended to present the responses of the k_e test to a wide variety of non-preselected chemicals that

Table VIII. Comparison of k_e- and Ames-test false-negative and false-positive responses to the chemicals listed in Table V[a]

False '−' responses to carcinogens[b]			False '+' responses to non-carcinogens[b]	
k_e test	Ames test		k_e test	Ames test
27	18	58	19	5
32	23	61	**25**	8
40	24	**67**	28	11
41	**32**	**77**	30	**25**
52	34	80	33	39
67	38	81		47
77	51	82		53
87	54	**87**		64
	55			74

[a]Numbers indicated chemical number in Table V.
[b]False responses common to the k_e and Ames tests are in bold type.

include a significant number of known carcinogens and non-carcinogens. Having done this in the Results section, we confine our discussion to four areas that evoke clarification.

Alternative bases for comparison of k_e and Ames-test predictivity

The four measures of screening-test predictivity for the k_e and Ames tests which are listed in Table VII include responses to 21 chemicals for which only the non-carcinogenicity classifications by Kawachi *et al.* (25) are available. Since the problem of characterizing chemical carcinogens (64,65) and especially non-carcinogens (56,66) is particularly vexing, a better measure of the k_e and Ames-test predictivity is, perhaps, obtainable if these 21 chemicals are excluded in calculations of k_e and Ames-test specificity, accuracy and predictive value (the sensitivity of the tests is unchanged). If the three chemicals having C(R) = +/− designations are again also excluded, i.e. V−15, 22 and 76, the revised k_e test specificity is (25/29) × 100 = 86%, the accuracy is (52/64) × 100 = 81% and the predictive value is (27/31) × 100 = 87%. For comparison, the revised Kawachi-determined Ames-test specificity is (22/29) × 100 = 76%, the accuracy is (40/64) × 100 = 63% and the predictive value is 72%.

The differences between these measures of predictivity and those calculated using the 85 chemicals in Table V is insignificant, but a second alternative analysis is also presented to circumvent the problem that Kawachi *et al.* did not adhere to standard Ames-test protocol in their screening of test chemicals. Rather than using the Kawachi-determined Ames-test responses listed in Table VII, those reported in the data base of Palajda and Rosenkranz (52) can be substituted and the corresponding k_e responses compared. In so doing, the number of screened chemicals listed in Table VII is reduced from 88 to 41 of which 24 are carcinogens and 17 are non-carcinogens. The recalculated k_e and Ames-test predictive criteria are, respectively, sensitivity: (17/24) × 100 = 71% versus (13/24) × 100 = 54%; specificity: (15/17) × 100 = 88% versus (15/17) × 100 = 88%; accuracy: (32/41) × 100 = 78% versus (28/41) × 100 = 68%; and predictive value: (17/19) × 100 = 89% versus (13/15) × 100 = 87%. Also, one additional comparison of these Ames-test responses can be made which is with the full data base in the Palajda−Rosenkranz survey. For the Ames test, this includes 170 carcinogens and 31 non-carcinogens for which the following were reported: sensitivity, 61%, specificity, 81%; and accuracy, 64% (52). From these results we calculate an Ames-test predictive value of 95%. The conclusion that we draw from these analyses is that the k_e test compares favorably with the Ames test as a screen for chemical carcinogens.

Use of the k_e test in a battery of short-term tests

Recognition that no single *in vitro* screening test for carcinogens can serve as a reliable surrogate for animal testing has prompted the development of methods to choose the most efficacious combination of short-term tests for use in a carcinogen-screening test battery. Chankong *et al.* recently reviewed this area in describing their application of Bayes' theorem to the problem and the Carcinogenicity Prediction and Battery Selection (CPBS) method that emerged (67). In both the CPBS method and other designs of test batteries (68,69), the need for component tests of the battery to be independent is important. One method of quantitatively estimating the test independence is through a χ^2 procedure, and applying such a statistical analysis to the 35 carcinogens and 50 non-carcinogens listed in Table V in a manner analogous to that done by Poulsen and Heinze (68) yields respective χ^2 values of 0.099 ($P \sim 75\%$) and 0.24 ($P \sim 50\%$). We caution that the limited sample size makes this analysis of questionable significance and, therefore, have attempted to demonstrate the independence of the k_e and Ames tests qualitatively with Table VIII. Since only four of 25 false-negative and one of 14 false-positive responses are common to the two tests, we surmise that the k_e and Ames tests exhibit a high degree of independence.

Rationale of the k_e−carcinogenicity correlation

Using electrons to probe the electrophilicity of biologically important chemicals is not a novel concept as is demonstrated by the gas-phase studies of Lovelock's group who measured the affinities of polyaromatic hydrocarbons (PAHs) (70) and steroids (71) for thermal electrons 25 years ago. From these studies, Lovelock *et al.* suggested that the electron-reaction properties of the PAHs were associated with their carcinogenic properties and that of the steroids with their role in oxidative metabolism. These gas-phase measurements and theoretical studies related to electron−carcinogen correlations (72,73) were based on Szent-Györgyi's theory concerning the involvement of electrons in the mechanism of carcinogenesis (74). Much of this work on the biological implications of electron-related studies has been reviewed by Christophorou who also suggested a toxicity index of chemicals based on their electron-capture properties (75−77).

Advances in chemical-purification and electron-monitoring techniques during the past 15 years have combined to permit the routine study of the transport and reaction properties of excess electrons in a variety of media (78). One of the most striking observations in such studies is the marked dependence of u_e and k_e on the structure of the medium; for example, in chemically similar methane and ethane at the same temperature, the u_es differ by a factor of >300 000 (79,80) and the k_es of SF_6 differ by >15 000 (14). Such structural dependence of u_e and k_e prompted conjecture that excess electrons in highly structured biological systems have u_es and k_es more than a 1000-fold greater than those of solvated electrons (81). Although numerous attempts have been made to measure the electron-transport properties of a variety of biomolecules (82), no physico-chemical technique is currently available to measure the u_e of cellular components in their native conformation. The best estimate of the u_e of structured biomolecules appears to be u_e in frozen DNA solutions which was measured to range between 0.3 and 15 cm^2/Vs by Van Lith *et al.* using a PC technique (83).

Several highly structured cellular components in addition to

nuclear DNA have been associated with the initiating step of carcinogenesis; these include mitochondrial DNA (84,85), chromatin and the nuclear matrix (86), intercellular junctions (87) and several components of the cytoskeleton (88). The electron-transport properties of these biomolecules are unknown but should more closely approximate frozen DNA than bulk water in which μ_e is 1.8×10^{-3} cm²/Vs (89). Thus, the microenvironment at the site at which interaction between a carcinogen and the target biomolecule occurs should be better simulated with regard to electron transfer by cyclohexane (μ_e = 0.22 cm²/Vs, ref. 32) than by water. Additional evidence that supports this hypothesis includes studies of enzyme catalysis in organic solvents (90,91), a new hypothesis concerning enzyme reactions (92) and recognition among organic chemists that single-electron-transfer processes are more ubiquitous than previously supposed (93).

Rationale of positive responses of the k_e test to unactivated procarcinogens

Among the 35 carcinogens tested in this study are many procarcinogens that require metabolic activation to a more electrophilic state in order for the initiating step of carcinogenesis to occur (1–3). In the standard Ames-test protocol, rat-liver microsomes perform this metabolic-activation function (5,50) with varying degrees of efficiency (94), and a number of other activating systems have been used in attempts to improve the Ames-test accuracy (95). The unactivated procarcinogen that is biologically inert with respect to mutagenesis in the Ames tester strains as well as with respect to carcinogenesis in the test animal yields a positive k_e test response.

Our explanation of this apparent paradox is that an excess electron in cyclohexane is simply a more sensitive probe for measuring the capacity of a procarcinogen to interact with a target biomolecule than is the biomolecule itself. Restating this with reference to the k_e and Ames tests, the excess electron in cyclohexane is a better nucleophile than is the DNA of the Ames histidine-deficient auxotrophs since the latter require that the procarcinogen be oxidatively metabolized to a more electrophilic state in order for DNA–carcinogen interaction to occur. In contrast, the excess electron in cyclohexane attaches to the procarcinogen at the first encounter. In chemical physics terminology, the conduction-band energy of the electron in cyclohexane matches the maximum in the distribution function of the unoccupied electronic levels of the acceptor molecule (96), which is the unactivated procarcinogen. This matching of the donor–acceptor levels does not occur in the Ames tester strains or the test animal until metabolic activation changes the distribution of the unoccupied electron levels in the acceptor molecule.

In conclusion, we again quote Bridges who stated in his review of short-term screening tests that 'even an empiricial "litmus paper" test, with no known theoretical basis, which gave an 80 to 90% predictiveness for carcinogenicity would be a powerful tool in the screening of chemicals for human toxicity' (12). The k_e test appears to meet Bridges' lower limit of predictiveness and progress is being made in applying this 'litmus paper' to screen a wider variety of carcinogens from which a better understanding of its theoretical basis should evolve.

Acknowledgements

We thank Dr F.K.Ennever for discussions pertaining to classifying the carcinogenicity of the chemicals tested, R.Daniels and V.Rodriguez for h.p.l.c. analyses and B.Aldridge for preparation of the manuscript. This work was supported by the National Institute for Occupational Safety and Health (Grant R01-OH-01331), the US Department of Energy (Contract DE-AC02-78EV04746) and the National Cancer Institute (2 P30 CA 19283-07).

References

1. Miller,J.A. and Miller,E.C. (1971) Chemical carcinogenesis: mechanisms and approaches to its control. *J. Natl. Cancer Inst.*, **47**, v–xiii.
2. Miller,E.C. and Miller,J.A. (1976) The metabolism of chemical carcinogens to reactive electrophiles and their possible mechanisms of action in carcinogenesis. In Searle,C.E. (ed.), *Chemical Carcinogens*, Am. Chem. Soc. Monog. 173. Am. Chem. Soc., Washington, DC, pp. 737–762.
3. Miller,E.C. and Miller,J.A. (1981) Mechanisms of chemical carcinogenesis. *Cancer*, **47**, 1055–1064.
4. Ames,B.N. (1972) A bacterial system for detecting mutagens and carcinogens. In Sutton,E. and Harris,M. (eds), *Mutagenic Effects of Environmental Contaminants*. Academic Press, New York, pp. 57–66.
5. Ames,B.N., McCann,J. and Yamasaki,E. (1975) Methods of detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test. *Mutat. Res.*, **31**, 347–364.
6. McCann,J. and Ames,B.N. (1976) Detection of carcinogens as mutagens in the *Salmonella*/microsome test: assay of 300 chemicals: discussion. *Proc. Natl. Acad. Sci. USA*, **73**, 950–954.
7. McCann,J., Choi,E., 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.
8. Sugimura,T., Sato,S., Nagao,M., Yahagi,T., Mutsushima,T., Sieno,Y., Takeuchi,M. and Kawachi,T. (1976) Overlapping of carcinogens and mutagens, In Magee,P.N., Takayama,S., Sugimura,T. and Matsushima,T. (eds), *Fundamentals in Cancer Prevention*. University Park Press, Baltimore, MD. pp. 191–215.
9. Purchase,I.F.H., Longstaff,E., Ashby,J., Styles,J.A., Anderson,D., Lefevre,P.A. and Westwood,R.F. (1978) An evaluation of 6 short-term tests for detecting organic chemical carcinogens. *Br. J. Cancer*, **37**, 873–903.
10. Rosenkranz,H.S. and Poirier,L.A. (1979) Evaluation of the mutagenicity and DNA-modifying activity of carcinogens and noncarcinogens in microbial systems. *J. Natl. Cancer Inst.*, **62**, 873–891.
11. Simnon,V. (1979) *In vitro* mutagenicity assays of chemical carcinogens and related compounds with *Salmonella typhimurium*. *J. Natl. Cancer Inst.*, **62**, 893–899.
12. Bridges,B.A. (1976) Short term screening tests for carcinogens. *Nature*, **261**, 195–200.
13. Bakale,G., Gregg,E.C. and McCreary,R.D. (1972) Decay of quasifree electrons in pulse-irradiated liquid hydrocarbons. *J. Chem. Phys.*, **57**, 4246–4254.
14. Bakale,G., Sowada,U. and Schmidt,W.F. (1975) Electron attachment to sulfur hexafluoride in nonpolar liquids. *J. Phys. Chem.*, **79**, 3041–3044.
15. Bakale,G., Sowada,U. and Schmidt,W.F. (1976) Effect of an electric field on electron attachment to SF_6, N_2O and O_2 in liquid argon and xenon. *J. Phys. Chem.*, **80**, 2556–2559.
16. Bakale,G., Gregg,E.C. and McCreary,R.D. (1977) Electron attachment to nitro compounds in liquid cyclohexane. *J. Chem. Phys.*, **67**, 5788–5794.
17. Bakale,G., Sowada,U. and Schmidt,W.F. (1977) Negative ion-molecule reactions in liquid argon following electron capture by N_2O. *Can. J. Chem.*, **55**, 2220–2224.
18. Bakale,G., McCreary,R.D. and Gregg,E.C. (1981) Quasifree electron attachment to carcinogens in liquid cyclohexane. *Cancer Biochem. Biophys.*, **5**, 103–109.
19. 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.
20. Bakale,G., McCreary,R.D. and Gregg,E.C. (1983) A pulse-conductivity screening test for carcinogens. *Environ. Mutagenesis*, **5**, 471–472.
21. Cooper,J.A.,II, Saracci,R. and Cole,P. (1979) Describing the validity of carcinogen screening tests. *Br. J. Cancer*, **39**, 87–89.
22. Bartsch,H , Malaveille,C., Camus,A.-M., Brun,G. and Hautefeuille,A. (1980) Validity of bacterial short-term tests for the detection of chemical carcinogens. In Norprath,K.H. and Garner,R.C. (eds), *Short Term Tests Systems for Detecting Carcinogens*. Springer-Verlag, Berlin, pp. 58–73.
23. Purchase,I.F.H. (1982) An appraisal of predictive tests for carcinogenicity. *Mutat. Res.*, **99**, 53–71.
24. Rosenkranz,H.S., Klopman,G., Chankong,V., Pet-Edwards,J. and Haimes, Y.Y. (1984) Prediction of environmental carcinogens: a strategy for the mid-1980s. *Environ. Mutagenesis*, **6**, 231–258.
25. Kawachi,T., Yahagi,T., Kada,T., Tazima,Y., Ishidate,M., Sasaki,M. and Sugiyama,T. (1980) Cooperative programme on short-term assays for carcinogenicity in Japan. In Montesano,R., Bartsch,H., Tomatis,L. and Davis,W. (eds), *Molecular and Cellular Aspects of Carcinogen Screening Tests*. International Agency for Research on Cancer, Lyon, pp. 323–330.
26. Bartsch,H., Malaveille,C., Camus,A.-M., Martel-Planche,G., Brun,G., Hautefeuille,A., Sabadie,N., Barbin,A., Kuroki,T., Drevon,C., Piccoli,C. and Montesano,R. (1980) Validation and comparative studies of 180 chemicals

with *S. typhimurium* strains and V79 Chinese hamster cells in the presence of various metabolizing systems. *Mutat. Res.*, **76**, 1–50.
27. Jortner,J. and Gaathon,A. (1977) Effects of phase density on ionization processes and electron localization in fluids. *Can. J. Chem.*, **55**, 1801–1819.
28. Kenney-Wallace,G.A. (1978) Electrons in molecular clusters: microscopic probes of fluids. *Acc. Chem. Res.*, **12**, 433–439.
29. Munoz,R.C., Holroyd,R.A. and Nishikawa,M. (1985) Effect of high pressure on the electron mobility in liquid *n*-hexane, 2,2-dimethylbutane and tetramethylsilane. *J. Phys. Chem.*, **89**, 2969–2972.
30. Bakale,G. and McCreary,R.D. (1984) A pulse-radiolysis technique for screening carcinogens. In *Book of Abstracts, 188th ACS National Meeting*. American Chemical Society, Washington, DC, abstract PHYS-98.
31. Bakale,G. (1986) A physico-chemical technique for identifying chemical carcinogens. In Politzer,P. and Parkanyi,C. (eds), *Meeting Report on Eighth Interdisciplinary Cancer Research Workshop. Cancer Res.*, **46**, 1566–1568.
32. Allen,A.O., Gangwer,T.E. and Holroyd,R.A. (1975) Chemical reaction rates of quasifree electrons. II. *J. Phys. Chem.*, **79**, 25–30.
33. Allen,A.O., de Haas,M.P. and Hummel,A. (1976) Measurement of ionic mobilities in dielectric liquids by means of concentric cylindrical electrodes. *J. Chem. Phys.*, **64**, 2587–2592.
34. Wada,T., Shinsaka,K., Namba,H. and Hatano,Y. (1977) Electron reactivity in liquid hydrocarbon mixtures. *Can. J. Chem.*, **55**, 2144–2155.
35. Beck,G. and Thomas,J.K. (1972) Dynamics of electrons in nonpolar liquids. *J. Chem. Phys.*, **57**, 3649–3653.
36 Baxendale,J.H. (1977) A pulse radiolysis study of solvated electrons in dilute solutions of polar liquids in nonpolar solvents. *Can. J. Chem.*, **55**, 1996–2002.
37. Poirier,L.A. and Weisburger,E.K. (1979) Selection of carcinogens and related compounds tested for mutagenic activity. *J. Natl. Cancer Inst.*, **62**, 833–840.
38. Stecher,P.G., Windholz,M., Leahy,D.S., Bolton,D.M. and Eaton,L.G. (1968) *The Merck Index*, 8th Edn., Merck, Rahaway, NJ.
39. Weast,R.C., Selby,S.M. and Hodgman,C.D. (1964) *Handbook of Chemistry and Physics*. 45th Edn., Chemical Rubber Co., Cleveland, OH.
40. International Agency for Research on Cancer (1976) *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man. Vol. 11. Cadmium, Nickel, Some Epoxides, Miscellaneous Industrial Chemicals and General Considerations on Volatile Anaesthetics*. IARC, Lyon, pp. 131–139.
41. International Agency for Research on Cancer (1972) *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man. Vol. 1. Some Inorganic Substances, Chlorinated Hydrocarbons, Aromatic Amines, N-Nitroso Compounds and Natural Products*. IARC, Lyon, pp. 125–134.
42. International Agency for Research on Cancer (1974) *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man. Vol. 4. Some Aromatic Amines, Hydrazine and Related Substances, N-Nitroso Compounds and Miscellaneous Alkylating Agents*. IARC, Lyon, pp. 253–265.
43. Weston,R.E.,Jr and Schwarz,H.A. (1972) Reactions in solution. In Johnston,H.S. (ed.), *Fundamental Topics in Physical Chemistry: Chemical Kinetics*. Prentice-Hall, Englewood Cliffs, NJ, pp. 151–187.
44. Hummel,A. (1974) Ionization in nonpolar molecular liquids by high-energy electrons. In Burton,M. and Magee,J.L. (eds), *Advances in Radiation Chemistry*, John Wiley, New York, Vol. 4, pp. 1–102.
45. Baird,J.K. (1977) Kinetics of electron capture by SF_6 in solution. *Can. J. Chem.*, **55**, 2133–2143.
45. Holroyd,R.A., Gangwer,T.E. and Allen,A.O. (1975) Chemical reaction rates of quasi-free electrons in non-polar liquids. The equilibrium $CO_2 + e^- \rightleftharpoons CO_2^-$. *Chem. Phys. Lett.*, **31**, 520–523.
47. Warman,J.M., de Haas,M.P., Zador,E. and Hummel,A. (1975) Concerning the equilibrium e^- + biphenyl ⇌ biphenyl$^-$ in liquid tetramethylsilane. *Chem. Phys. Lett.*, **35**, 383–386.
48. Holroyd,R.A. (1977) Equilibrium reactions of excess electrons with aromatics in non-polar solvents. *Ber. Bunseges, Phys. Chem.*, **81**, 298–304.
49. Holroyd,R.A., McCreary,R.D. and Bakale,G. (1979) Reversible reaction of excess electrons with *p*-difluorobenzene in *n*-hexane and cyclohexane. *J. Phys. Chem.*, **83**, 435–439.
50. Maron,D.M. and Ames,B.N. (1983) Revised methods for the *Salmonella* mutagenicity test. *Mutat. Res.*, **113**, 173–215.
51. DeFlora,S., Zanacchi,P., Camoirano,A., Bennicelli,C. and Badolati,G.S. (1984) Genotoxic activity and potency of 135 compounds in the Ames reversion test and in a bacterial DNA-repair test. *Mutat. Res.*, **133**, 161–198.
52. Palajda,M. and Rosenkranz,H.S. (1985) Assembly and preliminary analysis of a genotoxicity data base for predicting carcinogens. *Mutat. Res.*, **153**, 79–134.
53. Upton,A.C., Clayson,D.B., Jansen,J.D., Rosenkranz,H.S. and Williams, G.M. (1984) Report of ICPEMC Task Group 5 on the differentiation between genotoxic and non-genotoxic chemicals. *Mutat. Res.*, **133**, 1–49.
54. Vainio,H., Hemminki,K. and Wilbourn,J. (1985) Data on the carcinogenicity of chemicals in the *IARC Monographs* programme. *Carcinogenesis*, **6**, 1653–1665.
55. International Agency for Research on Cancer (1983) *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man. Vol. 32. Polynuclear Aromatic Compounds. Part 1. Chemical, Environmental and Experimental Data*. IARC, Lyon, pp. 105–121.
56. Shelby,M.D. and Stasiewicz,S. (1984) Chemicals showing no evidence of carcinogenicity in long-term, two-species rodent studies: the need for short-term test data. *Environ. Mutagenesis*, **6**, 871–878.
57. Chu,K.C., Cueto,C.,Jr and Ward,J.M. (1981) Factors in the evaluation of 200 National Cancer Institute carcinogen bioassays. *J. Toxicol. Environ. Health*, **8**, 251–280.
58. Svoboda,D.J. and Azarnoff,D.L. (1979) Tumors in male rats fed ethyl chlorophenoxyisobutyrate, a hypolipidemic drug. *Cancer Res.*, **39**, 3419–3428.
59. Tennant,R.W., Stasiewicz,S. and Spalding,J.W. (1986) Comparison of multiple parameters of rodent carcinogenicity and *in vitro* genetic toxicity. *Environ. Mutagenesis*, **8**, 205–227.
60. Haseman,J.K., Crawford,D.D., Huff,J.E., Boorman,G.A. and McConnell, E.E. (1984) Results from 86 two-year carcinogenicity studies conducted by the National Toxicology Program (1984). *J. Toxicol. Environ. Health*, **14**, 621–639.
61. International Agency for Research on Cancer (1982) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Vol. 30. Miscellaneous Pesticides*. IARC, Lyon, pp. 329–344.
62. International Agency for Research on Cancer (1978) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Vol. 18. Polychlorinated Biphenyls and Polybrominated Biphenyls*. IARC, Lyon, pp. 43–103.
63. International Agency for Research on Cancer (1983) *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man. Vol. 32. Polynuclear Aromatic Compounds. Part 1. Chemical, Environmental and Experimental Data*. IARC, Lyon, pp. 431–440.
64. Griesemer,R.A. and Cueto,C.Jr (1980) Toward a classification scheme for degree of experimental evidence for the carcinogenicity of chemicals in animals. In Montesano,R., Bartsch,H., Tomatis,L. and Davis,W. (eds), *Molecular and Cellular Aspects of Carcinogen Screening Tests*. IARC, Lyon, pp. 259–281.
65. Interdisciplinary Panel on Carcinogenicity (1984) Criteria for evidence of chemical carcinogenicity. *Science*, **225**, 682–687.
66. Clayson,D.B. and Crewski,D. (1986) The concept of negativity in experimental carcinogenesis. *Mutat. Res.*, **167**, 233–240.
67. Chankong,V., Haimes,Y.Y., Rosenkranz,H.S. and Pet-Edwards,J. (1985) The carcinogenicity prediction and battery selection (CPBS) method: a Bayesian approach. *Mutat. Res.*, **153**, 135–166.
68. Heinze,J.E. and Poulsen,N.K. (1983) The optimal design of batteries of short-term tests for detecting carcinogens. *Mutat. Res.*, **117**, 259–269.
69. Ashby,J., de Serres,F.J., Draper,M.H., Ishidate,M., Margolin,B., Matter,B.E. and Shelby,M.D. (1985) Evaluation of short-term tests for carcinogens. *Prog. Mutat. Res.*, **5**, 3–7, 117–174.
70. Lovelock,J.E., Zlatkis,A. and Becker,R.S. (1962) Affinity of polycyclic aromatic hydrocarbons for electrons with thermal energies: its possible significance in carcinogenesis. *Nature*, **193**, 540–541.
71. Lovelock,J.E., Simmonds,P.G. and Vandenheuvel,W.J.A. (1963) Affinity of steroids for electrons with thermal energies. *Nature*, **197**, 249–251.
72. Mason,R. (1958) Electron mobility in biological systems and its relation to carcinogenesis. *Nature*, **181**, 820–822.
73. Pullman,A. and Pullman,B. (1962) From quantum chemistry to quantum biochemistry. In Kasha,M. and Pullman,B. (eds), *Horizons in Biochemistry. Albert Szent-Gyorgyi Dedicatory Volume*. Academic Press, New York, pp. 553–599.
74. Szent-Györgyi,A. (1960) *Introduction to Submolecular Biology*. Academic Press, New York.
75. Christophorou,L.G. (1971) *Atomic and Molecular Radiation Physics*. Wiley-Interscience, New York.
76. Christophorou,L.G. (1980) Negative ions of polyatomic molecules. *Environ. Health Persp.*, **36**, 3–32.
77. Christophorou,L.G. and Hunter,S.R. (1984) From basic research to application. In Christophorou,L.G. (ed.), *Electron Molecule Interactions and Their Applications*. Academic Press, New York, Vol. 2, pp. 317–422.
78. Warman,J.M. (1982) The dynamics of electrons and ions in non-polar liquids. In Baxendale,J.H. and Busi,F. (eds), *The Study of Fast Processes and Transient Species by Electron Pulse Radiolysis*. Reidel, Dordrecht, pp. 433–533.
79. Bakale,G. and Schmidt,W.F. (1973) Excess electrons and positive charge carriers in liquid methane. *Z. Naturforsch.*, **28a**, 511–518.
80. Schmidt,W.F., Bakale,G. and Sowada,U. (1974) Excess electrons and positive charge carriers in liquid ethane. *J. Chem. Phys.*, **61**, 5275–5278.
81. Bakale,G. and Gregg,E.C. (1978) Conjecture on the role of dry charges in radiosensitization. *Br. J. Cancer*, **73** (Suppl. III), 24–28.
82. Pethig,R. (1979) *Dielectric and Electronic Properties of Biological Molecules*.

John Wiley, Chichester, UK.
83. Van Lith,D., de Haas,M.P., Warman,J.M. and Hummel,A. (1983) Highly mobile charge carriers in hydrated DNA and collagen formed by pulsed ionization. *Biopolymers*, **22**, 807–810.
84. Backer,J.M. and Weinstein,I.B. (1980) Mitochondrial DNA is a major cellular target for a dihydrodiol-epoxide derivative of benzo[a]pyrene. *Science*, **209**, 297–299.
85. Allen,J.A. and Coombs,M.M. (1980) Covalent binding of polycyclic aromatic compounds to mitochondrial and nuclear DNA. *Nature*, **287**, 244–245.
86. Obi,F.O., Ryan,A.J. and Billett,M.A. (1986) Preferential binding of the carcinogen benzo[a]pyrene to DNA in active chromatin and the nuclear matrix. *Carcinogenesis*, **7**, 907–913.
87. Tachikawa,T., Kohno,Y., Matsui,Y. and Yoshiki,S. (1986) *In vitro* early changes in intercellular junctions by treatment with a chemical carcinogen. *Carcinogenesis*, **7**, 885–892.
88. Ben-Ze'ev,A. (1985) The cytoskeleton in cancer cells. *Biochim. Biophys. Acta*, **780**, 197–212.
89. Schmidt,K.H. and Buck,W.L. (1966) Mobility of the hydrated electron. *Science*, **151**, 70–71.
90. Carnbou,B. and Klibanov,A.M. (1984) Preparative production of optically active esters and alcohols using esterase-catalyzed stereospecific transesterification in organic media. *J. Am. Chem. Soc.*, **106**, 2687–2692.
91. Zaks,A. and Klibanov,A.M. (1985) Enzyme-catalyzed processes in organic solvents. *Proc. Natl. Acad. Sci. USA*, **82**, 3192–3196.
92. Dewar,M.J.S. and Storch,D.M. (1985) Alternative view of enzyme reactions. *Proc. Natl. Acad. Sci. USA*, **82**, 2225–2229.
93. Pross,A. (1985) The single electron shift as a fundamental process in organic chemistry: the relationship between polar and electron-transfer pathways. *Acc. Chem. Res.*, **18**, 212–219.
94. Bigger,C.A.H., Tomaszewski,J.E., Dipple,A. and Lake,R.S. (1980) Limitations of metabolic activation systems used with *in vitro* tests for carcinogens. *Science*, **209**, 503–505.
95. Phillipson,C.E. and Ionnides,C. (1983) Activation of aromatic amines to mutagens by various animal species including man. *Mutat. Res.*, **124**, 325–336.
96. Henglein,A. (1977) Electron reactivity as a function of phase. *Can. J. Chem.*, **55**, 2112–2123.

Received on 18 July 1986; accepted on 30 October 1986