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Transformation of BALB/c-3T3 Cells: V. Transformation Responses of 168 Chemicals Compared with Mutagenicity in Salmonella and Carcinogenicity in Rodent Bioassays

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This report describes the activities of 168 chemicals tested in a standard transformation assay using A-31-1-13 BALB/c-3T3 cells. The data set includes 84 carcinogens, 77 noncarcinogens, and 7 research chemicals. Carcinogens included 49 mutagens and 35 nonmutagens; noncarcinogens included 24 mutagens and 53 nonmutagens. The transformation assay did not use an exogenous activation system, thus, all chemical responses depended on the inherent target cell metabolic capacity where metabolic activation was required. The upper dose limit was 100 milli-osmolar because the assay could not discriminate active and inactive chemicals tested above this concentration. Certain physicochemical properties resulted in technical problems that affected chemical biological activity. For example, chemicals that reacted with plastic were usually nonmutagenic carcinogens. Similarly, chemicals that were insoluble in medium, or bound metals, were usually nonmutagenic and nontransforming.

Multifactorial data analyses revealed that the transformation assay discriminated between nonmutagenic carcinogens and noncarcinogens; it detected 64% of the carcinogens and only 26% of the noncarcinogens. In contrast, the transformation assay detected most mutagenic chemicals, including 94% of the mutagenic carcinogens and 70% of the mutagenic noncarcinogens. Thus, transformation or Salmonella typuimurium mutagenicity assays could not discriminate mutagenic carcinogens from mutagenic noncarcinogens. Data analyses also revealed that mutagenic chemicals were more cytotoxic than nonmutagenic chemicals; 88% of the mutagens had an LD_{50} < 5 mM, whereas half of the nonmutagens had an LD_{50} > 5 mM. Binary data analyses of the same data set revealed that the transformation assay and rodent bioassay had a concordance of 71%, a sensitivity for carcinogens of 80.0%, and a specificity for detecting noncarcinogens of 60%. In contrast, Salmonella mutagenicity assays and rodent bioassays had a concordance of 63%, a sensitivity of 58%, and a specificity of 69%. The transformation assay complemented the Salmonella mutagenesis assay in the identification of nonmutagenic carcinogens; thus, the two assays had a combined 83% sensitivity for all carcinogens and a 75% specificity for nonmutagenic noncarcinogens.

Introduction

Recent investigations supported by the National Toxicology Program (NTP) have revealed that many chemical carcinogens were not detected in Salmonella typhimurium mutagenesis assays (*1–4*). These carcinogens have been operationally classified as either nongenotoxic or nonmutagenic carcinogens, based on their activity in the Salmonella assay (*1–4*). While some of the nonmutagenic carcinogens induced chromosomal aberrations (ABS) and sister chromatid exchanges (SCE) in Chinese hamster ovary cells (CHO), or $TK^{+/-}$ mutations in mouse lymphoma (ML) L5178Y cells, the chemicals were not consistently active in all three assays (*4*). Furthermore, the three genotoxicity assays all detected as many nonmutagenic noncarcinogens as nonmutagenic carcinogens (*4*). Thus, there is a continuing need to develop a short term, *in vitro* assay with which to selectively characterize nonmutagenic carcinogens.

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The National Institute of Environmental Health Sciences (NIEHS) has supported research programs using different cell transformation assay systems because such assays demonstrate phenotypes that respond to carcinogen treatments and mimic certain events in the multistep process of chemical carcinogenesis *in vivo* (*5–8*). The BALB/c-3T3 transformation assay was one of the assays evaluated because chemical-induced morphologically transformed cells are easily recognized and induced at relatively high frequencies in this assay (*7,9–12*). Furthermore, normal BALB/c-3T3 cells have been demonstrated to be biologically different from chemical-induced transformed cells isolated from a type III focus. Whereas normal BALB/c-3T3 cells were nonmalignant and grow at low frequencies in soft agar, transformed cells readily grew in soft agar and were tumorigenic *in vivo* (*7,11,13*).

This report summarizes the results we obtained in testing 168 chemicals in a standard BALB/c-3T3 cell transformation assay protocol. The protocol was developed in this laboratory (*10,14*), and it differed substantially from the method first described by Kakunaga (*7*) and that currently recommended by government agencies (*6,15*). Our method modified the standard assay design to enhance the sensitivity for detection of chemical-induced transformation (*14,16*). The improved sensitivity was achieved without using an exogenous activation system; thus, all chemical responses were dependent on the inherent metabolic capability of the BALB/c-3T3 cells. Each chemical was tested in two or more experiments, and a total of 110 experiments were conducted over a 2.5-year period. The majority of the 168 test chemicals were selected from the NTP database of 301 chemicals tested in rodent bioassay (*3*); thus, the chemical structures and biological activities of most of these chemicals in several assay systems was readily available for comparative analyses (*1–4*).

The 168 test chemicals in this data set included comparable numbers of chemicals with three different biological activities (carcinogenicity, cytotoxicity, and mutagenicity). For example, the data set contained roughly equal numbers of carcinogens and noncarcinogens, as well as mutagenic and nonmutagenic chemicals. Furthermore, this data set also included many examples of nonmutagenic carcinogens as well as mutagenic noncarcinogens. Currently both groups of chemicals reduce our ability to predict carcinogenicity in rodents using *in vitro* tests for genotoxicity. Finally, this set contained many examples of cytotoxic and noncytotoxic chemicals that differed in their carcinogenic and mutagenic activities. The only chemicals tested in the assay that were omitted from this paper were 24 chemicals with unknown carcinogenicity, which were reported separately (*14*), 10 polycyclic aromatic hydrocarbons (unpublished data), and 21 test chemicals with a unique technical problem. The 21 chemicals rapidly reacted with plastic culture vessels at treatment dose concentrations that were tested for transforming activity and will have to be evaluated separately in a chemical-resistant culture vessel. Taken together, none of the test chemical responses detected during this investigation were either selectively, or arbitrarily, omitted from this report.

This study included a major effort to determine the appropriate upper dose limit for the BALB/c-3T3 cell transformation assay and to investigate the relationship of chemical cytotoxicity to transformation, mutagenicity, and carcinogenicity. Currently most *in vitro* genotoxicity assays using cultured mammalian cells employ an arbitrary upper dose limit of 5–10 mg/mL. This decision creates two problems. Because test chemicals have widely different molecular weights, the 5–10 mg/mL limit represents a high physiological treatment dose for some chemicals and a relatively lower dose for other chemicals. We avoided this problem by analyzing chemical activities in terms of treatment doses expressed in millimolar (mM) concentrations. In addition, the use of an arbitrary dose limit inhibits one from determining the actual upper dose limit. For the purpose of this investigation, the actual upper dose limit of an assay was defined as the dose at which the assay could not discriminate active and inactive test chemicals. This upper dose limit can only be determined when all chemicals are tested at comparable ranges of cytotoxic responses. This report will provide evidence that the actual upper dose limit for noncytotoxic test chemicals was equivalent to a treatment dose of 100 milliosmolar (mOsM).

The statistical methods used in this report to evaluate the activities of chemicals in one or more experiments, as well as those used to weight and rank-order chemical transformation responses, have been described previously (*17–18*). These methods were developed because transformation experiments had different statistical sensitivities (*17*) and different detection sensitivities for chemical-induced transformation (*18*). The statistical weighting procedures used mean and rank t-statistics (*18*), and these methods solved three data analysis problems. First, statistically weighted chemical responses provided an unbiased method for comparing responses in two independent experiments and could be used to determine whether chemical activities detected in two consecutive experiments were reproducible. Second, the statistical weighting procedure provided an easy and unbiased method for combining the data for a chemical tested in two or more trials. Third, rank-ordered and statistically weighted chemical responses provide a very sensitive means of comparing biological activities of small sets of chemicals.

This report does not present a single table with all of the test chemicals and their transformation responses. A binary presentation of positive and negative test chemical responses was too simplistic and masked the multifactorial activities of chemicals in this database. Thus, binary procedures were only used to demonstrate that the data set had a comparable distribution of chemicals to that of other NTP data sets. In contrast, multifactorial procedures were used to compare the activities of chemicals that shared selected biological activities. Multifactorial comparisons of groups of chemicals were examined for many different correlations between biological properties before they were presented in the format of the tables contained herein.

Materials and Methods

Cell Culture

The investigations in this report used the A31-1-13 clone of BALB/c-3T3 cells (*19,20*). The materials and methods used to culture the cells have been previously reported in detail (*10*) and are summarized in part I of these investigations (*17*).

Standard Clonal Survival Assay

The standard clonal survival assay was used to *a*) estimate the cytotoxic activity of a test chemical, *b*) select treatment doses for the preliminary co-culture clonal survival assay, *c*) assess the reproducibility of the chemical-induced cytotoxic responses, and *d*) determine the relative shift in test chemical cytotoxic responses between high- and low-density cell cultures. The standard clonal survival assay using low-density cultures of BALB/c-3T3 cells was conducted according to our modification (*10,14*) of the method described by Kakunaga (*7*). Briefly, 200 wild type (WT) cells were seeded in either 60-mm culture dishes (or 25-cm^2 culture flasks), and chemical treatment doses were applied to triplicate cultures for 48 hr beginning 2 days after seeding. After a total culture period of 8 days, the vessels were washed, fixed in methanol, stained with Giemsa, and colonies of cells were hand tabulated according to the procedure described in part IV of these investigations (*14*).

Co-culture Clonal Survival Assay

The co-culture clonal survival assay was used to *a*) select chemical treatment doses for transformation assays, *b*) assess the reproducibility of chemical-induced cytotoxic responses, and *c*) verify that the test chemical and positive control treatment doses were cytotoxic in the transformation assay. The procedure used for the co-culture clonal survival assay has been previously reported in detail (*11,13*) and is summarized in part III of this series (*21*).

Transformation Assay

Chemical-induced transformation of BALB/c-3T3 cells was evaluated in a standard transformation assay protocol that has been reported in detail (*10*) and is summarized in part IV of this series (*14*). Briefly, each transformation assay contained three components: a standard clonal survival assay (*10,14*), a co-culture clonal survival assays (*21*), and a transformation assay (*10,14*). In each experiment, chemial-induced transformation was detected in 18–20 vessels/dose seeded with 3.2×10^4 cells/vessel. Chemical doses were applied to cell cultures for 48 hr, days 2–4, using standard procedures (*14*). A total of three to six test chemicals were included in each transformation experiment, and each chemical was tested at four treatment doses in two or more independent trials. The procedure for selecting the four doses has been described in part IV of these investigations (*14*), and the doses covered a range of cytotoxic responses of approximately 10–100% relative cloning efficiency (RCE).

Transformation Assay Acceptance and Evaluation Criteria

A complete explanation of the transformation assay acceptance and evaluation criteria for a test chemical evaluated in a single trial or in multiple trials is provided in part IV of this investigation (*14*). Briefly, a test chemical evaluated in one experiment had one of four possible transformation responses: sufficient positive (SP), limited activity (LA), sufficient negative (SN), and limited negative (LN). Briefly, an SP transformation response required that a test chemical response was statistically significant at two or more consecutive treatment doses. In contrast, an LA transformation response required that a test chemical response was statistically significant at either one treatment dose alone at the 99% confidence level or at two consecutive doses at the 95% confidence level. An SN transformation response required that a test chemical response did not have a statistically significant increase in transformation responses at any of the four treatment doses. An LN transformation response occurred under two different circumstances. First, the four test chemical treatment doses did not induce a statistically significant transformation response; however, in contrast to an SN transformation response, the test chemical treatments did not have a significant cytotoxic response. Therefore, higher concentrations of the test chemical could have induced a significant cytotoxic response, and this could have resulted in a statistically significant transformation response. Second, the test chemical had the equivalent of an SN transformation response; however, the positive control for the transformation experiment was inactive and did not induce a statistically significant response.

Evaluation of Transformed Foci

The method used to evaluate transformed foci of BALB/c-3T3 cells has been reported in detail (*10*) and is summarized in part IV (*14*) of these investigations. Briefly, the number of type I–III transformed foci of BALB/c-3T3 cells were identified microscopically using published criteria (*6–8,12,17*), and type III foci had three phenotypic properties: piling and overlapping cells, disorientation of cells at the periphery of the focus, and invasion of transformed cells into a contact-inhibited monolayer of WT cells. Type I and II foci also appeared in many different sizes, but they lacked one or more of the three phenotypic properties of the type III transformed focus.

Handling of Test Chemicals

Many chemicals in this investigation had physicochemical properties that could have potentially interfered with them being adequately tested in the BALB/c-3T3 cell transformation assay (Table 1). Therefore, procedures were developed to ensure that all test chemicals would be consistently and adequately evaluated, and the procedures are described in detail in part IV of these investigations (*14*).

Table 1. Cytotoxicity of 168 test chemicals.[a]

Group of chemicals	LD_{50}	No.
1. Cytotoxic	< 5mM	114
2. Noncytotoxic	5 mM–100 mOsM	43
3. Very noncytotoxic	> 100mOsM	11

Abbreviations: LD_{50}, lethal dose for 50% of the cells; mOsM, milliosmolar; no., number of chemicals in a subgroup of chemicals.

[a]Chemical-induced, cytotoxic response data for this table were obtained from Tables A1 and A4.

Statistical Analyses and Mathematical Models

Mathematical Transformation of Focus Data. The method used to determine the distribution of spontaneous transformed foci of BALB/c-3T3 cells has been previously reported (*10,11*) and is described in detail in part I of these investigations (*17*).

Significance of Transformation Responses. The methods used to determine the statistical significance of a chemical-induced transformation response has been described in detail in part IV of these investigation (*14*). Briefly, the significance was determined using analysis of variance (*F*-test) and modified Student's *t*-tests, and the computations were performed using SAS software (*22*).

Method for Rank-Ordering Test Chemical Transformation Responses. The method used to rank-order test chemical transformation responses on the basis of the significance of their activity in the transformation assay has been described in detail in part IV of these investigations (*14*). Briefly, the significance of the test chemical response was observed to vary proportionally to the magnitude of the *t*-statistic, and the *t*-statistic was independent of the absolute spontaneous transformation response of the solvent control. The average significance of each chemical transformation response, or mean *t*-statistic, was calculated by averaging the *t*-statistics of the four test chemical, (or two positive control) treatment doses. Treatment doses with <5% RCE and incomplete monolayers were deleted, and negative *t*-statistics were arbitrarily assigned the value of zero. This mean *t*-statistic was used to rank order chemical transformation responses in individual experiments. The test chemical activity in two or more experimental trials was assessed using a weighted the rank *t*-statistic. It was calculated using all the *t*-statistics for test chemical treatments in two or more experimental trials (see Tables A3 and A6 for actual and estimated rank *t*-statistics of 168 chemical transformation responses). Examples of these calculations are provided in Results.

Effect of Statistical Sensitivity on Detection Sensitivity for BaP. Both the magnitude of the spontaneous and the benzo[*a*]pyrene (BaP) transformation response varied among the 110 experiments included in this investigation (*17,18*). Variable spontaneous transformation responses resulted in experiments with different statistical sensitivity to detect test chemical responses (*17*) and different detection sensitivity for BaP (*18*).

Experiments with significantly low statistical sensitivity were demonstrated to have a low detection sensitivity for BaP (*18*). Therefore, these experiments had a high probability of underestimating the activity and rank *t*-statistics of test chemicals. In contrast, experiments with normal or significantly high statistical sensitivity had normal detection sensitivity for BaP (*18*). To compensate for the diminished sensitivity to detect chemical-induced transformation, the rank *t*-statistic was multiplied by a correction factor to obtain an estimated rank *t*-statistic (*14*). Example calculations using the actual rank *t*-statistic and the correction factor to determine the estimated rank *t*-statistics are provided in Tables A3 and A6.

Test Chemicals

The 43 cytotoxic, mutagenic carcinogens evaluated in this investigation were tested either as coded test chemicals (marked with an asterisk below) or as uncoded test chemicals. In addition, five chemicals were tested as both coded and uncoded (dichlorvos, C. I. basic red 9-HCl, HC red 3, dimethyl morpholinophosphoramidate, and methyl carbamate). The following 39 test chemicals were supplied by Radian Corporation (Houston, TX): *2-amino-4-nitrophenol; *2-amino-5-nitrophenol; benzidine-2HCl; 2-biphenylamine; 4-biphenylamine; 4-chloro-*o*-phenylenediamine; 3-(chloromethyl)pyridine-HCl; 4-chloro-*o*-toluidine-HCl; 5-chloro-*o*-toluidine; *C. I. acid orange 3; C. I. basic red 9-HCl; *C. I. basic red 9-HCl; *C. I. disperse blue 1; C. I. disperse yellow 3; C. I. solvent yellow 14; cytembena; 1,2-dibromo-3-chloropropane; 2,6-dichloro-*p*-phenylenediamine; 1,3-dichloropropene; dichlorvos; *dichlorvos; diglycidyl resorcinol ether; 2,4-dinitrotoluene; epichlorohydrin; *1,2-epoxybutane; 1,2-epoxypropane; ethylene dibromide; HC blue 1; *iodinated glycerol; melphalan; *N-methyl-*o*-acrylamide; 4,4-methylenedianiline; 2-naphthylamine; *nitrofurantoin; *nitrofurazone; 2-nitro-*p*-henylenediamine; 4,4-oxydianiline; quinoline; selenium sulfide; *o*-toluidine; and ziram. Three chemicals were purchased from Sigma Chemical Company (St. Louis, MO): acetylaminofluorene, 5-azacytidine, and N-methyl-N′-nitro-N-nitrosoguanidine. One chemical, acrylonitrile, was purchased from Aldrich Chemical Company (Milwaukee, WI).

The 21 cytotoxic, mutagenic, noncarcinogens evaluated in this investigation were all supplied by Radian Corporation (Houston, TX): 4-acetylaminofluorene; 4′-(chloroacetyl)acetanilide; 2(chloromethyl)pyridine-HCl; 3-chloro-*p*-toluidine; coumaphos; dimethoate; 2,4-dimethoxyaniline-HCl; HC blue 2; HC red 3; *HC red 3; 8-hydroxyquinoline; malaoxon; 1-naphthylamine; N-(1-naphthyl)ethylenediamine-2HCl; 1-nitronaphthalene; 4-nitro-*o*-phenylenediamine; 3-nitropropionic acid; *p*-phenylenediamine-2HCl; *N-phenyl-2-naphthylamide; 2,3,5,6-tetrachloro-4-nitroanisole; tetraethylthiuram disulfide; and 2,6-toluenediamine-2HCl.

Nineteen of 20 cytotoxic, nonmutagenic carcinogens evaluated in this investigation were supplied by Radian Corporation: allyl isothiocyanate; allyl isovalerate; *chlorendic acid; *chlorinated paraffins C23, 43% chlorine (also

chlorowax 40); *chlorinated paraffins 60% chlorine (also chlorowax 500c); 3-chloro-2-methylpropene; *dimethylvinyl chloride; cinnamyl anthranilate; ethyl acrylate; isophorone; *D-limonene; *malonaldehyde, sodium salt; *2-mercaptobenzothiazole; methapyrilene-HCl; polybrominated biphenyl mixture; reserpine; tris(2-ethylhexyl)-phosphate; and *4-vinylcyclohexene. One chemical, diethylstilbestrol, was purchased from Aldrich, and one chemical, trisodium salt, was purchased from Sigma.

The 30 cytotoxic, nonmutagenic noncarcinogens evaluated in this investigation were all supplied by Radian: anilazine; L-ascorbic acid; bisphenol A; carbromal; *chlorpheniramine-maleate; C. I. acid red 14; C. I. acid yellow 73; *ephedrine sulfate; *erythromycin stearate; ethoxylated dodecyl alcohol; ethylenediamine tetraacetic acid, trisodium salt; eugenol; geranyl acetate; *4-hexylresorcinol; D,L-menthol; methoxychlor; *methyldopa sesquihydrate; methylphenidate-HCL; *oxytetracycline-HCl; phenol; *phenylephrine-HCl; propyl gallate; *rotenone; sodium diethyldithiocarbamate; stannous chloride; *tetracycline-HCl; *tetrakis(hydroxymethyl)-phosphonium chloride; *tetrakis(hydroxymethyl)phosphonium sulfate; triphenyltin hydroxide; and *xylenes (mixed).

Fourteen of 21 noncytotoxic, carcinogens evaluated in this investigation were supplied by Radian: 11-aminoundecanoic acid; DC red no. 9; *decabromodiphenyl-oxide; di(2-ethylhexyl)adipate; di(2-ethylhexyl)phthalate; diethanolnitrosamine; dimethyl hydrogen phosphite; dimethyl methyl phosphonate; dimethylmorpholinophosphoramidate; *dimethylmorpholinophosphoramidate; ethylene thiourea; melamine; methyl carbamate; *methyl carbamate; monuron; and 2,4- and 2,6-toluene diisothiocyanate. Six chemicals were purchased from Sigma: 3-amino-1,2,4-triazole; cyclamate, sodium salt; diethylnitrosamine; dimethylnitrosamine; phenobarbital, sodium salt; and saccharin, sodium salt. One chemical, hexamethylphosphoramide, was purchased from Aldrich.

The 26 noncytotoxic noncarcinogens evaluated in this investigation were supplied by Radian: aldicarb; *ampicillin trihydrate; *o*-anthranilic acid; benzoin; *benzyl alcohol; caprolactam; 2-chloroethanol; (2-chloroethyl)-trimethylammonium chloride; C. I. acid orange 10; dimethyl terephthalate; diphenylhydantoin; FD&C yellow no. 6; D-mannitol; *methyl methacrylate; molybdenum trioxide; 4-nitroanthranilic acid; *penicillin VK + ; phthalamide; phthalic anhydride; *roxarsone; sodium(2-ethylhexyl) alcohol sulfate; sulfisoxazole; 3-sulfolene; tetrahydrofuran; titanium dioxide; and witch hazel.

The seven very noncytotoxic chemicals evaluated in this investigation were all supplied by three companies: Sigma, Fisher Scientific, and U.S. Industrial Products.

Results

Range of Cytotoxic Responses of 168 Chemicals

A co-culture clonal survival assay was used to measure the cytotoxic responses of 168 chemicals (*21*), and each chemical was tested in two or more experiments. The cytotoxic responses of individual chemicals are presented in detail in Tables A1 and A4. The data set had a range of cytotoxic responses of over 7 logs. The most cytotoxic chemical was ziram, and it had an average cytotoxic response, or LD_{50}, of 0.0000373 mM. Based on a molecular weight of 305.81, this concentration was equivalent to approximately 0.0114 μg/mL. The least cytotoxic chemical was witch hazel, and it had an LD_{50} estimated at approximately 540 mM.

The 168 chemicals were arbitrarily divided into three groups according to their relative cytotoxic responses: group 1, cytotoxic chemicals with an $LD_{50} < 5$ mM; group 2 noncytotoxic chemicals with an LD_{50} 5 mM–100 mOsM; and group 3, very noncytotoxic chemicals with an $LD_{50} >$ 100 mOsM (Table 1). There were 114 cytotoxic chemicals, 43 noncytotoxic chemicals and 11 very noncytotoxic chemicals (see Table 1). Chemical cytotoxic responses were divided into groups 1–3 based on three empirical observations. First, using the appropriate solvent vehicles, nearly all cytotoxic chemicals could be tested at treatment doses either at or below their solubility limit in culture medium. In contrast, many noncytotoxic chemicals had to be tested at treatment doses above their solubility limit to obtain cytotoxicity to the BALB/c-3T3 cells. Second, many cytotoxic chemicals ($LD_{50} < 5$ mM) were consistently inactive in the transformation assay; however, few noncytotoxic chemicals ($LD_{50} > 5$ mM) were inactive if they were fully soluble in culture medium. Thus, the solubility of noncytotoxic test chemicals clearly correlated their potential activity in the transformation assay, and nearly all of the noncytotoxic chemicals that were inactive in the transformation assay had solubility problems in culture medium. Third, mutagenic and nonmutagenic test chemicals had very different profiles of cytotoxic responses. Most mutagenic chemicals were cytotoxic chemicals, while only half of the nonmutagenic chemicals were cytotoxic. Data supporting this observation will be presented later in this report.

Distribution of Cytotoxic Responses among Carcinogens and Noncarcinogens

The cytotoxic responses of carcinogenic and noncarcinogenic chemicals were compared in the data set of 168 chemicals (Table 2). This set of chemicals included 84 carcinogens and 77 noncarcinogens, and the remaining 7 test chemicals were model chemicals that had not been evaluated in the NTP rodent bioassay. These analyses of the data revealed that the data set contained a balanced distribution of cytotoxic responses among the carcinogens and noncarcinogens. Furthermore, the data set contained many examples of cytotoxic and noncytotoxic carcinogens and noncarcinogens (Table 2). Thus, these data demonstrated that *in vitro* cytotoxicity of chemicals to BALB/c-3T3 cells neither correlated with nor predicted their *in vivo* carcinogenic activity.

Table 2. Cytotoxicity of carcinogens versus noncarcinogens.[a]

Type of chemical	LD_{50}	No.	%
Cytotoxic chemicals			
Carcinogens	< 5 mM	63	55.3
Noncarcinogens	< 5 mM	51	44.7
Noncytotoxic chemicals			
Carcinogens	5 mM–100 mOsM	21	44.7
Noncarcinogens	5 mM–100 mOsM	26	55.3
Total chemicals			
Carcinogens		84	52.2
Noncarcinogens		77	47.8

Abbreviations: LD_{50}, lethal dose for 50% of the cells; mOsM, milliosmolar; no., number of chemicals in a subgroup; %, percentage of chemicals in a subgroup (e.g., 63/63 + 51 = 52.2%).

[a]Chemical-induced, cytotoxic response data for this table were obtained from Tables A1 and A4.

Upper Dose Limit of the Transformation Assay

This investigation did not use an arbitrary upper dose limit of 5–10 mg/mL for the BALB/c-3T3 cell transformation assay. All chemicals were tested over a comparable range of cytotoxicity of 0–100% RCE, and the data from these experiments were retrospectively used to determine an empirical upper dose limit. In addition, the concentration of test chemical treatment doses was expressed in millimoles, and not in micrograms per milliliter because the 168 test chemicals had molecular weights that ranged from 46.07 for ethanol to approximately 1200 for ethoxylated dodecyl alcohol.

The upper dose limit of the BALB/c-3T3 cell transformation assay was set at 100 mOsM based on two empirical observations in this investigation. First, we observed that the test chemicals that were the least cytotoxic to the target cells all had an LD_{50} over a narrow range of 240–504 mOsM (see Tables 3 and A4). Second, all of the very noncytotoxic chemicals were active in the transformation assay (Appendix H). Furthermore, each of these chemicals began to induce significant transforming activity at an average concentration of 134 mOsM (Table 3). Optimal induction of transforming activity occurred at slightly higher treatment dose concentrations that were close to the chemical's LD_{50} dose. Taken together, the BALB/c-3T3 cell transformation assay could not discriminate active and inactive chemicals when they were tested at concentrations above about 134 mOsM; thus, the actual dose limit for the data set of 168 chemicals was set at 100 mOsM.

Table 3. Cytotoxic and transformation responses of seven very noncytotoxic test chemicals.[a]

Name	Cytotoxicity response, LD_{50}, mOsM	Transformation response, mOsM	
		Maximum	Minimum
Acetone	257	176	102
Dimethyl sulfoxide	507	563	141
Ethanol	429	257	150
Glycerol	401	340	136
Sodium chloride	288	262	154
Sucrose	240	300	150
Urea	254	208	104
Average	339	301	134

Abbreviations: LD_{50}, lethal dose for 50% of the cells; mOsM, milliosmolar.

[a]Chemical cytotoxic response data for this table were obtained by plotting cytotoxic and transformation response data contained in Appendix H.

Physicochemical Properties of 168 Chemicals

We were concerned in this investigation that uncontrolled test chemical technical problems could affect the activity of a chemical in the transformation assay. This concern arose because most of the 168 chemicals in this investigation had physicochemical properties that could potentially have caused technical problems when they were tested in an *in vitro* assay using cultured mammalian cells (refer to chemical technical problems listed in Tables A1 and A4). Fortunately, the majority of the technical problems were avoided by using specific techniques to handle the test chemicals [see Materials and Methods in part IV of this series (*14*)].

Nevertheless, six types of technical problems were difficult to control in this investigation, and each of these problems could have influenced the results in these experiments (Table 4). First, 21 chemicals reacted with plastic polystyrene culture vessels; thus, treatment times were reduced from 48 hr to minutes. The chemical reaction with plastic was unusual in that it occurred after the chemical was completely dissolved in the aqueous environment. Because this problem could only be overcome through the use of chemical-resistant culture vessels such as glass bottles, these chemicals were not included in this investigation. A complete list of the 21 chemicals is provided in the Discussion. Second and third, 56 chemicals were oxidized by air and 15 chemicals reacted with water; thus, the BALB/c-3T3 cells were exposed to not only the parent test chemical, but also its oxidized and hydrolyzed byproducts. Fourth, eight chemicals reacted with biochemicals; thus, they could have combined with biochemicals in the culture medium or biochemicals within the target cells. Fifth, seven chemicals bound different metal salts; thus, they could have complexed with critical metals in either the culture medium or the target cell. Finally, over half of the chemicals had solubility problems in an aqueous environment. Fortunately, the use of organic solvents in conjunction with the nonionic surfactant pluronic F68 (*14,23*) resulted in most of these chemicals being soluble at concentrations that induced cytotoxicity to the BALB/c-3T3 cells. Nevertheless, 14 test chemicals could not be solubilized and were insoluble at a portion or all of the treatment dose concentrations used to test for cytotoxic and transforming activities.

Thus, we predicted that any one of the six technical problems could have affected detection of chemical-induced transformation of BALB/c-3T3 cells. Furthermore, we anticipated that the same six technical problems might also have affected detection of mutagenicity in

Salmonella assays and carcinogenicity in rodent bioassay. Therefore, we examined sets of chemicals with the six technical problems to determine whether any of the problems correlated with the expression of carcinogenicity, mutagenicity, and transformation. If a chemical technical problem had either no effect or a random effect on a biological activity, then there would be equal distributions of active and inactive chemicals with this problem (i.e., ratio of active/inactive chemicals = 1.00). Conversely, if a technical problem had a consistent effect on the biological activity, then the distribution of active and inactive chemicals would be altered (i.e., ratio of active/inactive chemicals <1.00 or >1.00).

The results of these comparisons are summarized in Table 4. It was found that two of the technical problems, reaction with air and water, had no significant effect on all three biological activities. Three additional technical problems had no effect on carcinogenicity, but they were correlated with suppressed detection of transformation and mutagenic activities. For example, chemicals with severe solubility problems and chemicals that bound metal salts tended to be inactive in both BALB/c-3T3 transformation and Salmonella mutagenicity assays. Conversely, chemicals that reacted with biochemicals tended to be active in both mutagenicity and transformation assays. In contrast, only one of the technical problems had an effect on all three biological properties of carcinogenicity, transformation, and mutagenicity. Nearly all of the 21 chemicals that reacted with plastic culture vessels in BALB/c-3T3 cytotoxicity assays (unpublished observations) were carcinogenic, and they did not induce either transformation or mutagenicity in Salmonella. Thus, the presence of this technical problem significantly correlated with these chemicals being nonmutagenic carcinogens in rodent bioassay.

Table 4. Effect of test chemical technical problems on biological activities of carcinogenicity, mutagenicity and transformation.

Biological activity[a]	Test chemical technical problems[b]	Relative effect[c]
Carcinogenicity	Reacts with plastic	3.49[d]
	Reacts with water	1.47
	Reacts with biochemicals	1.38
	Reacts with air	1.22
	Solubility problem	1.09
	Binds metal salts	1.08
Transformation	Reacts with biochemicals	3.00[d]
	Reacts with water	1.77
	Binds metal salts	1.29
	Reacts with air	1.26
	Solubility problem	0.393[d]
	Reacts with plastic	0.000[d,e]
Mutagenicity	Reacts with biochemicals	2.43[d]
	Reacts with air	1.38
	Reacts with water	1.08
	Solubility problem	0.574[d]
	Binds metal salts	0.246[d]
	Reacts with plastic	0.105[d]

[a]The three biological activities included carcinogenicity in rodent bioassay, mutagenicity in Salmonella, and transformation in BALB/c-3T3 cells.

[b]Test chemicals in this investigation had several difficult problems: 56 chemicals were oxidized upon exposure to air; 21 chemicals reacted with plastic; 15 chemicals reacted with water; 14 chemicals had severe solubility problems in culture medium that was not corrected by the use of pluronic F68; 8 chemicals reacted with biochemicals (i.e., alkylating agents and chemicals that reacted with alcohols and amine groups); and 7 chemicals bound metal salts.

[c]When a technical problem had no effect on the biological property, it resulted in a relative effect of 1.00 (i.e., equal ratio of inactive and active chemicals). When a technical problem correlated with an enhanced biological activity, it resulted in a relative effect > 2.00. Conversely, when a technical problem correlated with a decreased biological activity, it resulted in a relative effect < 0.500.

[d]Chemicals with relative effects either >2.00 or <0.500.

[e]Because the 21 chemicals that reacted with plastic could not be tested for transformation, they were all arbitrarily considered inactive to get a relative effect of 0.000.

Transformation Responses of 168 Chemicals

Variability among spontaneous transformation responses resulted in experiments with different statistical sensitivities to detect chemical-induced transformation responses (*17*). Likewise, variability among BaP responses demonstrated that individual experiments had different detection sensitivities for BaP (*18*). Thus, individual experiments had different sensitivities to measure test chemical-induced transformation responses. Therefore, the responses of test chemicals in the BALB/c-3T3 cell transformation assay were evaluated in terms of the rank-ordered sensitivity of individual experiments to detect both spontaneous and BaP-induced transformation responses (*14,17,18*).

In the current study, the 168 chemicals were tested in two or more transformation assay experiments. The results of individual experiments for each test chemical are provided in detail in Appendices B–H. In addition, a summary of transformation responses of all the chemicals are presented in summary Tables A2 and A5. Explanations for the different response calls and evaluation criteria for a single transformation assay experiment have been reported (*14*) and are summarized in Materials and Methods. The final determination of the rank-ordered activity of each chemical is summarized in Tables A3 and A6. The method used for combining the activities of chemicals tested in two or more experiments has been discussed in detail in part IV of these investigations (*14*). For the reader who is interested in the cumulative data associated with an individual test chemical, a narrative description of the activities of individual chemicals is provided in Appendix A. To facilitate comparative analyses of chemicals with different biological activities, the same sequence of chemicals has been presented within each of the tables of Appendix A.

Comparison of Carcinogenicity with Mutagenicity and Transformation Responses

The data set of 161 carcinogens and noncarcinogens was compared to the activities of different sets of chemicals tested in other NTP investigations (*1–4*). In these binary analyses, the concordance of each assay was compared to

Table 5. Correlation of rodent bioassay carcinogenicity and Salmonella mutagenicity data.[a]

Carcinogenicity	Mutagenicity	No.
Carcinogenic	Mutagenic	49
Noncarcinogenic	Nonmutagenic	53
Carcinogenic	Nonmutagenic	35
Noncarcinogenic	Mutagenic	24

Concordance = 49 + 53/161 = 63.4%
Sensitivity = 49/ 84 = 58.3%
Specificity = 53/ 77 = 68.8%

No., number of chemicals in a subgroup.
[a]The computations for this table were made using data obtained from Tables A3 and A6.

Table 6. Correlation of rodent bioassay carcinogenicity and BALB/c-3T3 transformation data.[a]

Carcinogenicity	Transformation	No.
Carcinogenic	Transforming	64
Noncarcinogenic	Nontransforming	40
Carcinogenic	Nontransforming	16
Noncarcinogenic	Transforming	27

Concordance = 64 + 40/147 = 70.7%
Sensitivity = 64/80 = 80.0%
Specificity = 40/67 = 59.7%

No., number of chemicals in a subgroup.
[a]The computations in this table excluded 4 carcinogens and 10 noncarcinogens that had an indeterminate transformation response (Tables A3 and A6).

the rodent bioassay using a chi-square method. In this database the concordance of Salmonella mutagenicity data with rodent bioassay was 63.4% (Table 5). Using the same group of chemicals, Salmonella assays had a sensitivity to detect carcinogens of 58.3% and a specificity for detecting noncarcinogens of 68.8%. Thus, this database was comparable to other NTP data sets (*1–4*), and it contained a large number of nonmutagenic carcinogens and mutagenic noncarcinogens.

Transformation data were also analyzed using the same method, and the concordance of BALB/c-3T3 transformation responses was compared to carcinogenicity data from rodent bioassay (Table 6). The transformation assay exhibited a concordance with the rodent bioassay of 70.7%, which was 7.3% higher than Salmonella (i.e., 70.7 versus 63.4%). Likewise, the transformation assay also had a 21.7% higher sensitivity for carcinogens (i.e., 80.0% versus 58.3%) and a 9.2% lower specificity for detecting noncarcinogens (i.e., 59.7% versus 68.9%) compared to Salmonella assays.

Correlation of Test Chemical Cytotoxicity with Mutagenicity

Binary comparisons of the responses of 147 chemicals in BALB/c-3T3 transformation and 161 chemicals in Salmonella mutagenicity assays revealed that the data from both assays had a high concordance with rodent bioassay (Tables 5 and 6). However, this database contained a disproportionate number of cytotoxic, versus noncytotoxic, test chemicals (see Table 2). Thus, the concordance of the transformation and Salmonella mutagenesis assays might have been affected by the relative cytotoxicity of the test chemicals. Because the number of carcinogens and noncarcinogens was roughly equal in both of these groups of chemicals, the correlation of test chemical cytotoxicity with mutagenicity in Salmonella and rodent bioassay carcinogenicity could be directly compared.

The correlation of test chemical cytotoxicity to BALB/c-3T3 cells with mutagenicity in Salmonella assays was examined first (Table 7). These multifactorial analyses revealed that Salmonella mutagenicity was highly correlated with chemical cytotoxicity. About 88% of the mutagenic chemicals had an $LD_{50} < 5$ mM, including both mutagenic carcinogens and noncarcinogens. In contrast, chemical cytotoxicity was not correlated with carcinogenicity; about 57% of both carcinogens and noncarcinogens were cytotoxic. Thus, cytotoxicity of the test chemical to BALB/c-3T3 cells correlated most with its capacity to induce mutations in Salmonella (Table 8). In contrast, cytotoxicity did not correlate with either the induction of transformation in BALB/c-3T3 cells or carcinogenicity in the rodent bioassay (Table 8). Thus, the *in vivo* capability of a chemical to induce tumors in rodents was not correlated with its *in vitro* cytotoxicity to a cultured mammalian cell.

Taken together, these data showed that among the four biological variables in this investigation (i.e., carcinogenicity, cytotoxicity, mutagenicity, and transformation), the highest correlation of variables was observed for results from BALB/c-3T3 transformation assays with rodent bioassay (70.7% concordance) and Salmonella mutagenicity assays (69.8% concordance) (Table 8). A less significant correlation was noted for BALB/c-3T3 cytotoxicity and Salmonella mutagenicity (63.4% concordance) and carcinogenicity and Salmonella mutagenicity (63.4% concordance). All other binary comparison of variables were not significantly correlated.

Comparison of Mutagenicity and Transformation

Because BALB/c-3T3 cell transformation and Salmonella mutagenicity assay data both exhibited a high concordance with rodent bioassay data, it was of interest to see whether the two assays detected the same profile of chemicals. If the two assays were to detect the same chemicals, this result would imply, but not prove, that the BALB/c-3T3 transformation assay was detecting primarily mutagenic test chemicals. Thus, a mutation at a gene for the transformed cell phenotype would be the most likely explanation of the activity of chemicals in the assay.

When the BALB/c-3T3 transformation response data was compared to the Salmonella assay data, the transformation assay was observed to detect 92.5% of the mutagenic carcinogens and approximately 70% of the mutagenic noncarcinogens (Table 9). These data demonstrated that the transformation assay detected a high

Table 7. Correlation of BALB/c-3T3 cytotoxicity with Salmonella mutagenicity and rodent bioassay carcinogenicity.[a]

Cytotoxicity	Mutagenicity	Carcinogenicity	No.	%
73 Mutagens				
Cytotoxic	Mutagenic	43 Carcinogens + 21 noncarcinogens	64	87.7
Noncytotoxic	Mutagenic	6 Carcinogens + 3 noncarcinogens	9	12.3
88 Nonmutagens				
Cytotoxic	Nonmutagenic	20 Carcinogens + 30 noncarcinogens	50	56.8
Noncytotoxic	Nonmutagenic	15 Carcinogens + 23 noncarcinogens	38	43.2

Cytotoxicity versus mutagenicity
Concordance = 43 + 21 + 15 + 23 / 64 + 9 + 50 + 38 = 102/161 = 63.4%
Sensitivity = 43 + 21 / 64 + 50 = 64/114 = 56.1%
Specificity = 15 + 23 / 9 + 38 = 38/ 47 = 80.9%

Cytotoxicity versus carcinogenicity
Concordance = 43 + 20 + 3 + 23 / 64 + 9 + 50 + 38 = 89/161 = 55.3%
Sensitivity = 43 + 20 / 43 + 6 + 20 + 15 = 63/ 84 = 75.0%
Specificity = 3 + 23 / 21 + 3 + 30 + 23 = 26/ 77 = 33.8%

Abbreviations: No., number of chemicals in a subgroup; %, percentage of the chemicals in a subgroup (e.g., 43 + 21/64 = 87.7%).
[a]The data for this table were obtained from Tables A3 and A6.

percentage of mutagenic carcinogens and mutagenic noncarcinogens. Most of the mutagenic noncarcinogens in this group were analogues of known carcinogens, and they all had DNA reactive structural alerts (*1–4*). Thus, neither the BALB/c-3T3 assay nor the Salmonella mutagenesis assays were able to distinguish mutagenic carcinogens from mutagenic noncarcinogens. Fortunately, the frequency of mutagenic noncarcinogens in rodent bioassays has been relatively small.

Table 8. Concordance of carcinogenicity, transformation, mutagenicity, and cytotoxic data.[a]

Biological property	%	Concordance (relative significance)
Carcinogenicity versus		
Transformation	70.7	XXXXXXXXXXXXXXXXXXXX
Mutagenicity	63.4	XXXXXXXXXXXX
Cytotoxicity	55.3	XXXXX
Control	50.0	–
Transformation versus		
Carcinogenicity	70.7	XXXXXXXXXXXXXXXXXXXX
Mutagenicity	69.8	XXXXXXXXXXXXXXXXXX
Cytotoxicity	55.3	XXXXXX
Control	50.0	–
Mutagenicity versus		
Transformation	69.8	XXXXXXXXXXXXXXXXXX
Carcinogenicity	63.4	XXXXXXXXXXXX
Cytotoxicity	63.4	XXXXXXXXXXXX
Control	50.0	–
Cytotoxicity versus		
Mutagenicity	63.4	XXXXXXXXXXXX
Transformation	56.0	XXXXXX
Carcinogenicity	55.3	XXXXX
Control	50.0	–

Abbreviations: %, the percentage of concordance between the two biological properties.
[a]The concordance of each biological activity with the remaining three biological activities is presented as percentage and as a bar graph. A concordance of 50% is the control (–) and each X is equivalent to 1% concordance over the control.

Detection of Nonmutagenic Carcinogens

Because the BALB/c-3T3 transformation assay did not detect all of the mutagenic carcinogens (Table 9) but it had a higher sensitivity to detect carcinogens than Salmonella (Table 8), the transformation assay must have detected a substantial number of nonmutagenic carcinogens. This data set included 35 nonmutagenic carcinogens, which was 41.7% of the total of 84 carcinogens. The nonmutagenic carcinogens were approximately equally divided between cytotoxic and noncytotoxic chemicals. A total of 20 of 35 carcinogens were cytotoxic, and 15 of 35 chemicals were noncytotoxic chemicals.

Table 9. Detection of mutagenic chemicals by the standard BALB/c-3T3 transformation assay.[a]

Mutagenicity/carcinogenicity	Transformation	No.	%
Cytotoxic chemicals (LD_{50} < 5 mM)			
Mutagenic/carcinogenic	Transforming	37/40	92.5
Mutagenic/noncarcinogenic	Transforming	13/20	65.5
Noncytotoxic chemicals (LD_{50} ≥ 5mM)			
Mutagenic/carcinogenic	Transforming	6/6	100.
Mutagenic/noncarcinogenic	Transforming	3/3	100.
Total chemicals			
Mutagenic/carcinogenic	Transforming	43/46	93.5
Mutagenic/noncarcinogenic	Transforming	16/23	69.6

Concordance = 43 + 7/69 = 72.5%
Sensitivity = 43/46 = 93.5%
Specificity = 7/23 = 30.4%[b]

Abbreviations: no., ratio of the number of chemicals in a subgroup that induced significant transformation responses versus the total number of chemicals in the subgroup; %, the ratio of chemicals expressed as a percentage (e.g., 37/40 = 92.5%); LD_{50}, lethal dose for 50% of the cells.
[a]Data for this table were obtained from Tables A3 and A6. The computations in this table excluded 4 carcinogens and 10 noncarcinogens that had an indeterminate transformation response.
[b]A total of only 7 chemicals were mutagenic, noncarcinogenic, and nontransforming (i.e., 23 – 16 = 7).

Table 10. Detection of nonmutagenic carcinogens by the Standard BALB/c-3T3 transformation assay.[a]

Mutagenicity/carcinogenicity	Transformation	No.	%
Cytotoxic chemicals (LD_{50} < 5 mM)			
Nonmutagenic/carcinogenic	Transforming	10/19	52.6
Nonmutagenic/noncarcinogenic	Transforming	6/26	23.1
Noncytotoxic chemicals (LD_{50} ≥ 5 mM)			
Nonmutagenic/carcinogenic	Transforming	11/14	78.6
Nonmutagenic/noncarcinogenic	Transforming	5/16	31.3
Total chemicals			
Nonmutagenic/carcinogenic	Transforming	21/33	63.6
Nonmutagenic/noncarcinogenic	Transforming	11/42	26.2
Concordance = 21 + 31/75 = 69.3%			
Sensitivity = 21/33 = 63.6%			
Specificity = 31/42 = 73.8%[b]			

Abbreviations: no., ratio of the number of chemicals in a subgroup that induced significant transformation responses versus the total number of chemicals in the subgroup; %, the ratio of chemicals expressed as a percentage (e.g., 10/19 = 52.6%); LD_{50}, lethal dose for 50% of the cells.

[a]Data for this table were obtained from Tables A3 and A6. The computations in this table excluded carcinogens and noncarcinogens that had an indeterminate transformation response.

[b]A total of 31 chemicals were nonmutagenic, noncarcinogenic and nontransforming (i.e., 42 − 11 = 31).

The capability of the BALB/c-3T3 assay to detect nonmutagenic carcinogens is summarized in Table 10. These data revealed that there was a high concordance of 69.3% between nonmutagenic carcinogens detected in rodent bioassay and transformation responses measured in the transformation assay. In addition, the transformation assay had a sensitivity for detecting nonmutagenic carcinogens of 63.6% (21/33), and a high specificity for not detecting noncarcinogens of 73.8% (31/42). The number of nonmutagenic carcinogens used in these analyses was 33 out of a total of 35 because 2 chemicals had an indeterminate activity (Tables A3 and A6).

Comparison of the Relative Carcinogenic Activity of Mutagenic and Nonmutagenic Carcinogens

The relative carcinogenic activity of chemicals in rodent bioassay has been evaluated in terms of their level of effect (*1–3*). The most active carcinogens induced tumors at one or more tissue sites in both species of rodents and were defined as having a level A effect (Table 11). In contrast, carcinogens with lower activities induced tumors in only one species, and they were evaluated as having level B, C, or D effects. Finally, chemicals that did not induce a significant tumor response were evaluated as having an equivocal activity (level E) or as being inactive (level F). Occasionally, a chemical was evaluated as having an indeterminate activity, because it has not been evaluated in a rodent bioassay that fulfilled all of the required prerequisites.

The relative level of activity of mutagenic and nonmutagenic carcinogens in rodent bioassay has also been compared (*1,3*). Ashby and Tennant (*1,3*) concluded that mutagenic carcinogens in general induced more multi-site and trans-species effects in the rodent bioassay than nonmutagenic carcinogens. Furthermore, they found evidence that mutagenic carcinogens induced tumors in a different profile of tissues sites than nonmutagenic carcinogens (*1*). Thus, it was of interest to determine whether the mutagenic and nonmutagenic carcinogens included in the these investigations had a comparable profile of activities as previously reported. It was also of interest to determine whether the BALB/c-3T3 cell transformation assay selectively detected carcinogens of either high or low activity.

Table 11. Relative activity of carcinogens in rodent bioassays.[a]

Activity	Level of effect	Species	Tissues
Carcinogenic			
High	A	2	1 or more
High	B	1	2 or more
Low	C	1	1 tissue/both sexes
Low	D	1	1 tissue/1 sex
Noncarcinogenic			
Equivocal	E		
Inactive	F		

[a]A method for estimating the relative activity of carcinogens in rodent bioassay as reported by Ashby and Tennant (*1,3*).

The results of these analyses are presented in Table 12. They confirmed the reported observation that the majority of the 49 mutagenic carcinogens in this investigation had a relatively high level of effect in the rodent bioassay (i.e., 37 were A or B versus 12 that were C or D; Tables A3 and A6). In addition, a total of 74% of the carcinogens detected by Salmonella and in the BALB/c-3T3 transformation assay had a level A or B effect. In contrast, the 35 nonmutagenic carcinogens in this investigation contained roughly equal numbers of chemicals with a high or low level of effect (i.e., 19 were A or B and 16 were C or D). In this group the BALB/c-3T3 transformation assay prefer-

Table 12. Correlation of level of effect of carcinogenicity with BALB/c-3T3 transformation responses.[a]

	Level of effect		
Transformation	No. AB	No. CD	% AB
46 Mutagenic carcinogens			
Transforming	32	11	74.4
Nontransforming	3	0	–
33 Nonmutagenic carcinogens			
Transforming	13	8	61.9
Nontransforming	6	6	–
79 Total carcinogens			
Transforming	45	19	70.3
Nontransforming	9	6	–

Abbreviations: no. AB, number of the chemicals with a level of effect A or B (C or D) of the subgroup of chemicals; % AB, percentage of chemicals with level of effect A or B (e.g., 32/26 + 11 = 74.4%).

[a]The data for this table were obtained from Tables A3 and A6. (Note: three of the mutagenic carcinogens and two of the nonmutagenic carcinogens had indeterminate activity and were not included in these analyses.)

entially detected 62% of the carcinogens with a high A or B level of effect.

Discussion

There were five accomplishments of this investigation. First, we were able to validate the use of the BALB/c-3T3 transformation assay and demonstrate that it selectively detected carcinogenic, versus noncarcinogenic, test chemicals. The data from this study show that the BALB/c-3T3 cell transformation assay exhibits a somewhat higher concordance with the rodent bioassay than Salmonella mutagenicity data, i.e., 70.7 versus 63.4% (Tables 5 and 6). Thus, both of these assays selectively detected carcinogens versus noncarcinogens in this data set. However, the BALB/c-3T3 transformation assay also detected a large number of noncarcinogenic chemicals that were active in Salmonella mutagenesis assays (i.e., mutagenic noncarcinogens). Thus, neither assay could discriminate most matched pairs of carcinogens and noncarcinogens, which have very similar chemical structures. Nearly all of the matched pairs of carcinogens and noncarcinogens, such as 2- and 3-chloromethylpyridine, were active in both assays. The only matched pairs which were discriminated by BALB/c-3T3 transformation assays were the active carcinogens 2-acetylaminofluorene and BaP and the inactive noncarcinogens 4-acetylaminofluorene and benzo[*e*]-pyrene (unpublished observation). One matched pair, HC blue 1 and 2, had either an inactive or an indeterminate activity in the transformation assay.

Second, the data obtained in this investigation demonstrate that the BALB/c-3T3 cell transformation assay can be used to selectively detect some carcinogens that were inactive in the Salmonella mutagenesis assays (i.e., nonmutagenic carcinogens). There were a total of 53 nonmutagenic carcinogens selected for evaluation in the transformation assay; however, only 35 chemicals were tested. The activities of the remaining 18 chemicals will be discussed below. Among the 35 chemicals that were tested in the standard transformation assay, 21 chemicals were active, including 10 of 19 cytotoxic and 11 of 14 noncytotoxic chemicals and 2 chemicals that had an indeterminate activity (Table 10). Of the remaining 12 inactive, nonmutagenic carcinogens, 3 carcinogens (i.e., cinnamyl anthraniliate, methapyrilene, and reserpine) have been demonstrated to be active in a new BALB/c-3T3 cell transformation assay that uses noncytotoxic treatment doses of the test chemical (unpublished data). In this protocol the BALB/c-3T3 cells are exposed continuously to multiple chemical treatment doses, and the assay is only used to evaluate the activities of cytotoxic test chemicals (LD_{50} < 5 mM). The remaining six cytotoxic, nonmutagenic carcinogens that were inactive (e.g., allyl isovalerate, chlorowax 40, chlorowax 500, D-liminone, tris(2-ethylhexyl)phthalate, and 4-vinylcyclohexene) and one equivocal (e.g., 2-mercaptobensothiazole) transformation response await further testing with the multiple treatment (MTA) assay. In contrast, the four noncytotoxic, nonmutagenic, carcinogens could not be evaluated in this assay: decabromodiphenyloxide, di(2-ethylhexyl)adipate, di(2-ethylhexyl)phthalate, and monuron. These carcinogens had severe solubility problems in culture medium, and they were noncytotoxic at treatment dose well above their solubility limit.

Taken together, the Salmonella mutagenesis assays and the standard BALB/c-3T3 transformation assay were complementary and detected 83.3% (70/84) of the carcinogens in this investigation, including 21 nonmutagenic carcinogens. Of the remaining 14 nonmutagenic carcinogens, 10 were cytotoxic and were eligible for evaluation in the BALB/c-3T3 MTA assay. Three of these 10 chemicals have already been shown to be active in a MTA protocol that has a high sensitivity to detect carcinogenic test chemicals. Thus, only four noncytotoxic, nonmutagenic carcinogens with severe solubility problems in culture medium would be predicted to lack activity in the two assays. The sacrifice in specificity in using the two assays could be to detect all 24 mutagenic noncarcinogens and approximately 26% of the nonmutagenic noncarcinogens.

An additional group of 18 nonmutagenic carcinogens were originally selected to be tested in the BALB/c-3T3 cell transformation assay. However, these chemicals were part of a group of 21 chemicals that reacted with polystyrene, plastic culture vessels (Table 4); thus, these chemicals could not be evaluated in the standard transformation assay. This reaction occurred at concentrations that were completely soluble in culture medium and used as treatment doses to detect cytotoxic and transforming activity (Table 4). While most of these chemicals had severe solubility problems in culture medium, they were all completely soluble in culture medium supplemented with pluronic F68. Thus, these chemicals reacted with polystyrene while they were in solution in water. These chemicals are distinguishable from chemicals such as acetone that react with polystyrene as a neat chemical, but not when it is dissolved in culture medium.

Among this group of 21 test chemicals that reacted with polystyrene, Salmonella detected only one weak positive (1,2-dichloropropane). An additional chemical, *bis*(2-chloro-1-methylethyl)ether, had a minor structural alert (*3*). Of the remaining 19 chemicals, 17 were nonmutagenic carcinogens: benzene; benzyl acetate; bromodichloromethane; bromoform; butyl benzyl phthalate; *p*-chloroaniline; chlorobenzene; chlorodibromomethane; diallyl phthalate; 1,4-dichlorobenzene; methylene chloride; pentachloroethane; safrole; 1,1,1,2-tetrachloroethane; tetrachloroethylene; 1,1,1-trichloroethane; and trichloroethylene (*1–3*). There were only 2 noncarcinogens in the group of 21 chemicals (*N*-butyl chloride and 1,2-dichlorobenzene). It should be noted that one of these chemicals, benzene, has been reported by Fitzgeral et al. to induce significant transformation of BALB/c-3T3 cells when the cells were treated in chemical-resistant glass dishes (*25*).

Third, we were able to determine the actual upper dose limit for testing chemicals in the BALB/c-3T3 cell transformation assay. To achieve this goal, we tested all of the noncytotoxic chemicals at very high treatment doses to determine the point at which the assay could not dis-

tinguish active and inactive chemicals. Furthermore, we tested a number of chemicals with solubility problems in culture medium at concentrations far exceeding their solubility limit. The results of these experiments revealed that the upper dose limit for the standard transformation assay was 100 mOsM because all of the least cytotoxic test chemicals induced significant transforming activity at treatment dose concentrations of about 134 mOsM or higher (Table 3). In the process of conducting these experiments we discovered that many of the chemicals which were tested at doses far above their solubility limit in culture medium were inactive in the transformation assay. In fact, noncytotoxic chemicals with solubility problems in culture medium were far less likely to be active in the transformation assay then chemicals that were freely soluble in culture medium (Table 4).

Fourth, we were able to ascertain that most of the chemicals tested in the standard BALB/c-3T3 transformation assay induced reproducible transformation responses. To accomplish this goal we tested all chemicals in two or more experiments. In addition, five chemicals were tested as both coded and uncoded test chemicals: C. I. basic red 9, dichlorvos, dimethylmorpholinophosphoramidate, HC red 3, and methyl carbamate. The results of these experiments showed that the cytotoxic responses of the paired chemicals were nearly identical (Tables A1 and A4). Likewise, the transformation responses of all 5 pairs of chemicals were not significantly different from one another (Tables A2 and A5). Both sources of dimethylmorpholinophosphoramidate, HC red 3, and methyl carbamate were active in the transformation assay, and C. I. basic red no 9 was inactive. The uncoded source of dichlorvos was inactive in the transformation assay, and the coded source of the chemical was evaluated as having an equivocal response.

Test chemical transformation responses were also observed to be very reproducible for the total group of 168 chemicals tested to at least two consecutive trials. A total of 82.7% (139/168) chemicals were clearly active or inactive in the transformation assay (Tables A3 and A6). Of the remaining 29 test chemicals, 8.9% (15/168) of the chemicals were evaluated as having weakly active or equivocal activities in the transformation assay. Thus, only 8.3% (14/168) of the chemicals had an indeterminate activity which resulted from different transformation responses being detected in two consecutive experiments. Therefore, the majority of the chemicals tested in the transformation assay had reproducible activities detected in two consecutive experiments.

The fifth accomplishment of this investigation has been to use the computer-automated structural evaluation software system (CASE) to investigate quantitative structure–activity relationships (QSAR) for BALB/c-3T3 transformation response data (unpublished observations). Because a combined database of 205 chemicals tested in a standard BALB/c-3T3 transformation assay was available [i.e., 168 chemicals in the current study, 24 chemicals in part IV of this series (*14*), and 13 polycyclic aromatic hydrocarbons (unpublished observations)], sufficient data were available to investigate a possible correlation of induction of transformation with specific portions of the chemical structure (i.e., biophores). This investigation revealed that the induction of transformation response data was significantly correlated with the presence of only 13 biophores; conversely, just four biophobes were associated with the inhibition of transformation. In addition, the study showed that the four biophobes were present on many of the 14 chemicals which had indeterminate activity in the transformation assay. In a companion investigation, CASE utilized the BALB-c-3T3 cell cytotoxicity data from the co-culture clonal survival assay to investigate QSAR for chemical-induced cytotoxic responses. This investigation revealed that a limited number of biophores were highly correlated with certain chemicals being cytotoxic to BALB/c-3T3 and other cultured mammalian cells [unpublished observations (*26*)]. QSAR investigations have determined that a limited number of biophores are highly correlated with the induction of cytotoxicity and transformation, and this information can be used to predict cytotoxic and transformation responses of chemicals untested in the BALB/c-3T3 transformation assay.

In conclusion, one of the major goals of the NTP Genetic Toxicology program during the 1980s has been to develop and evaluate *in vitro* assays that selectively detect carcinogenic chemicals that were inactive in Salmonella mutagenesis assays. If such assays could be developed, they could be used to investigate *in vitro* biological activities in common among the active chemicals and thereby lead to a clearer understanding of the mechanism(s) by which nonmutagenic carcinogens are carcinogenic in rodent bioassays. This report and the companion investigations have demonstrated progress in achieving this goal. The data in this report show that the majority of the 35 nonmutagenic carcinogens (21/35) were selectively detected in a standard BALB/c-3T3 transformation assay. In addition, CASE has identified chemical fragments of each of the nonmutagenic carcinogens that significantly correlated with the effects of the chemical in the transformation assay. Therefore, it now feasible to investigate the several different nonmutagenic carcinogens to determine the mechanism(s) by which they induced a permanent change in the transformed phenotype of BALB/c-3T3 cells. It is hoped that these investigations will help to close the current gap in our understanding of *in vitro* and *in vivo* chemical carcinogenesis.

The opinions expressed in this paper are solely those of the authors and do not necessarily reflect the positions of the U.S. Food and Drug Administration.

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Appendix A

Cytotoxic, Mutagenic Carcinogens

2-Acetylaminofluorene. 2-Acetylaminofluorene was a potent level *A* carcinogen (Table A3) with no serious technical problems reported (Table A1). It was cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 0.171 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 83 and 89/110, respectively; the detection sensitivities for BaP of trails 1 and 2 were 70 and 87/110, respectively (Table A2). The test chemical had an SP transformation response in two consecutive experiments. 2-Acetylaminofluorene was evaluated as very active in the transformation assay, and its actual and estimated rank *t*-statistics were 3.12 and 4.67, respectively (Table A3).

Acrylonitrile. Acrylonitrile was a level *B* carcinogen (Table A3). This chemical had one serious technical problem, because it was reported to be oxidized upon exposure to air (Table A1). It was cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 0.337 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 52 and 41/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 79 and 83/110, respectively (Table A2). In both trials the chemical had an LA transformation responses. Acrylonitrile was evaluated as having had weak activity in the transformation assay. Its actual and estimated rank *t*-statistics were 3.75 and 4.35, respectively (Table A3).

2-Amino-4-Nitrophenol. 2-Amino-4-nitrophenol was a relatively weak level *D* carcinogen (Table A3). It had one difficult technical problem, because it is oxidized upon exposure to air (Table A1). It was cytotoxic to the BALB/c-3T3 cells with an average LD_{50} of 0.933 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 13 and 56/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 66 and 109/110, respectively (Table A2). In trials 1 and 2 the chemical had SN transformation responses. However, there were two problems with the second transformation experiment. The test chemical cytotoxic response in the second experiment did not have a large cytotoxic shift as noted in previous experiments, and the detection sensitivity was very low in the experiment. Therefore, the test chemical should be tested in a third experiment to properly evaluate its activity in the transformation assay. 2-Amino-5-nitrophenol was therefore evaluated as having had an indeterminate activity in the transformation assay. Its actual and estimated rank *t*-values were both 0.00 (Table A3).

2-Amino-5-Nitrophenol. 2-Amino-5-nitrophenol was a relatively weak level *D* carcinogen (Table A3). It had one difficult technical problem, because it is oxidized upon exposure to air (Table A1). It was cytotoxic to the BALB/c-3T3 cells with an average LD_{50} of 0.409 mM (Table A1). The statistical sensitivities of transformation assay trials 1-3 were 71, 43 and 21/110, respectively; the detection sensitivites for BaP of trials 1-3 were 23, 9 and 53/110, respectively (Table A2). In a preliminary trial 1 the chemical had a SN transformation response. In trials 2 and 3 the chemical had SP transformation responses. 2-Amino-5-nitrophenol was therefore evaluated as active in the transformation assay. Its actual and estimated rank *t*-statistics were both 5.94 (Table A3).

5-Azacytidine. 5-Azacytidine was a level *D* carcinogen (Table A3) with no serious technical problems reported (Table A1). It was very cytotoxic to the BALB/c-3T3 cells with an average LD_{50} of 0.00463 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 45 and 101/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 38 and 104/110, respectively (Table A2). In trials 1 and 2 the chemical had an SP transformation response. 5-Azacytidine was evaluated as one of the most active chemicals in the transformation assay. Its actual and estimated rank *t*-statistics were 12.8 and 16.8, respectively (Table A3).

Benzidine-2HCl. Benzidine-2HCl was a level *A* carcinogen (Table A3). It had one serious technical problem because it was reported to become oxidized upon exposure to air (Table A1). It was cytotoxic to the BALB/c-3T3 cells with an average LD_{50} of 0.121 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 30 and 34/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 4 and 45/110, respectively (Table A2). In trial 1 and 2 the chemical had an SP transformation response. Benzidine-2HCl was evaluated as active in the transformation assay. Its actual and estimated rank *t*-statistics were both 3.38 (Table A3).

Table A1. Cytotoxic responses of 114 cytotoxic chemicals.

Test Chemical[a]			Physicochemical Properties			Cytotoxic Responses[b] (millimolar LD_{50}) Co-culture
Name	CAS No.	M.W.	1	2	3	Assay
43 Mutagenic Carcinogens						
Group I. Moderately Cytotoxic Chemicals						
5-chloro-*o*-toluidine	95-79-4	141.61	S	DFC	a. ac, ai, o	1.69
C. I. disperse yellow 3	2832-40-8	269.31	S	DFC	o	1.50
1,2-epoxybutane	106-88-7	72.11	L	C	ai, a, b, bc, ls, mh, o, p, ts	1.45
1,2-epoxypropane	75-56-9	58.08	L	C	a, ai, b, bc, mc, mh, msc, o, p, r	1.60
ethylenedibromide	106-93-4	187.88	L	DFC	b, ls, met, o, p, r	1.69
HC blue 1	2784-94-3	256.31	S	DFC	ai,	1.96
iodinated glycerol	5634-39-9	260.	L	C	ls	3.47
4,4-methylenedianiline	101-77-9	271.21	S	DFC	ai, ls	1.56
N-methyl-*o*-acrylamide	924-42-5	101.11	S	FC	ls, ts, o	1.75
2-naphthylamine	91-59-8	143.18	S	CF	a, ai, o,	1.59
quinoline	91-22-5	129.16	L	DFC	a, ls, msc, o, p	4.09
o-toluidine	95-53-4	107.16	L	DFC	a, ai, o, p, r	4.33
Group II. Cytotoxic Chemicals						
2-acetylaminofluorene	53-96-3	223.3	S	DFC		0.171
acrylonitrile	107-13-1	53.06	L	DC	ai, v,	0.337
2-amino-4-nitrophenol	99-57-0	154.13	S	DFC	a, ac, ai, ls, o, ts	0.933
2-amino-5-nitrophenol	121-88-0	154.13	S	DFC	ai, ls, ts,	0.409
benzidine-2HCl	531-85-1	257.18	S	DFC	ai, ls	0.121
2-biphenylamine	90-41-5	169.22	S	DFC	mel, o,	0.421
4-biphenylamine	92-67-1	169.23	S	DFC	ai, o,	0.479
4-chloro-*o*-toluidine-HCl	3165-93-3	178.07	L	DFC	a, ac, ai, o	0.650
C. I. acid orange 3	6373-74-6	453.41	S	DFC		0.102
C. I. disperse blue 1	2475-45-8	268.3	S	FC	sp, ts	0.240
C. I. solvent yellow 14	842-07-9	248.30	S	AFC	ls, o, sp	0.199
1,2-dibromo-3-chloropropane	96-12-8	236.35	L	DFC	b, met, r	0.401
2,6-dichloro-*p*-phenylenediamine	609-20-1	177.0	S	DFC	ai, ls, ts	0.921
1,3-dichloropropene	542-75-6	110.98	L	DFC	a, hc, met, o, tc	0.280
dichlorvos uncoded	62-73-7	220.98	L	DC	a, b, met, p, ru, w	0.145
(676384)						0.140
2,4-dinitrotoluene	121-14-2	182.14	S	DCF	o, r, ts	0.917
epichlorohydrin	106-89-8	92.53	L	DC	ai, a, b, c, msc, o, w	0.364
nitrofurantoin	67-20-9	238.16	S	DFC	a, b, ls, met, o, ts	0.106
2-nitro-*p*-phenylenediamine	5307-14-2	153.16	S	DFC	ai, ls, o	0.947
4,4-oxydianiline	101-80-4	200.24	S	DFC	ai, ls, o,	0.270
selenium sulfide	7446-34-6	111.02	S	CF		0.125
Group III. Very Cytotoxic Chemicals						
5-azacytidine	320-67-2	244.2	S	DC	ts,	0.00463
4-chloro-*o*-phenylenediamine	95-83-0	142.59	S	DFC	ai, k, l, o	0.0318
3-(chloromethyl)pyridine-HCl	6959-48-4	164.04	S	C	a, o,	0.0756
C. I. basic red 9-HCl uncoded	569-61-9	323.83	S	FC		0.00281
(947733)						0.00216
cytembena	21739-91-3	307.09	S	C		0.153
diglycidyl resorcinol ether	101-90-6	222.26	L	DCF	alk	0.00416
melphalan	148-82-3	305.23	S	FC	ls, ts, w	0.00120
N-methyl-N-nitro-N'-nitrosoguanidine	70-25-7	147.1	S	DC	ls, ts, w	0.0154
nitrofurazone	59-87-0	198.14	S	DFC	ls	0.0515
ziram	137-30-4	305.81	S	DFC	a, b, met	0.0000373
21 Mutagenic Non-Carcinogens						
Group I. Moderately Cytotoxic Chemicals						
4-acetylaminofluorene	28322-02-3	223.29	S	DFC	sp	4.07
3-chloro-*p*-toluidine	95-74-9	141.60	S	DFC	a, ach, ai, l, o	1.17
2,4-dimethoxyaniline-HCL	54150-69-5	189.66	S	DFC	-	1.13
HC blue 2	33229-34-4	285.34	S	C	ai	5.21
HC red 3 (uncoded)	2871-01-4	197.22	S	FC	-	3.72
(coded)						4.50

(Continued on next page)

Table A1. *Continued.*

Test Chemical[a]			Physicochemical Properties			Cytotoxic Responses[b] (millimolar LD_{50}) Co-culture
Name	CAS No.	M.W.	1	2	3	Assay
3-nitropropionic acid	504-88-1	119.08	S	C	a, ai, b, o	1.23
2,6-toluenediamine-2HCl	15481-70-6	195.11	S	C	-	4.11
Group II. Cytotoxic Chemicals						
2-(chloromethyl)pyridine-HCl	6959-47-3	164.04	S	DC	o	0.118
coumaphos	56-72-4	362.78	S	DFC	b, o	0.218
dimethoate	60-51-5	229.27	L	DFC	b, v	0.602
malaoxon	1634-78-2	314.32	L	DC	ai, v	0.468
1-naphthylamine	134-32-7	143.18	S	DFC	a, ai l, o	0.506
N-(1-naphthyl)ethylenediamine -2HCl	1465-25-4	259.18	S	DFC	a, ach, l, o	0.125
1-nitronaphthalene	86-57-7	173.17	S	DFC	a, o, r	0.464
4-nitro-*o*-phenylenediamine	99-56-9	153.14	S	DFC	a, ac, ai, ls r	0.292
N-phenyl-2-naphthylamide	135-88-6	219.30	S	DFC	ai, l	0.195
Group III. Very Cytotoxic Chemicals						
4'-(chloroacetyl)acetanilide	140-49-8	211.66	S	DFC	-	0.00336
8-hydroxyquinoline	148-24-3	145.16	S	DC	l, m, o	0.00251
p-phenylenediamine-2HCl	624-18-0	181.07	S	C	ai, l, o	0.0712
2,3,5,6-tetrachloro-4-nitroanisole	2438-88-2	290.91	S	DFC	-	0.0437
tetraethylthiuram disulfide	97-77-8	296.54	S	DFC	0	0.0000583
20 Non-Mutagenic Carcinogens						
Group I. Moderately Cytotoxic Chemicals						
allyl isovalerate	2835-39-4	142.22	L	DFC	-	4.51
chlorendic acid	115-28-6	388.83	S	DFC	m	4.07
chlorowax 40	108171-27-3	560.	L	FC	b, l, o, r, ts	1.43
chlorowax 500	108171-26-2	415.	L	AFC	l, o, r, ts	1.58
dimethylvinyl chloride	513-37-1	90.55	L	DFC	ai, b, o, l, t, v	4.74
isophorone	78-59-1	138.23	L	DFC	o, t, v	5.18
malonaldehyde, sodium salt	24382-04-5	94.05	S	C	l, vts	3.74
nitrilotriacetic acid	139-13-9	257.1	S	C	m	5.98
4-vinylcyclohexene	100-40-3	108.20	L	DFC	ai, o, ts	3.88
Group II. Cytotoxic Chemicals						
3-chloro-2-methylpropene	563-47-3	99.55	L	DFC	b, o , l, t, v, W	0.662
D-limonene	5989-27-5	136.24	L	DFC	a, ai, l, o, ts	0.988
2-mercaptobenzothiazole	149-30-4	167.25	S	DFC	a, o, W	0.130
methapyrilene-HCl	135-23-9	297.88	S	C	-	0.812
polybrominated biphenyl mixture	67774-32-1	627.59	S	DFC	l, sp	0.291
tris(2-ethylhexyl)phosphate	78-42-2	434.65	L	DFC	o	0.338
Group III. Very Cytotoxic Chemicals						
allyl isothiocyanate	57-06-7	99.16	L	DFC	a, al, b, o, v, W	0.00712
cinnamyl anthranilate	87-29-6	253.32	S	DFC	ai, ald, l	0.0947
diethylstilbestrol	56-53-1	268.34	S	DFC		0.0858
ethyl acrylate	140-88-5	100.12	L	DC	a, b, o, pi, v, W	0.0746
reserpine	50-55-5	608.70	S	DC	ai, l, o, r	0.0133
30 Non-Mutagenic Non-Carcinogens						
Group I. Moderately Cytotoxic Chemicals						
carbromal	77-65-6	237.10	S	DFC	ai, l	3.60
C. I. acid red 14	3567-69-9	502.44	S	C	-	3.38
C. I. acid yellow 73	518-47-8	376.	S	C	o	4.65
ephedrine sulfate	134-72-5	428.54	S	C	l, ts	1.53
ethylenediamine tetraacetic acid, trisodium salt	150-38-9	358.22	S	FC	m	1.89
D,L-menthol	15356-70-4	156.27	S	DFC	o, oc	4.63
methylphenidate-HCl	298-59-9	269.80	S	C	-	5.63
phenol	108-95-2	94.11	S/L	C	a, ai, b, l, o, oc, s, ts	3.29

(Continued on next page)

Table A1. *Continued.*

Test Chemical[a]			Physicochemical Properties			Cytotoxic Responses[b] (millimolar LD_{50}) Co-culture Assay
Name	CAS No.	M.W.	1	2	3	
phenylephrine-HCl	61-76-7	203.67	S	C	a, ai, ach, l, o, ts	3.52
tetracycline-HCl	64-75-5	480.94	S	FC	ai, l, o, ts	3.24
xylenes, mixed	1330-20-7	106.17	L	DFC	o, ts	3.20
Group II. Cytotoxic Chemicals						
L-ascorbic acid	50-81-7	176.14	S	C	ai, l, m, oc, r	0.363
bisphenol A	80-05-7	228.29	S	DFC	ach, o	0.147
chlorpheniramine-maleate	113-92-8	390.87	S	C	-	0.287
eugenol	97-53-0	164.20	L	DFC	ai, b, l, o, ts, v	0.875
geranyl acetate	105-87-3	196.32	L	DFC	-	0.302
4-hexylresorcinol	136-77-6	194.27	L	DFC	ach, l, o, ts	0.103
oxytetracycline-HCl	2058-46-0	496.90	S	FC	l, ts, w	0.523
Group III. Very Cytotoxic Chemicals						
anilazine	101-05-3	275.53	S	DFC	b, mo	0.0475
erythromycin stearate	643-22-1	1018.59	S	AFC	a	0.0746
ethoxylated dodecyl alcohol	9002-92-0	~1200.00	L	DC	b, o, v	0.0172
methoxychlor	72-43-5	345.66	S	DFC	l, mo, o	0.0978
methyldopa sesquihydrate	555-30-6	238.24	S	DFC	b, ts	0.0810
propyl gallate	121-79-9	212.20	S	DFC	a, b, i, r	0.0631
rotenone	83-79-4	394.43	S	AFC	ai, b, l, o, ts	0.000464
sodium diethyldithiocarbamate	148-18-5	171.27	S	C	a, o	0.000142
stannous chloride	7772-99-8	189.60	S	DC	al, am, b, mo, o	0.0285
tetrakis(hydroxymethyl) phosphonium chloride	124-64-1	190.58	L	C	b, o, ts	0.00825
tetrakis(hydroxymethyl) phosphonium sulfate	55566-30-8	406.32	L	C	b, o	0.00438
triphenyltin hydroxide	76-87-9	367.03	S	FC	a, l, ts	0.000134

Abbreviations: CAS No., Chemical Abstract Service registry number; LD_{50}, lethal dose for 50% of the cells; M.W., molecular weight.

Abbreviations for Test Chemical Physiochemical Properties: Physicochemical considered in this study included: [1] physical state (S = solid; L = liquid); [2] solvent vehicle (D = dimethyl sulfoxide, C = culture medium, F = pluronic F68, A = acetone, E = ethanol) and [3] technical problems. The technical problems included test chemicals that were a = reactive with acids; ac = reactive with acid chlorides and acid anhydrides; ai = reactive with air; al = reactive with alcohols; alk = alkylating agent and reacts with labile hydrogen; b = reacts with bases; bc = reacts with biochemicals (amino, hydroxyl, and carboxyl groups); hc = reacts with halogenated chemicals; k = reacts with alpha keto acids; ls = light sensitive; m = binds metals; mel = reacts with hexachloro- and trichloromelamine; met = reacts with metals (aluminum, iron, magnesium, potassium, sodium, tin or zinc); mh = metal halides; msc = reacts with miscellaneous organic chemicals (i.e., alpha-aminoethanol, chlorosulfonic acid, ethylene imine, linseed oil, maleic anhydride, oleum, or K-tert-butyloxide); o = reacts with oxidizing agents; p = reacts with plastics; pi = polymerization initiators; r = reacts with reducing agents; ru = reacts with rubber; sp = solubility problem in culture medium; tc = reacts with thiocyanates; ts = temperature sensitive; vts = very temperature sensitive; v = volatile at 37°C; and w = reacts with water [refer to MATERIALS and METHODS].

[a]Test Chemical: Tables A1 and A3 contain 168 chemicals along with their individual CAS registry number and molecular weight. The chemicals were divided into groups of chemicals that correspond to the groups of chemicals that were compared in different text Tables 1–12. Thus, the chemicals were divided into two groups, including 114 cytotoxic test chemicals (LD_{50} < 5.0 mM) presented in Table A1, and 53 noncytotoxic chemicals (LD_{50} > 5.0 mM) presented in Table A4. The 114 cytotoxic chemicals in Table A1 were subdivided into groups of 43 mutagenic carcinogens, 21 mutagenic noncarcinogens, 20 nonmutagenic carcinogens, and 30 nonmutagenic noncarcinogens. The 53 noncytotoxic test chemicals in Table A4 were subdivided into groups of 21 carcinogens, 26 noncarcinogens and 7 model very noncytotoxic chemicals. In addition, all of the cytotoxic test chemicals were separated into three groups, including: group I, moderately cytotoxic test chemicals [LD_{50} 1–5 mM]; group II, cytotoxic chemicals [LD_{50} 0.1–1.0 mM]; and group III, very cytotoxic chemicals [LD_{50} < 0.1 mM]. In addition, this table presents important physicochemical properties that influenced the procedure used in testing the chemicals.

[b]Cytotoxic Response: The co-culture clonal survival assay design used to detect the cytotoxic response of the test chemical is described in Materials and Methods. The cytotoxic responses of chemicals in individual experiments are summarized in terms of the millimolar (mM) LD_{50} treatment dose that resulted in 50% survival of the chemically-treated cells relative to the survival of untreated or solvent control treated cell cultures. The LD_{50} cytotoxic response of each chemical in Tables A1 and A4 is an average of two or more experiments with the chemical. The molecular weight of each chemical is provided in order that treatment doses could be converted from mM to μg/mL. For example, based upon the molecular weight of 141.61, the LD_{50} detected for the first chemical in Table A1, 5-chloro-*o*-toluidine, was 1.69 mM or 239 μg/mL.

Table A2. Transformation responses of 114 cytotoxic chemicals.

Chemical[a] Name	Exp. No.	Spontaneous[c] Foci/Vessel: Rank Order	Transformation Response[b] Benzo(a)pyrene[d] Call : Rank Order	Test Chemical[e] Call : mean *t*-statistic
43 Mutagenic Carcinogens				
Active Chemicals				
2-acetylaminoflourene	1 (17)	.327 83	SP 70	SP 4.15
	2 (24)	.308 89	SP 87***	SP 2.08
acrylonitrile	1 (86)	.464 52	SP 79*	LA 5.36
	2 (92)	.579 41	SP 83*	LA 2.14
2-amino-5-nitrophenol	1 (62)	6.02 3***	SN 110***	LN .000
	2 (83)	.351 71	SP 23**	SN .575
	3 (93)	.416 43	SP 9***	SP 5.41
	4 (103)	.874 21	SP 53	SP 11.8
5-azacytidine	1 (6)	.431 45	SP 38	SP 16.7
	2 (11)	.301 101	SP 104***	SP 9.03
benzidine-2HCl	1 (43)	1.05 30	SP 4***	SP 4.54
	2 (52)	1.09 34	SP 45	SP 2.22
2-biphenylamine	1 (33)	1.04 49	SP 24**	SP 2.63
	2 (52)	1.09 34	SP 45	SP 3.79
4-biphenylamine	1 (35)	1.97 14	SP 72	LA 2.21
	2 (53)	2.78 11*	SP 39	SP 2.51
3-(chloromethyl)pyridine-HCl	1 (14)	.213 99	SP 82*	LA 1.35
	2 (22)	.893 36	SP 28	SP 2.77
4-chloro-*o*-phenylenediamine	1 (13)	.201 96	SP 86***	LN .870
	2 (20)	.368 81	SP 22***	SP 2.76
	3 (101)	.260 62	SP 48	SP 8.92
4-chloro-*o*-toluidine-HCl	1 (81)	7.36 2***	SP 2***	SP 7.18
	2 (92)	.597 41	SP 83*	SP 4.44
5-chloro-*o*-toluidine	1 (81)	7.36 2***	SP 2***	SP 3.57
	2 (92)	.597 41	SP 83*	SP 5.38
C. I. acid orange 3	1 (70)	.526 47	SP 43	LA 1.55
	2 (87)	.346 63	SP 101***	SP 4.23
C. I. disperse blue 1	1 (73)	.274 80	SP 89**	SP 3.53
	2 (97)	.414 54	SP 44	SN .405
	3 (107)	2.95 5**	SP 46	SN .000
C. I. solvent yellow 14	1 (7)	.135 105*	SP 69	SP 3.52
	2 (67)	.085 107**	SP 106***	SP 4.64
	3 (IP17)	.411 ~99	SP ~37	LA 2.16
	4 (IP18)	.189 ~105*	SP ~39	SP 3.43
cytembena	1 (70)	.526 47	SP 43	LA 4.34
	2 (83)	.351 71	SP 23**	SP 7.12
1,2-dibromo-3-chloropropane	1 (23)	.661 66	SP 81*	LN .333
	2 (27)	.555 78	SP 58	SP 6.36
	3 (102)	.697 27	SP 63	SP 4.83
selenium sulfide	1 (7)	.135 105*	SP 69	SP 3.34
	2 (11)	.301 101	SP 104***	SN .223
	3 (97)	.414 54	SP 44	SP 2.24

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Table A2. *Continued.*

Chemical[a] Name	Exp. No.	Spontaneous[c] Foci/Vessel: Rank Order	Transformation Response[b] Benzo(a)pyrene[d] Call : Rank Order	Test Chemical[e] Call : mean *t*-statistic
O-toluidine	1 (16)	.344 87	SP 64	LA .900
	2 (25)	.101 106*	SP 51	SP 9.28
	3 (98)	.618 59	SP 17***	SP 4.31
ziram	1 (77)	.972 17	SP 6***	LA 1.17
	2 (91)	.322 56	SP 109***	SP 4.10
Chemical with an Equivocal Activity				
dichlorvos	1 (68)	.226 98	SP 60	SN .460
(uncoded)	2 (DRI5)	1.27 ~72	NA	LA 3.51
	3 (98)	.618 59	SP 17***	SN .000
dichlorvos	1 (78)	3.28 9*	SP 37	SN .697
(coded)	2 (90)	1.95 15	SP 21**	SP 2.66
Inactive Chemicals				
C. I. basic red 9-HCl	1 (48)	.537 64	SP 84*	SN .353
(uncoded)	2 (66)	.056 108**	SP 99***	SN .700
	3 (DRI4)	.668 ~82	NA	SN .000
C. I. basic red 9-HCl	1 (73)	.274 80	SP 89**	SN .098
(coded)	2 (95)	2.84 8*	SP 29	SN .000
2,4-dinitrotoluene	1 (46)	.384 88	SP 77	SN 1.57
	2 (55)	.129 104*	SP 90***	SN .478
	3 (DRI2)	.503 ~88	NA	SN .000
Chemicals with an Indeterminate Activity				
2-amino-4-nitrophenol	1 (63)	1.92 13	SP 66	SN .000
	2 (91)	.322 56	LA 109	SN .000
	3 (NA)			
C. I. disperse yellow 3	1 (71)	1.06 24	SP 10***	SN .000
	2 (91)	.322 56	LA 109***	SN .155
	3 (NA)			
Chemicals with an Indeterminate Activity				
HC blue 1	1 (15)	.186 100	SP 42	SN 1.51
	2 (21)	.347 79	SP 75	SN .725
	3 (DRI2)	.503 88	NA	SP 3.09
	4 (NA)			
21 Mutagenic Non-Carcinogens				
Active Chemicals				
2-(chloromethyl)pyridine-	1 (14)	.213 99	SP 82	SP 2.36
HCl	2 (22)	.893 36	SP 28*	SP 2.77
3-chloro-*p*-toluidine	1 (81)	7.36 2***	SP 2***	SP 2.60
	2 (92)	.597 41	SP 83*	SP 3.92
coumaphos	1 (30)	.787 40	SP 54	SN .000
	2 (95)	2.847 8*	SP 29	SP 3.75
	3 (99)	.586 33	SP 14**	SP 7.60
dimethoate	1 (41)	.274 90	SP 13***	LA 1.24
	2 (94)	1.52 18	SP 31	SP 5.39
HC red 3	1 (40)	.533 60	SP 27*	SP 2.50
	2 (57)	.278 86	SP 74	SP 2.77

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Table A2. *Continued.*

Chemical[a] Name	Exp. No.	Spontaneous[c] Foci/Vessel: Rank Order		Transformation Response[b] Benzo(a)pyrene[d] Call : Rank Order		Test Chemical[e] Call : mean *t*-statistic	
HC red 3 (260886)	1 (61)	.222	93	SP	100***	LA	1.61
	2 (99)	.586	33	SP	14**	SP	9.58
malaoxon	1 (16)	.344	87	SP	64	LA	4.17
	2 (25)	.101	106*	SP	51	SP	9.46
1-naphthylamine	1 (13)	.201	96	SP	86***	SP	2.66
	2 (19)	.357	77	SP	67	SP	2.97
4-nitro-*o*-phenylenediamine	1 (14)	.213	99	SP	82	SP	3.83
	2 (18)	.663	46	SP	80*	LA	1.27
3-nitropropionic acid	1 (39)	.427	82	SP	18***	SP	3.90
	2 (85)	.588	67	SP	78	SP	5.47
p-phenylenediamine-2HCl	1 (37)	.631	58	SP	55	SP	3.81
	2 (89)	.492	51	SP	50	SP	2.27
N-phenyl-2-naphthylamide	1 (61)	.222	93	SP	100***	SP	4.79
	2 (87)	.346	63	SP	101***	SP	3.71
tetraethylthiuram disulfide	1 (80)	3.024	10*	SP	5***	SP	4.60
	2 (93)	.416	43	SP	9***	SP	2.92
2,6-toluenediamine	1 (29)	.606	68	SP	40	SP	3.89
	2 (44)	1.52	23	SP	11***	SP	10.0
4'-(chloroacetyl)-acetanilide	1 (37)	.631	58	SP	55	LA	1.85
	2 (95)	2.84	8*	SP	29	LA	1.46
2,4-dimethoxyaniline	1 (34)	2.51	7*	SP	56	SP	5.95
	2 (87)	.346	63	SP	101***	SN	1.32
	3 (97)	.414	54	SP	44	SN	.960
8-hydroxyquinoline	1 (3)	.285	94	SP	91***	SP	2.46
	2 (9)	.149	102*	SP	95***	SN	.170
	3 (28)	.818	39	SP	68	SN	.128
N-(1-naphthyl)ethylene-diamine-2HCl	1 (38)	.496	69	SP	12***	LA	.957
	2 (87)	.346	63	SP	101***	LA	2.31
2,3,7,8-tetrachloro-4-nitroanisole	1 (29)	.606	68	SP	40	LA	2.47
	2 (93)	.416	43	SP	9***	LA	1.70
Inactive Chemicals							
HC blue 2	1 (5)	.035	110***	SP	107***	LA	4.31
	2 (10)	.053	109**	SP	103***	SN	1.43
Chemicals with an Indeterminate Activity							
4-acetylaminofluorene	1 (12)	.160	103*	SP	73	LN	.963
	2 (17)	.327	83	SP	70	LN	1.64
1-nitronaphthalene	1 (33)	1.04	49	SP	24**	SN	.000
	2 (87)	.346	63	SP	101***	SP	2.53

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Table A2. *Continued.*

Chemical[a]		Spontaneous[c]	Transformation Response[b]	
		Foci/Vessel:	Benzo(a)pyrene[d]	Test Chemical[e]
Name	Exp. No.	Rank Order	Call : Rank Order	Call : mean *t*-statistic
2,6-dichloro-*p*-phenylene-diamine	1 (32)	1.99 19	SP 34	SP 2.50
	2 (54)	.265 91	SP 85	SP 2.03
1,3-dichloropropene	1 (79)	5.12 4**	SP 1***	SP 3.11
	2 (94)	1.52 18	SP 31	SN .000
	3 (104)	.878 26	SP 93**	LA 1.75
diglycidyl resorcinol ether	1 (6)	.348 45	SP 38	SP 15.2
	2 (12)	.160 103*	SP 73	SP 6.66
epichlorohydrin	1 (68)	.226 98	SP 60	LA 2.38
	2 (DRI5)	1.27 ~72	NA	SP 3.47
1,2-epoxybutane	1 (79)	5.12 4**	SP 1***	LN NA
	2 (104)	.878 26	SP 93***	SP 3.64
	3 (108)	1.17 20	SP 19**	SP 2.50
1,2-epoxypropane	1 (72)	.289 70	SP 41	SP 4.04
	2 (88)	.406 57	SP 88***	SP 4.93
ethylene dibromide	1 (74)	.657 32	SP 108***	SP 8.53
	2 (92)	.597 41	SP 83*	SP 2.83
iodinated glycerol	1 (74)	.657 32	SP 108***	SP 6.04
	2 (106)	1.30 28	SP 15***	SN 1.31
melphalan	1 (80)	3.02 10*	SP 5***	LA 1.37
	2 (96)	.660 31	SP 52	SP 4.81
N-methyl-*o*-acrylamide	1 (70)	.526 47	SP 43	LA 1.53
	2 (85)	.313 67	SP 78	SP 3.88
4,4-methylenedianiline	1 (40)	.533 60	SP 27*	SP 1.93
	2 (55)	.129 104*	SP 90***	SP 1.82
N-methyl-N′-nitro-N-nitrosoguanidine	1 (93)	.416 43	SP 9***	SP 10.1
	2 (IP2)	1.13 ~54	NA	SP 7.52
2-naphthylamine	1 (13)	.201 96	SP 86***	SP 3.33
	2 (26)	.907 37	SP 20***	SP 3.99
nitrofurantoin	1 (61)	.222 93	SP 100***	SN .778
	2 (93)	.416 43	SP 9***	SP 4.25
nitrofurazone	1 (71)	1.06 24	SP 10***	LN .000
	2 (77)	.972 17	SP 6***	SP 3.26
	3 (87)	.346 63	SP 101***	SP 4.99
2-nitro-*p*-phenylene-diamine	1 (15)	.186 100	SP 42	SP 4.21
	2 (21)	.347 79	SP 75	SN 1.38
	3 (96)	.660 31	SP 52	SP 4.38
4,4-oxydianiline	1 (1)	1.44 25	SP 36	SP 1.81
	2 (8)	2.19 16	SP 7***	SP 3.72
quinoline	1 (16)	.344 87	SP 64	LN .000
	2 (27)	.555 78	SP 58	LN .000
	3 (31)	.930 42	SP 25*	LA 1.93
	4 (104)	.878 26	SP 93***	SP 3.96

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Table A2. *Continued.*

Chemical[a] Name	Exp. No.	Spontaneous[c] Foci/Vessel: Rank Order		Transformation Response[b] Benzo(a)pyrene[d] Call : Rank Order		Test Chemical[e] Call : mean *t*-statistic	
20 Non-Mutagenic Carcinogens							
Active Chemicals							
allyl isothiocyanate	1 (41)	.274	90	SP	13***	LA	1.62
	2 (98)	.226	98	SP	60	SP	4.09
chlorendic acid	1 (63)	1.92	13	SP	66	SP	1.96
	2 (83)	.351	71	SP	23**	LA	2.01
3-chloro-2-methylpropene	1 (29)	3.28	9*	SP	37	LA	1.62
	2 (31)	.406	57	SP	88***	SP	2.66
diethylstilbestrol	1 (42)	.861	55	SP	8***	LA	2.22
	2 (96)	.660	31	SP	52	SP	3.38
dimethylvinyl chloride	1 (76)	1.79	12*	SP	98***	LA	1.45
	2 (102)	.697	27	SP	63	SP	3.74
ethyl acrylate	1 (23)	.661	66	SP	81	LA	2.49
	2 (36)	.424	74	SP	35	SP	6.02
isophorone	1 (25)	.101	106*	SP	51	LN	.203
	2 (36)	.424	74	SP	35	SP	3.46
	3 (104)	.878	26	SP	93***	SP	2.06
malonaldehyde, sodium salt	1 (75)	.882	21	SP	26*	SP	1.92
	2 (97)	.414	54	SP	44	LA	1.55
nitrilotriacetic acid	1 (73)	.274	80	SP	89**	SP	2.46
	2 (99)	.586	33	SP	14**	SP	8.41
polybrominated biphenyl mixture	1 (20)	.368	81	SP	22***	SP	2.22
	2 (28)	.818	39	SP	68	SP	1.64
Chemical with an Equivocal Activity							
2-mercaptobenzothiazole	1 (62)	6.02	3*	LA	110***	LN	.000
	2 (77)	.970	17	SP	6**	LA	1.23
	3 (89)	.492	51	SP	50	LA	1.22
Inactive Chemicals							
allyl isovalerate	1 (23)	.661	66	SP	81*	LN	.080
	2 (27)	.555	78	SP	58	LN	.055
	3 (31)	.930	42	SP	25*	SN	.591
	4 (102)	.697	27	SP	63	LA	2.35
chlorowax 40	1 (76)	1.79	12*	SP	98***	LA	.850
	2 (104)	.878	26	SP	93***	SN	.078
chlorowax 500	1 (74)	.657	32	SP	108***	SN	.185
	2 (90)	1.95	15	SP	21***	SN	.000
cinnamyl anthranilate	1 (2)	.660	53	SP	30	SN	.010
	2 (9)	.149	102*	SP	95***	SN	.000
	3 (DFR3)	.424	~92	NA		SN	.000
D-limonene	1 (72)	.289	70	SP	41	LN	1.02
	2 (76)	1.79	12*	SP	98***	SN	.473
	3 (88)	.406	57	SP	88***	SN	.000
methapyrilene-HCl	1 (40)	.533	60	SP	27*	SN	.000
	2 (54)	.265	91	SP	85	SN	.417
	3 (DRI4)	.668	~82	NA		SN	.000

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Table A2. *Continued.*

Chemical[a] Name	Exp. No.	Spontaneous[c] Foci/Vessel: Rank Order		Transformation Response[b] Benzo(a)pyrene[d] Call : Rank Order		Test Chemical[e] Call : mean *t*-statistic	
reserpine	1 (1)	1.44	25	SP	36	LA	1.38
	2 (8)	2.19	16	SP	7***	SN	.000
	3 (DR3)	.424	~92	NA		SN	.887
tris(2-ethylhexyl)phosphate	1 (88)	.406	57	SP	88***	SN	.000
	2 (98)	.618	59	SP	17***	SN	.000
4-vinylcyclohexene	1 (74)	.657	32	SP	108***	SN	.000
	2 (110)	.609	38	SP	16***	SN	.000
30 Non-Mutagenic Non-Carcinogens							
Active Chemicals							
C. I. acid red 14	1 (30)	.787	40	SP	54	SP	2.26
	2 (45)	.732	35	SP	57	SP	2.05
phenol	1 (76)	1.79	12*	SP	26*	SP	10.5
	2 (90)	1.95	15	SP	21**	SP	4.60
propyl gallate	1 (3)	.285	94	SP	91***	SP	2.42
	2 (9)	.149	102*	SP	95***	LA	.958
sodium diethyldithio-carbamate	1 (38)	.496	69	SP	12***	SP	3.37
	2 (96)	.660	31	SP	52	SP	2.52
Weakly Active Chemicals							
carbromal	1 (35)	1.97	14	SP	72	SN	.673
	2 (44)	1.52	23	SP	11***	SP	3.94
chlorpheniramine-maleate	1 (70)	.526	47	SP	43	SN	.000
	2 (85)	.313	67	SP	78	SP	1.97
Chemicals with an Equivocal Activity							
anilazine	1 (29)	.606	68	SP	40	LA	1.09
	2 (85)	.313	67	SP	78	LA	2.81
Chemicals with an Equivocal Activity							
tetrakis(hydroxymethyl) phosphonium chloride	1 (72)	.289	70	SP	41	SN	.288
	2 (90)	1.95	15	SP	21**	SP	3.53
	3 (98)	.618	59	SP	17***	SN	.000
Inactive Chemicals							
L-ascorbic acid	1 (4)	1.51	50	SP	59	SN	.300
	2 (11)	.301	101	SP	104***	SN	.348
bisphenol A	1 (2)	.660	53	SP	30	SN	.060
	2 (8)	2.196	16	SP	7***	LA	1.15
	3 (IP17)	.411	~99	SP	~37	SN	.393
	4 (IP18)	.789	~105*	SP	~39	SN	.000
C. I. acid yellow 73	1 (77)	.972	17	SP	6***	SN	.000
	2 (83)	.351	71	SP	23***	SN	.065
ephedrine sulfate	1 (71)	1.061	24	SP	10***	LN	.810
	2 (77)	.972	17	SP	6***	SN	.000
	3 (89)	.492	51	SP	50	SN	.320
erythromycin stearate	1 (71)	1.06	24	SP	10***	SN	.000
	2 (89)	.492	51	SP	50	SN	.163

(Continued on next page)

Table A2. *Continued.*

Chemical[a] Name	Exp. No.	Spontaneous[c] Foci/Vessel: Rank Order		Transformation Response[b] Benzo(a)pyrene[d] Call : Rank Order		Test Chemical[e] Call : mean *t*-statistic	
Inactive Chemicals Continued							
ethoxylated dodecyl alcohol	1 (82)	8.01	1***	SP	3***	SN	.000
	2 (90)	1.95	15	SP	21**	SN	.583
ethylenediamine tetraacetic acid, trisodium salt	1 (73)	.274	80	SP	89***	LA	2.49
	2 (85)	.313	67	SP	78	SN	.810
geranyl acetate	1 (84)	.511	44	SP	65	SN	.530
	2 (92)	.597	41	SP	83*	SN	.090
4-hexylresorcinol	1 (63)	1.92	13	SP	66	SN	.000
	2 (85)	.313	67	SP	78	LA	.920
D,L-menthol	1 (18)	.663	46	SP	80*	SN	.215
	2 (24)	.308	89	SP	87***	SN	.083
methoxychlor	1 (37)	.631	58	SP	55	SN	.705
	2 (89)	.492	51	SP	50	LA	1.26
methyldopa sesquihydrate	1 (75)	.882	21	SP	26*	LA	1.67
	2 (91)	.322	56	SP	109***	SN	.600
methylphenidate-HCl	1 (48)	.537	64	SP	84*	SN	1.31
	2 (57)	.278	86	SP	74	SN	.780
oxytetracycline-HCl	1 (73)	.274	80	SP	89***	SN	.000
	2 (103)	.874	22	SP	53	SN	.000
	3 (107)	2.95	5***	SP	46	SN	.000
phenylephrine-HCl	1 (73)	.274	80	SP	89**	SN	.385
	2 (105)	.581	29	SP	97***	SN	.260
rotenone	1 (75)	.882	21	SP	26*	SN	.000
	2 (96)	.660	31	SP	52	SN	.510
stannous chloride	1 (19)	.357	77	SP	67	SN	.175
	2 (26)	.907	37	SP	20***	SN	1.38
tetracycline-HCl	1 (71)	1.06	24	SP	10***	SN	.000
	2 (89)	.492	51	SP	50	SN	.043
tetrakis(hydroxymethyl) phosphonium sulfate	1 (72)	.289	70	SP	41	SN	.125
	2 (84)	.511	44	SP	65	SN	.000
xylenes, mixed	1 (72)	.289	70	SP	41	SN	.000
	2 (100)	.268	73	SP	49	SN	.945
Chemicals with an Indeterminate Activity							
eugenol	1 (74)	.657	32	SP	108***	LN	1.88
	2 (94)	1.52	18	SP	31	SN	.000
triphenyltin hydroxide	1 (39)	.427	82	SP	18***	SN	.828
	2 (93)	.416	43	SP	9***	SP	3.27

General Abbreviations: Exp. No., experiment number; NA, not available.

Abbreviations for Transformation Responses: SP, sufficient positive; LA, limited activity; SN, sufficient negative; LN, limited negative.

[a]The 114 cytotoxic chemicals in Table A2 are identical to those in Table A1, and they are subdivided into groups of 43 mutagenic carcinogens, 21 mutagenic noncarcinogens, 20 nonmutagenic carcinogens, and 30 nonmutagenic noncarcinogens.

[b]Transformation Response: This table presents a summary of the spontaneous, BaP, and test chemical transformation responses detected in two or more experiments per test chemical. The assay design and procedures used in the standard transformation assay are described in the Materials and Methods. The transforming activities of individual chemical treatment doses (i.e., focus data), as well as the individual transformation responses (i.e., type III foci/vessel), are provided in detail in the Appendices B–H. Appendices B, C, D, E, F, G and H contain the activities of the 43 cytotoxic, mutagenic carcinogens; 21 cytotoxic, mutagenic noncarcinogens; 20 cytotoxic, nonmutagenic carcinogens; 30 cytotoxic, nonmutagenic, noncarcinogens; 21 noncytotoxic carcinogens; 26 noncytotoxic noncarcinogens; and 7 very noncytotoxic model test chemicals.

(Continued on next page)

Table A2. *Continued.*

[c]Spontaneous Transformation Response: The method used to calculate the spontaneous transformation response, as well as the positive control and test chemical responses, is explained in the Materials and Methods. The transformation responses are expressed as type III foci/vessel and were calculated using a $\log_{10}$ mathematical transformation procedure. The arithmetic value for foci/vessel in this table is the antilog of the $\log_{10}$ mean transformation response minus one. The procedure for rank ordering the spontaneous responses from 110 experiments is based upon the different statistical sensitivities of transformation experiments with different spontaneous responses is explained in the Statistical Sensitivity versus Spontaneous Response section of the Materials and Methods. Experiments with high spontaneous responses had a high statistical sensitivity and have relatively low rank-order numbers. For example, 2-amino-5-nitrophenol had a high spontaneous response of 6.02 foci/vessel in experiment 62, which had a high statistical sensitivity and rank order number 3/110. Conversely, experiments with a low statistical sensitivity had high rank-order numbers. For example, C.I. solvent yellow 14 had a low spontaneous response of 0.085 foci/vessel in experiment 67, which had a low statistical sensitivity and high rank-order number 107/110.

*Significant spontaneous or BaP transformation response, $0.01 < p \leq 0.05$.

**Significant transformation response, $0.001 < p \leq 0.01$.

***Significant transformation response, $p \leq 0.001$.

[d]Benzo(a)pyrene Transformation Response. The method used to call individual transformation experiments is described in detail in Materials and Methods. The method used to rank order the BaP transformation responses from the 110 experiments is based upon statistical comparison of the BaP transformation at the two treatment doses detected in an individual experiment with the median historical activity of the assay. This procedure is described in the Detection Sensitivity versus Benzo(a)pyrene Transformation Response section of the Materials and Methods. The rational for rank-ordering the experiments is analogous to that described for the spontaneous transformation responses (refer to footnote c above).

[e]Test Chemical Transformation Response: The method used to call individual experiments is described in detail in Materials and Methods, and the abbreviations for the calls are provided above. The significance of the transformation responses of individual chemical treatment doses were calculated using SAS statistical software (*22*). The mean t-statistic represents the average of the t-statistics of the four test chemical treatment doses in the experiment. The t-statistics for individual chemical treatment doses which were used to calculate the mean t-statistic are provided in the Appendices B–H.

Table A3. Rank-ordered potency of the transformation responses of 114 cytotoxic chemicals compared to rodent bioassay activities.

Test Chemical	Rodent Bioassay[b]			Transformation Response[c]	
	Level of Activity			Rank *t*-statistic	
Name	High	Low	None	Actual	Estimated[d]
43 Mutagenic Carcinogens					
Total Active Chemicals [92.5%][e]					
Active Chemicals					
5-azacytidine		D		12.8	16.8
diglycidyl resorcinol ether	A			9.31	11.0
N-methyl-N-nitro-N'-nitrosoguanidine	A			10.3	10.3
ethylenedibromide	A			5.68	6.82
2-amino-5-nitrophenol		D		5.94	5.94
cytembena	B			5.93	5.93
4-chloro-*o*-phenylenediamine	A			5.84	5.84
1,2-dibromo-3-chloropropane	A			5.59	5.74
o-toluidine	A			4.83	5.62
4-chloro-*o*-toluidine-HCl		C		5.61	5.61
C. I. solvent yellow 14		C		3.52	5.34
1,2-epoxypropane	A			4.48	5.21
2-acetylaminofluorene	A			3.12	4.67
acrylonitrile	B			3.75	4.35
epichlorohydrin	A			3.03	4.22
2-nitro-*p*-phenylenediamine		D		3.60	4.13
nitrofurazone	A			4.12	4.12
2-naphthylamine	B			3.66	3.98
5-chloro-*o*-toluidine	B			3.92	3.92
iodinated glycerol	A			3.68	3.68
melphalan	A			3.66	3.66
benzidine-2HCl	A			3.38	3.38
C. I. acid orange 3		D		2.89	3.34
N-methyl-*o*-acrylamide	B			3.10	3.31
2-biphenylamine		D		3.21	3.21
1,2-epoxybutane	B			2.99	2.99
quinoline		C		2.94	2.94
selenium sulfide	A			1.92	2.78
ziram		D		2.72	2.72
4,4-oxydianiline	A			2.60	2.60
nitrofurantoin	A			2.26	2.52
4,4-methylenedianiline	A			1.87	2.39
4-biphenylamine	A			2.38	2.38
2,6-dichloro-*p*-phenylenediamine		C		2.26	2.35
3-(chloromethyl)pyridine-HCl	A			2.06	2.29
1,3-dichloropropene	A			1.77	1.77
C. I. disperse blue 1		C		1.31 (7.08)	1.31
Total Inactive Chemicals [7.5%][e]					
Chemicals with Equivocal Activity					
dichlorvos (uncoded)	A		F	1.49	1.66
(coded)	A		F	1.68	1.68
Inactive Chemicals					
C. I. basic red 9-HCl (uncoded)	A			.41	.65
(coded)	A			.05	.05
2,4-dinitrotoluene	B			.73	1.19
Chemicals with an Indeterminate Activity[e]					
2-amino-4-nitrophenol		D		.00	.00
C. I. disperse yellow 3	A			.07	.07
HC blue 1	A			1.72	2.40

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Table A3. *Continued.*

Test Chemical	Rodent Bioassay[b]			Transformation Response[c]	
	Level of Activity			Rank *t*-statistic	
Name	High	Low	None	Actual	Estimated[d]
21 Mutagenic Non-Carcinogens					
Total Active Chemicals [65.0%]					
Active Chemicals					
malaoxon			F	8.14	11.4
2,6-toluenediamine-2HCl			F	6.95	6.95
N-phenyl-2-naphthylamine		E		4.25	6.90
3-nitropropionic acid		E		4.69	5.22
HC red 3 (AVG.)				4.12	4.54
(coded)		E		5.60	6.11
(uncoded)		E		2.64	2.96
1-naphthylamine			F	2.82	4.18
coumaphos			F	4.12	4.12
tetraethylthiuram disulfide			F	3.88	3.88
4-nitro-*o*-phenylenediamine			F	2.54	3.54
dimethoate			F	3.31	3.31
3-chloro-*p*-toluidine			F	3.26	3.26
p-phenylenediamine-2HCl			F	3.04	3.04
2-(chloromethyl)pyridine-HCl			F	2.56	2.85
Total Inactive Chemicals [35.0%]					
Chemicals with Equivocal Activity					
2,4-dimethoxyaniline-HCL			F	3.06	3.06
2,3,5,6-tetrachloro-4-nitroanisole			F	2.08	2.08
N-(1-naphthyl)ethylenediamine-2HCl			F	1.64	1.83
4'-(chloroacetyl)acetanilide			F	1.65	1.65
8-hydroxyquinoline			F	.78	1.16
Inactive Chemical					
HC blue 2			F	1.80	3.51
Inactive Chemical (Indeterminate Activity)					
4-acetylaminofluorene			F (I)	1.30	1.94
Chemicals with an Indeterminate Activity					
1-nitronaphthalene			F	1.45	1.56
20 Non-Mutagenic Carcinogens					
Total Active Chemicals [52.6%]					
Active Chemicals					
nitrilotriacetic acid	A			5.86	5.86
ethyl acrylate	A			4.51	5.25
allyl isothiocyanate		D		2.86	3.39
diethylstilbestrol	A			2.91	2.91
isophorone		D		2.76	2.86
dimethylvinyl chloride	A			2.59	2.59
3-chloro-2-methylpropene	A			2.14	2.14
chlorendic acid	A			1.98	1.98
polybrominated biphenyl mixture	A			1.93	1.93
malonaldehyde, sodium salt		C		1.87 (5.81)	1.87
Total Inactive Chemicals [47.4%]					

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Table A3. *Continued.*

Test Chemical	Rodent Bioassay[b]			Transformation Response[c]	
	Level of Activity			Rank *t*-statistic	
Name	High	Low	None	Actual	Estimated[d]
Chemical with Equivocal Activity					
2-mercaptobenzothiazole		C		1.23	1.23
Inactive Chemicals					
allyl isovalerate	A			1.65	1.65
D-limonene		D		.24	.27
reserpine	A			.24	.24
methapyrilene-HCl		C		.14	.17
chlorowax 500	A			.06	.06
cinnamyl anthranilate	A			.004	.005
tris(2-ethylhexyl)phosphate		D		.00	.00
4-vinylcyclohexene		D		.00	.00
Chemicals with an Indeterminate Activity					
chlorowax 40		D		.46	.48

30 Non-Mutagenic Non-Carcinogens

Total Active Chemicals [20.0%]

Active Chemicals					
phenol		F		7.60	7.60
propyl gallate	E			1.70	2.95
sodium diethyldithiocarbamate		F		2.94	2.94
C. I. acid red 14		F		2.15	2.15
Weakly Active Chemicals					
carbromal		F		2.26	2.26
chlorpheniramine-maleate		F		1.18	1.26

Total Inactive Chemicals [80.0%]

Chemicals with Equivocal Activity					
anilazine		F		1.82	2.09
tetrakis(hydroxymethyl) phosphonium chloride		F		1.27	1.27
Inactive Chemicals					
ethylenediamine tetraacetic acid, Na^3		F		1.41	2.01
methylphenidate-HCl			I	1.05	1.47
methyldopa sesquihydrate	E			1.21	1.21
methoxychlor		F		1.08	1.08
bisphenol A	E			.79	.79
stannous chloride	E			.78	.78
xylenes, mixed		F		.47	.50
4-hexylresorcinol	E			.46	.47
L-ascorbic acid		F		.32	.46
phenylephrine-HCl		F		.31	.42
geranyl acetate		F		.35	.37
ethoxylated dodecyl alcohol		F	(I)	.29	.29
rotenone	E			.26	.26
D,L-menthol		F		.16	.22
ephedrine sulfate		F		.16	.16
erythromycin stearate		F		.08	.08
tetrakis(hydroxymethyl)phosphonium SO_4		F		.083	.083
C. I. acid yellow 73			I	.030	.030
tetracycline-HCl		F		.021	.021
oxytetracycline-HCl	E			.000	.000

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Table A3. *Continued.*

Test Chemical	Rodent Bioassay[b]			Transformation Response[c]	
	Level of Activity			Rank *t*-statistic	
Name	High	Low	None	Actual	Estimated[d]
Chemicals with an Indeterminate Activity					
triphenyltin hydroxide			F	1.64	1.64
eugenol		E		1.07	1.07

[a]Test Chemical: The 114 cytotoxic chemicals in Table A3 are identical to those in Table A1, and they are subdivided into groups of 43 mutagenic carcinogens, 21 mutagenic noncarcinogens, 20 nonmutagenic carcinogens, and 30 nonmutagenic noncarcinogens.

[b]Rodent Bioassay Level of Activity: The relative carcinogenic activity of chemicals in rodent bioassay has been described in terms of the chemical's level of effect (*1,3*). The highest level A corresponds to chemicals that cause cancer in both mice and rats at one or more sites, and level B refers to chemicals that cause cancer at multiple sites in one species of rodent. Level C includes chemicals carcinogenic at one site in both sexes of one species, and D includes chemicals carcinogenic at one site in only one sex of a single species. Level E chemicals that only equivocal evidence of carcinogenic activity. Finally, level F includes both noncarcinogens and chemicals that had inadequate carcinogenicity studies.

[c]Transformation Response Rank *t*-statistic: The method used to calculate the significance of test chemical transformation responses employed SAS statistical software (*22*) and is described in detail in Materials and Methods. The correct *t*-statistics of each treatment dose of the test chemical in a single experiment are presented in the Appendices B–H, and these *t*-statistics were averaged to determine the mean *t*-statistic of the test chemical for the experiment (refer to Table A2). The mean *t*-statistics for two or experiments for each chemical was weighted according to the number of treatment doses evaluated and averaged to determine the actual rank *t*-statistic presented in this table. For example, the actual rank *t*-statistic of 5-azacytidine transformation responses in experiments 6 and 11 is equal to 12.8 [i.e., 8.36 + 9.11 + 15.3 + 33.9 (Exp. 6) + 14.7 + 16.5 + 4.21 + .70 (Exp. 11)/8 = 12.8; Appendix B].

[d]Estimated Rank *t*-statistic: The estimated rank *t*-statistic is used to estimate both the historical behavior of the test chemical in the transformation assay, as well as predicting the future behavior or the chemical. It is calculated by correcting the actual rank *t*-statistic. The data presented in Table A2 showed that individual experiments had very different rank-ordered sensitivities to detect chemical-induced transformation. Therefore, the estimated rank *t*-statistic modified the actual rank *t*-statistic to correct for differences in the sensitivities of individual experiments. The method uses the rank ordered sensitivity of individual experiments to detect spontaneous and BaP-induced transformation, and an example calculation is provided below.

The most active test chemical, 5-azacytidine, had statistical sensitivities for spontaneous transformation responses of 45 and 101/110 for experiments 6 and 11, respectively, and detection sensitivities for BaP of 38 and 104/110 (Table A2). Therefore, the average rank order of the two experiments was 72.0 (i.e., 45 + 101 + 38 + 104/4 = 72). For a total of 110 experiments, the median experiment has an automatic average rank order of 55.0 (i.e., 110/2 = 55.0). Therefore, the correction factor for the experimental sensitivity to detect chemical transformation was 72.0/55.0 or 1.31. Because the correction factor had been more than one, the actual rank *t*-statistic would have been multiplied by the correction factor to obtain the estimated rank *t*-statistic of 16.8. A justification for this correction factor has been reported (*18*), and it is explained in the Materials and Methods.

[e]Percentage (%) of Active Chemicals: Active chemicals included chemicals with active and weakly active transformation responses. In contrast, inactive chemicals included chemicals with equivocal and inactive transformation responses. Chemicals with an indeterminate activity have to be retested in an additional experiment in order to determine their activity in the standard transformation assay. Therefore, chemicals with indeterminate transformation responses were omitted from the computation of the percentage (%) of the total chemicals that were either active or inactive in the assay.

Mutagenic Carcinogens

2-Biphenylamine. 2-Biphenylamine was a relatively weak level D carcinogen (Table A3) with no serious technical problems reported (Table A1). However, an isomer of the chemical, 4-biphenylamine, has been reported to be oxidized upon exposure to air. It was cytotoxic to the BALB/c-3T3 cells with an average LD_{50} of 0.421 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 49 and 34/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 24 and 45/110, respectively (Table A2). In trial 1 and 2 the chemical had a SP transformation response. 2-Biphenylamine was evaluated as active in the transformation assay. Its actual and estimated rank *t*-statistics were both 3.21 (Table A3).

4-Biphenylamine. 4-Biphenylamine was a level A carcinogen (Table A3). It had one difficult technical problem because it was reported to become oxidized upon exposure to air (Table A1). It was cytotoxic to the BALB/c-3T3 cells with an average LD_{50} of .479 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 14 and 11/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 72 and 39/110, respectively (Table A2). In a preliminary trial 1 the chemical had an LA transformation response. In trial 2 the chemical had an SP transformation response. 4-Biphenylamine was evaluated as active in the transformation assay. Its actual and estimated rank *t*-statistics were both 2.38 (Table A3).

4-Chloro-o-Phenylenediamine. 4-Chloro-*o*-phenylenediamine was a potent level *A* carcinogen (Table A3). It had one difficult technical problem because it was reported to become oxidized upon exposure to air (Table A1). It was very cytotoxic to the BALB/c-3T3 cells with an average LD_{50} of 0.0318 mM (Table A1). The statistical sensitivities of transformation assay trials 1-3 were 96, 81 and 62/110, respectively; the detection sensitivities for BaP of trials 1-3 were 86, 22 and 48/110, respectively (Table A2). In a preliminary trial 1, the chemical had a LN transformation response because the test chemical treatment doses did not induce significant cytotoxic activity. In trials 2 and 3 the chemical had an SP transformation response. 4-Chloro-*o*-phenylenediamine was evaluated as active in the transformation assay. Its actual and estimated rank *t*-statistics were both 5.84 (Table A3).

3-(Chloromethyl)pyridine-HCl. 3-(Chloromethyl)pyridine-HCl was a potent level *A* carcinogen (Table A3) with no serious technical problems reported (Table A1). It was very cytotoxic to the BALB/c-3T3 cells with an average LD_{50} of 0.0756 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 99 and 36/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 82 and 28/110, respectively (Table A2). In a preliminary trial 1 the chemical had an LA transformation response. In trial 2 the chemical had an SP transformation response. 3-(Chloromethyl)pyridine-HCl was evaluated as active in the transformation assay. Its actual and estimated rank *t*-statistics were 2.06 and 2.29, respectively (Table A3).

4-Chloro-o-Toluidine-HCl. 4-Chloro-*o*-toluidine-HCl was a relatively weak level *C* carcinogen (Table A3) with no serious technical problems reported (Table A1). However, an isomer of the chemical, 5-chloro-o-toluidine, has been reported to be oxidized upon exposure to air (Table A1). It was cytotoxic to the BALB/c-3T3 cells with an average LD_{50} of 0.650 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 2 and 41/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 2 and 83/110, respectively (Table A2). In trial 1 and 2 the chemical had an SP transformation response. 4-Chloro-*o*-toluidine-HCl was evaluated as active in the transformation assay. Its actual and estimated rank *t*-statistics were both 5.61 (Table A3).

5-Chloro-o-Toluidine. 5-Chloro-*o*-toluidine was a potent level *B* carcinogen (Table A3). It had one serious technical problem because it was reported to become oxidized upon exposure to air (Table A1). It was moderately cytotoxic to the BALB/c-3T3 cells with an average LD_{50} of 1.69 mM (Table A1). The stalistical sensitivities of transformation assay trials 1 and 2 were 2 and 41/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 2 and 83/110, respectively (Table A2). In trial 1 and 2 the chemical had an SP transformation response. 5-Chloro-*o*-toluidine was evaluated as active in the transformation assay. Its actual and estimated rank *t*-statistics were both 3.92 (Table A3).

C. I. Acid Orange 3. C. I. Acid orange 3 was a relatively weak level *D* carcinogen (Table A3) with no serious technical problems reported (Table A1). It was cytotoxic to the BALB/c-3T3 cells with an average LD_{50} of 0.102 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 47 and 63/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 43 and 101/110, respectively (Table A2). In a preliminary trial 1 the chemical had an LA transformation response. In trial 2 the chemical had an SP transformation response. C. I. acid orange 3 was evaluated as active in the transformation assay. Its actual and estimated rank *t*-statistics were 2.89 and 3.34, respectively (Table A3).

C. I. Basic Red 9-HCl. C. I. Basic red 9-HCl was one of five chemicals that was tested as both a coded and an uncoded test chemical in this investigation. It was a potent level *A* carcinogen (Table A3) with no serious technical problems reported (Table A1). Both chemical samples were very cytotoxic to the BALB/c-3T3 cells with an average LD_{50} of 0.00281 and 0.00216 mM (Table A1). For the uncoded test chemical, the statistical sensitivities of transformation assay trials 1-3 were 64, 108 and 82/110, respectively; the detection sensitivities for BaP of trials 1-3 were 84, 99 and NA/110, respectively (Table A2). For the coded test chemical, the statistical sensitivities of trials 1 and 2 were 80 and 8/110; the detection sensitivities for BaP were 89 and 29/110, respectively. The coded and uncoded test chemical had SN transformation responses in a total of 5 trials. Therefore, C. I. basic red 9-HCl was evaluated as inactive in the transformation assay. The actual and estimated rank *t*-statistics of the uncoded test chemical were 0.41 and 0.65, respectively (Table A3). The actual and estimated rank *t*-statistics of the coded test chemical were both 0.05 (Table A3). Taken together, the coded and uncoded test chemicals had nearly identical cytotoxic and transforming activities in the BALB/c-3T3 cell transformation assay.

C. I. Disperse Blue 1. C. 1. Disperse blue 1 was a level *C* carcinogen (Table A3). It had one difficult technical problem. It was insoluble in culture medium at a portion of the treatment doses that were used to evaluate both cytotoxic and transforming activity (Table A1). In addition, this test chemical was observed to bind to the target cells, and it could not be removed using the standard washing procedure. It was cytotoxic to the BALB/c-3T3 cells with an average LD_{50} of 0.240 mM (Table A1). The statistical sensitivities of transformation assay trials 1-3 were 80, 54 and 5/110, respectively; the detection sensitivities for BaP of trials 1-3 were 89, 44 and 46/110, respectively (Table A2). In a preliminary trial 1, the chemical had a SP type III focus transformation response. In trials 2 and 3, the chemical had a SN type III transformation response. In contrast, the test chemical had an SP type I-III transformation response for all three trials. Thus, this test chemical had the unusual and consistent capability of inducing very significant levels of type I and II foci, but not for type III foci. This type of transformation response is shared by two other carcinogens, asbestos and polybrominated biphenyl mixture. Taken together, C. I. acid orange 3 was evaluated as weakly active in the transformation assay. Its actual and estimated rank *t*-statistics for the type III transformation response were both 1.31; however, the actual and estimated rank *t*-statistics for the type I-III response were both 7.08 (Table A3).

C. I. Disperse Yellow 3. C. I. Disperse yellow 3 was a level *A* carcinogen (Table A3) with no serious technical problems reported (Table A1). It was moderately cytotoxic to the BALB/c-3T3 cells with an average LD_{50} of 1.50 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 24 and 56/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 10 and 109/110, respectively (Table A2). In trials 1 and 2 the chemical had an SN transformation response. C. I. disperse yellow 3 was evaluated as having had an indeterminate activity in the transformation assay. Its actual and estimated rank *t*-statistics were both 0.07 (Table A3).

C. I. Solvent Yellow 14. C. I. Solvent yellow 14 was a relatively weak level *C* carcinogen (Table A3). It was insoluble at a portion of the treatment doses that were

evaluated for both cytotoxic and transforming activities (Table A1). It was cytotoxic to the BALB/c-3T3 cells with an average LD_{50} of 0.199 mM (Table A1). The statistical sensitivities of transformation assay trials 1-4 were 105, 107, 99 and 105/110, respectively; the detection sensitivities for BaP of trials 1-4 were 69, 106, 37, and 39/110, respectively (Table A2). In trials 1, 2 and 4 the chemical had SP transformation responses. In trial 3 the chemical had an LA transformation response. The test chemical was evaluated in more than two experiments, because it was used as a model test chemical in the development of additional assay protocols. C. I. solvent yellow 14 was evaluated as active in the transformation assay. Its actual and estimated rank *t*-statistics were 3.52 and 5.34, respectively (Table A3).

Cytembena. Cytembena was a level *B* carcinogen (Table A3) with no serious technical problems reported (Table A1). It was cytotoxic to the BALB/c-3T3 cells with an average LD_{50} of 0.153 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 47 and 71/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 43 and 23/110, respectively (Table A2). In a preliminary trial 1 the chemical had an LA transformation response. In trial 2 the chemical had an SP transformation response. Cytembena was evaluated as active in the transformation assay. Its actual and estimated rank *t*-statistics were both 5.93 (Table A3).

1,2-Dibromo-3-Chloropropane. 1,2-Dibromo-3-chloropropane was a potent level *A* carcinogen (Table A3) with no serious technical problems reported (Table A1). It was cytotoxic to the BALB/c-3T3 cells with an average LD_{50} of 0.401 mM (Table A1). The statistical sensitivities of transformation assay trials 1-3 were 66, 78 and 27/110, respectively; the detection sensitivities for BaP of trials 1-3 were 81, 58 and 63/110, respectively (Table A2). In a preliminary trial 1, the test chemical had an LN transformation response, because the chemical treatment doses were noncytotoxic to the target cells. In trials 2 and 3 the chemical had an SP transformation response. 1,2-Dibromo-3-chloropropane was evaluated as active in the transformation assay. Its actual and estimated rank *t*-statistics were 5.59 and 5.74, respectively (Table A3).

2,6-Dichloro-p-Phenylenediamine. 2,6-Dichloro-p-phenylenediamine was a relatively weak level *C* carcinogen (Table A3). It had one difficult technical problem because it was reported to become oxidized upon exposure to air (Table A1). It was cytotoxic to the BALB/c-3T3 cells with an average LD_{50} of 0.921 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 19 and 91/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 34 and 85/110, respectively (Table A2). In trials 1 and 2 the chemical had an SP transformation response. 2,6-Dichlorop-phenylenediamine was evaluated as active in the transformation assay. Its actual and estimated rank *t*-statistics were 2.26 and 2.35, respectively (Table A3).

1,3-Dichloropropene. 1,3-Dichloropropene was a potent level *A* carcinogen (Table A3) with no serious technical problems reported (Table A1). It was cytotoxic to the BALB/c-3T3 cells with an average LD_{50} of 0.280 mM (Table A1). The statistical sensitivities of transformation assay trials 1-3 were 4, 18 and 26/110, respectively; the detection sensitivities for BaP of trials 1-3 were 1, 31 and 93/110, respectively (Table A2). In a preliminary trial 1 the chemical had a SP transformation response, and in trial 2 the chemical had a SN transformation response. Because these responses were disparate and significantly different from one another, the test chemical was evaluated in a third trial. In the third experiment the chemical had an LA transformation response. 1,3-Dichloropropene was evaluated as weakly active in the transformation assay. Its actual and estimated rank *t*-statistics were both 1.77, respectively (Table A3).

Dichlorvos. Dichlorvos was one of five chemicals that was tested as both a coded and an uncoded test chemical in this investigation. It was evaluated as a noncarcinogen is its first rodent bioassay trial; however, it was determined in a second trial using a different route of exposure to be a potent level *A* carcinogen (Table A3). This test chemical had one difficult technical problem because it was rapidly hydrolyzed in water (Table A1). Both chemical samples were cytotoxic to the BALB/c-3T3 cells with an average LD_{50} of 0.145 and 0.140 mM (Table A1). For the uncoded test chemical, the statistical sensitivities of transformation assay trials 1-3 were 98, 72 and 59/110, respectively; the detection sensitivities for BaP of trials 1-3 were 60, NA and 17/110, respectively (Table A2). For the coded test chemical, the statistical sensitivities of trials 1 and 2 were 9 and 15/110, the detection sensitivities for BaP were 37 and 21/110, respectively. The uncoded test chemical had SN transformation responses in 2 trials and an LA transformation response in one trial. The coded test chemical had an SN transformation response in a preliminary trial and an SP transformation response in trial 2. The mean *t*-statistics of the SP and LA transformation responses were not significantly different from the corresponding SN responses, which showed that the test chemical activity in the assay was relatively weak. Taken together, dichlorvos was evaluated as having had equivocal activity in the transfonnation assay. The actual and estimated rank *t*-statistics of the uncoded test chemical were 1.49 and 1.66, respectively (Table A3). The actual and estimated rank *t*-statistics of the coded test chemical were both 1.68 (Table A3). Taken together, the coded and uncoded test chemicals had nearly identical cytotoxic and transforming activities in the BALB/c-3T3 cell transformation assay.

Diglycidyl Resorcinol Ether. Diglycidyl resorcinol ether was a potent level *A* carcinogen (Table A3). It is an alkylating chemical; thus, it could react with labile hydrogen atoms not only on DNA, but also on a variety of biochemicals in the culture medium (Table A1). It was very cytotoxic to the BALB/c-3T3 cells with an average LD_{50} of 0.00416 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 45 and 103/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 38 and 73/110, respectively (Table A2). In trials 1 and 2 the chemical had an SP transformation response. Diglycidyl resorcinol ether was evaluated as one of the most active chemicals in the transformation assay. Its actual and estimated rank *t*-statistics were 9.31 and 11.0, respectively (Table A3).

2,4-Dinitrotoluene. 2,4-Dinitrotoluene is a relative potent level *B* carcinogen (Table A3) with no serious technical problems reported (Table A1). It was cytotoxic to the BALB/c-3T3 cells with an average LD_{50} of 0.917 mM (Table A1). The statistical sensitivities of transformation assay trials 1-3 were 88, 104 and 88/110, respectively; the detection sensitivities for BaP of trials 1-3 were 77, 90 and NA/110, respectively (Table A2). In a preliminary trial 1 the chemical had an LA transformation response. In trials 1-3 the chemical had SN transformation responses. 2,4-Dinitrotoluene was evaluated as inactive in the transformation assay. Its actual and estimated rank *t*-statistics were 0.73 and 1.19, respectively (Table A3).

Epichlorohydrin. Epichlorohydrin was a level *A* carcinogen (Table A3). It had two serious technical problems, because it was reported to become oxidized upon exposure to air and it reacts with water (Table A1). It was cytotoxic to the BALB/c-3T3 cells with an average LD_{50} of .364 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 98 and 72/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 60 and NA/110, respectively (Table A2). In a preliminary trial 1 the chemical had an LA transformation response. In trial 2 the chemical had an SP transformation response. Epichlorohydrin was evaluated as active in the transformation assay. Its actual and estimated rank *t*-statistics were 3.03 and 4.22, respectively (Table A3).

1,2-Epoxybutane. 1,2-Epoxybutane was a relatively weak level D carcinogen (Table A3). It was reported to be a highly reactive chemical (Table A1), and it reacts with carboxyl and hydroxyl groups found on constituent biochemicals in culture medium, as well as in the target cells. It was moderately cytotoxic to the BALB/c-3T3 cells with an average LD_{50} of 1.45 mM (Table A1). The statistical sensitivities of transformation assay trials 1-3 were 4, 26 and 20/110, respectively; the detection sensitivities for BaP of trials 1-3 were 1, 93 and 19/110, respectively (Table A2). In a preliminary trial 1 the chemical had an LN transformation response because the chemical treatment doses were too high and completely cytotoxic to the cells. In trials 2 and 3 the chemical had SP transformation responses. 1,2-Epoxybutane was evaluated as active in the transformation assay. Its actual and estimated rank *t*-statistics were both 2.99 (Table A3).

1,2-Epoxypropane. 1,2-Epoxypropane was a potent level *A* carcinogen (Table A3). It had one difficult technical problem because it was reported to become oxidized upon exposure to air (Table A1). It was moderately cytotoxic to the BALB/c-3T3 cells with an average LD_{50} of 1.60 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 70 and 57/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 41 and 88/110, respectively (Table A2). In trials 1 and 2 the chemical had an SP transformation response. 1,2-Epoxypropane was evaluated as active in the transformation assay. Its actual and estimated rank *t*-statistics were 4.48 and 5.21, respectively (Table A3).

Ethylene Dibromide. Ethylene dibromide was a level *A* carcinogen (Table A3). It was reported to be highly reactive chemical, but none of these problems were serious (Table A1). It was moderately cytotoxic to the BALB/c-3T3 cells with an average LD_{50} of 1.69 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 32 and 41/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 108 and 83/110, respectively (Table A2). In trials 1 and 2 the chemical had an SP transformation response. Ethylene dibromide was evaluated as active in the transformation assay. Its actual and estimated rank *t*-statistics were 5.68 and 6.82, respectively (Table A3).

HC Blue 1. HC Blue 1 was a level *A* carcinogen (Table A3). It had one difficult technical problem because it was reported to become oxidized upon exposure to air (Table A1). It was cytotoxic to the BALB/c-3T3 cells with an average LD_{50} of 1.96 mM (Table A1). The statistical sensitivities of transformation assay trials 1-3 were 100, 79 and 88/110, respectively; the detection sensitivities for BaP of trials 1-3 were 42, 75 and NA/110, respectively (Table A2). In trials 1 and 2 the chemical had an SN transformation response. Ordinarily this chemical would not have been tested in a third experiment, but it was selected as a model chemical with an inactive response in the transformation assay. In the third experiment, the test chemical had an SP transformation response. This disparate transformation response could have been caused by the different sample batches of test chemicals that were tested in experiments 1 and 2 versus experiment 3. Therefore, the test chemical has to be tested in a fourth experimental trial. HC blue 1 was evaluated as having had an indeterminate activity in the transformation assay. Its actual and estimated rank *t*-statistics were 1.72 and 2.40, respectively (Table A3).

Iodinated Glycerol. Iodinated glycerol was a level *A* carcinogen (Table A3) with no serious technical problems reported (Table A1). It was moderately cytotoxic to the BALB/c-3T3 cells with an average LD_{50} of 3.47 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 32 and 28/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 108 and 15/110, respectively (Table A2). In a preliminary trial 1 the chemical had an SP transformation response. In trial 2 the chemical had an SN transformation response. The disparate transformation responses were examined further, and the mean *t*-statistics of the two experiments were not significantly different from one another. In addition, there was a dose-related increase in test chemical activity in the experiment with an SN response at treatment doses that were comparable to that inducing an SP response. Taken together, iodinated glycerol was evaluated as weakly active in the transformation assay. Its actual and estimated rank *t*-statistics were both 3.68, respectively (Table A3).

Melphalan. Melphalan was a level *A* carcinogen (Table A3). It had one serious technical problem because it was reported to react with water (Table A1). It was very cytotoxic to the BALB/c-3T3 cells with an average LD_{50} of 0.00120 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 10 and 31/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 5 and 52/110, respectively (Table A2). In a

preliminary trial 1 the chemical had an LA transformation response. In trial 2 the chemical had an SP transformation response. Melphalan was evaluated as active in the transformation assay. Its actual and estimated rank *t*-statistics were both 3.66, respectively (Table A3).

N-Methyl-o-Acrylamide. N-Methyl-*o*-acrylamide is a level *B* carcinogen (Table A3) with no insurmountable technical problems (Table A1). It was moderately cytotoxic to the BALB/c-3T3 cells with an average LD_{50} of 1.75 mM (Table A1). The statistical sensitivities of trials 1 and 2 were 47 and 67/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 43 and 78/110, respectively (Table A2). In a preliminary trial 1 the chemical had an LA transformation response. In trial 2 the chemical had an SP transformation response. N-Methyl-*o*-acrylamide was evaluated as active in the transformation assay. Its actual and estimated rank *t*-statistics were 3.10 and 3.31, respectively (Table A3).

4,4-Methylenediamine. 4,4-Methylenediamine was a potent level *A* carcinogen (Table A3). It had one difficult technical problem because it was reported to become oxidized upon exposure to air (Table A1). It was moderately cytotoxic to the BALB/c-3T3 cells with an average LD_{50} of 1.56 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 60 and 104/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 27 and 90/110, respectively (Table A2). In trials 1 and 2 the chemical had an SP transformation response. 4,4-Methylenedianiline was evaluated as active in the transformation assay. Its actual and estimated rank *t*-statistics were 1.87 and 2.39, respectively (Table A3).

N-Methyl-N′-Nitro-N-Nitrosoguanidine. N-Methyl-N′-Nitro-N-Nitrosoguanidine was a potent level *A* carcinogen (Table A3). It had one serious technical problem because it reacts with water (Table A1). It was very cytotoxic to the BALB/c-3T3 cells with an average LD_{50} of 0.0154 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 43 and 54/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 9 and NA/110, respectively (Table A2). In trials 1 and 2 the chemical had an SP transformation response. N-Methyl-N′-nitro-N-nitrosoguanidine was one of the most active chemicals in the transformation assay. Its actual and estimated rank *t*-statistics were both 10.3 (Table A3).

2-Naphthylamine. 2-Naphthylamine was a level *B* carcinogen (Table A3). It had one serious technical problem because it was reported to become oxidized upon exposure to air (Table A1). It was moderately cytotoxic to the BALB/c-3T3 cells with an average LD_{50} of 1.59 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 96 and 37/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 86 and 20/110, respectively (Table A2). In trials 1 and 2 the chemical had an SP transformation response. 2-Naphthylamine was evaluated as active in the transformation assay. Its actual and estimated rank *t*-statistics were 3.66 and 3.98, respectively (Table A3).

Nitrofurantoin. Nitrofurantoin was a potent level A carcinogen (Table A3). It was reported to be a highly reactive chemical, but none of the problems were serious (Table A1). It was cytotoxic to the BALB/c-3T3 cells with an average LD_{50} of 0.106 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 93 and 43/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 100 and 9/110, respectively (Table A2). In a preliminary trial 1 the chemical had an SN transformation response. In trial 2 the chemical had an SP transformation response. The disparate transformation responses were examined further, and the mean *t*-statistics of the two experiments were not significantly different from one another. In addition, there was a dose-related increase in test chemical activity in the experiment with an SN response at treatment doses that were comparable to that inducing an SP response. Taken together, nitrofurantoin was evaluated as having had weak activity in the transformation assay. Its actual and estimated rank *t*-statistics were 2.26 and 2.52, respectively (Table A3).

Nitrofurazone. Nitrofurazone was a potent level *A* carcinogen (Table A3) with no serious technical problems reported (Table A1). It was very cytotoxic to the BALB/c-3T3 cells with an average LD_{50} of 0.0515 mM (Table A1). The statistical sensitivities of transformation assay trials 1-3 were 24, 17 and 63/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 10, 6 and 101/110, respectively (Table A2). In a preliminary trial 1 the chemical had an LN transformation response, because the chemical treatment doses were too cytotoxic. In trials 2 and 3 the chemical had an SP transformation response. Nitrofurazone was evaluated as active in the transformation assay. Its actual and estimated rank *t*-statistics were both 4.12, respectively (Table A3).

2-Nitro-p-Phenylenediamine. 2-Nitro-*p*-phenylenediamine was a relatively weak level D carcinogen (Table A3). It had one difficult technical problem because it was reported to become oxidized upon exposure to air (Table A1). It was cytotoxic to the BALB/c-3T3 cells with an average LD_{50} of 0.947 mM (Table A1). The statistical sensitivities of transformation assay trials 1-3 were 100, 79 and 31/110, respectively; the detection sensitivities for BaP of trials 1-3 were 42, 75 and 52/110, respectively (Table A2). In a preliminary trial 1 the chemical had an SP transformation response. In trial 2 the chemical had an SN transformation response. The disparate transformation responses were examined further, and the mean *t*-statistics of the two experiments were significantly different from one another. Therefore, the chemical was tested in a third trial, and the activity in this experiment was evaluated as an SP. There was no obvious reason for the absence of activity of the test chemical in the second experiment. Taken together, 2-nitro-*p*-phenylenediamine was evaluated as active in the transformation assay. Its actual and estimated rank *t*-statistics were 3.60 and 4.13, respectively (Table A3).

4,4-Oxydianiline. 4,4-0xydianiline was a potent level *A* carcinogen (Table A3). It had one serious technical problem, because it was reported to become oxidized upon exposure to air (Table A1). It was cytotoxic to the BALB/c-3T3 cells with an average LD_{50} of 0.270 mM (Table A1). The statistical sensitivities of trials 1 and 2 were 25 and

16/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 36 and 7/110, respectively (Table A2). In trials 1 and 2 the chemical had an SP transformation response. 4,4-Oxydianiline was evaluated as active in the transformation assay. Its actual and estimated rank *t*-statistics were both 2.60 (Table A3).

Quinoline. Quinoline was a level *C* carcinogen (Table A3). It was reported to be a highly reactive chemical, but none of the problems were serious (Table A1). It was moderately cytotoxic to the BALB/c-3T3 cells with an average LD_{50} of 4.09 mM (Table A1). The statistical sensitivities of transformation assay trials 1-4 were 87, 78, 42 and 26/110, respectively; the detection sensitivities for BaP of trials 1-4 were 64, 58, 25 and 93/110, respectively (Table A2). In trials 1 and 2 the chemical had an LN transformation response because the chemical treatment doses did not induce a significant cytotoxic activity. In trial 3 the chemical had an LA transformation response, and trial 4 the response was evaluated as an SP. Quinoline was evaluated as active in the transformation assay. Its actual and estimated rank *t*-statistics were both 2.94 (Table A3).

Selenium Sulfide. Selenium sulfide was a potent level *A* carcinogen (Table A3) with no serious technical problems reported (Table A1). It was cytotoxic to the BALB/c-3T3 cells with an average LD_{50} of 0.125 mM (Table A1). The statistical sensitivities of transformation assay trials 1-3 were 105, 101 and 54/110, respectively; the detection sensitivities for BaP of trials 1-3 were 69, 104 and 44/110, respectively (Table A2). In a preliminary trial 1, the chemical had an SP transformation response. In trial 2, the chemical had an SN transformation response. The disparate transformation responses were examined further, and the mean *t*-statistics of the two experiments were significantly different from one another. Therefore, the test chemical had to be tested in a third experiment, and the activity in this trial was evaluated as an SP. There was no obvious explanation for the disparate transformation responses in second experiment, verses the experiments 1 and 3. Taken together, selenium sulfide was evaluated as weakly active in the transformation assay. Its actual and estimated rank *t*-statistics were 1.92 and 2.78, respectively (Table A3).

o-Toluidine. o-Toluidine was a potent level *A* carcinogen (Table A3). It had one difficult technical problem because it was reported to become oxidized upon exposure to air (Table A1). It was moderately cytotoxic to the BALB/c-3T3 cells with an average LD_{50} of 4.33 mM (Table A1). The statistical sensitivities of transformation assay trials 1-3 were 87, 106 and 59/110, respectively; the detection sensilivities for BaP of trials 1 and 2 were 64, 51 and 17/110, respectively (Table A2). In a preliminary trial 1 the chemical had an LA transformation response. In trial 2 the chemical had an SP transformation response, but it did not induce a significant increase in type I and II foci (data not presented). Because of this unusual activity, the test chemical was tested in a third trial. In the third experiment the chemical response was evaluated as an SP. Taken together, o-toluidine was evaluated as active in the transformation assay. Its actual and estimated rank *t*-statistics were 4.83 and 5.62, respectively (Table A3).

Ziram. Ziram is a relatively weak level *D* carcinogen (Table A3) with no serious technical problems reported (Table A1). It was the most cytotoxic chemical in this group of test chemicals, and it had an average LD_{50} of 0.0000373 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 17 and 56/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 6 and 109/110, respectively (Table A2). In a preliminary trial 1 the chemical had an LA transformation response. In trial 2 the chemical had an SP transformation response. Ziram was evaluated as active in the transfonnation assay. Its actual and estimated rank *t*-statistics were both 2.72 (Table A3).

Cytotoxic, Mutagenic Noncarcinogens

4-Acetylaminonuorene. 2-Acetylaminofluorene is a level *F(1)* noncarcinogen because it has not been evaluated in a complete rodent bioassay (Table A3). It had one difficult technical problem, because it had a solubility limit in culture medium supplemented with pluronic F68 of 200 μg/ml (Table A1). The test chemical was moderately cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} estimated to be over 900 μg/ml or about 0.07 mM (Table A1). Thus, the LD_{50} was considerably above the solubility limit of the test chemical. The statistical sensitivities of transformation assay trials 1 and 2 were 103 and 83/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 73 and 70/110, respectively (Table A2). The test chemical had an LN transformation response in both experiments, and it was tested at treatment doses that were both above and below its solubility limit. Taken together, 4-acetylaminofluorene was evaluated as an inactive chemical with an indeterminate activity in the transformation assay. Its actual and estimated rank *t*-statistics were 1.30 and 1.49, respectively (Table A3).

4'-(Chloroacetyl)acetanilide. 4'-(Chloroacetyl)-acetanilide is a level *F* noncarcinogen (Table A3). It had no insurmountable technical problems (Table A1). The test chemical was very cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 0.00336 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 58 and 8/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 55 and 29/110, respectively (Table A2). In trials 1 and 2 the test chemical had LA transformation responses. 4'-(Chloroacetyl)-acetanilide evaluated as having equivocal activity in the transformation assay, and its actual and estimated rank *t*-statistics were both 1.65 (Table A3).

2-(Chloromethyl)pyridine-HCl. 2-(Chloromethyl)-pyridine is a level *F* noncarcinogen (Table A3). It had no insurmountable technical problems (Table A1). The test chemical was cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 0.118 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 99 and 36/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 82 and 28/110, respectively (Table A2). In trials 1 and 2 the test chemical had SP transformation responses. 2-(Chloromethyl)pynidine was evaluated as active in the transformation assay, and its actual and

estimated rank *t*-statistics were 2.56 and 2.85, respectively (Table A3).

3-Chloro-p-Toluidine. 3-Chloro-*p*-toluidine is a level *F* noncarcinogen (Table A3). It had one difficult technical problem. It was reported to become oxidized upon exposure to air, and it was exposed to air during the treatment period (Table A1). The test chemical was moderately cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 1.17 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 2 and 41/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 2 and 83/110, respectively (Table A2). In trials 1 and 2 the test chemical had SP transformation responses. 3-Chloro-*p*-toluidine was evaluated as active in the transformation assay, and its actual and estimated rank *t*-statistics were both 3.26 (Table A3).

Coumaphos. Coumaphos is a level *F* noncarcinogen (Table A3). It had no insurmountable technical problems (Table A1). The test chemical was cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 0.218 mM (Table A1). The statistical sensitivities of transformation assay trials 1-3 were 40, 8 and 33/110, respectively; the detection sensitivities for BaP of trials 1-3 were 54, 29 and 14/110, respectively (Table A2). The test chemical had an SN transformation response in the first experiment, and an SP response in the second experiment. Because the mean *t*-statistics of the transformation responses in the first two experiments were significantly different from one another, the test chemical was evaluated in a third trial. In the third experiment the test chemical had an SP transformation response. Coumaphos was evaluated as active in the transformation assay, and its actual and estimated rank *t*-statistics were both 4.12 (Table A3).

Dimethoate. Dimethoate is a level *F* noncarcinogen (Table A3). It had no insurmountable technical problems (Table A1). The test chemical was cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 0.602 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 90 and 18/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 13 and 31/110, respectively (Table A2). The test chemical had an LA transformation response in the first experiment, and an SP response in the second experiment. Dimethoate was evaluated as active in the transformation assay, and its actual and estimated rank *t*-statistics were both 3.31 (Table A3).

2,4-Dimethoxyaniline-HCl. 2,4-Dimethoate is a level *F* noncarcinogen (Table A3). It had no insurmountable technical problems (Table A1). The test chemical was moderately cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 1.13 mM (Table A1). The statistical sensitivities of transformation assay trials 1-3 were 7, 63 and 54/110, respectively; the detection sensitivities for BaP of trials 1-3 were 56, 101 and 44/110, respectively (Table A2). The test chemical had a SP transformation response in the first experiment, and a SN response in the second experiment. Because the mean *t*-statistics of the transformation responses in the first two experiments were significantly different from one another, the test chemical was evaluated in a third trial. The test chemical had a SN transformation response in the third experiment. 2,4-Dimethoxyaniline was evaluated as having had equivocal activity in the transformation assay, and its actual and estimated rank *t*-statistics were both 3.06 (Table A3).

HC Red 3. HC red 3 was one of five chemicals that was tested as both a coded and an uncoded test chemical in this investigation. It is a level *E* noncarcinogen (Table A3). It had no insurmountable technical problems (Table A1). The test chemical was moderately cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 3.72 mM as a uncoded test chemical and 4.50 mM as a coded chemical (Table A1). For the uncoded test chemical the statistical sensitivities of transformation assay trials 1 and 2 were 60 and 86/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 27 and 74/110, respectively (Table A2). In trials 1 and 2 the uncoded test chemical had SP transformation responses. For the coded test chemical the statistical sensitivities of transformation assay trials 1 and 2 were 93 and 33/110, respectively; the detection sensitivities for BaP were 100 and 14, respectively. The coded test chemical had an LA transformation response in the first experiment, and an SP transformation response in the second experiment. Both the uncoded and the coded HC Red 3 were evaluated as active in the transformation assay. The coded test chemical actual estimated rank *t*-statistics were 5.60 and 6.11, respectively; the uncoded test chemical actual and estimated rank *t*-statistics were 2.64 and 2.96, respectively (Table A3).

8-Hydroxyquinoline. 8-Hydroxyquinoline is a level *F* noncarcinogen (Table A3). It had one difficult technical problem because it was reported to combine with different metal salts (Table A1). Thus, it could have combined with metal salts in FBS and EMEM medium. The test chemical was very cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 0.00251 mM (Table A1). The statistical sensitivities of transformation assay trials 1-3 were 94, 102 and 39/110, respectively; the detection sensitivities for BaP of trials 1-3 were 91, 95 and 68/110, respectively (Table A2). The test chemical had an SP transformation response in the first experiment, and an SN response in the second experiment. Because the mean *t*-statistics of the transformation responses of the first two experiments were significantly different from one another the chemical was evaluated in a third trial. The test chemical had an SN transformation response in the third experiment. 8-Hydroxyquinoline was evaluated as having equivocal activity in the transformation assay, and its actual and estimated rank *t*-statistics were 0.78 and 1.16, respectively (Table A3).

Malaoxon. Malaoxon is a level *F* noncarcinogen (Table A3). It had one difficult technical problem. It was reported to become oxidized upon exposure to air, and it was exposed to air during the treatment period (Table A1). The test chemical was cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 0.468 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 87 and 106/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 64 and 51/110, respectively (Table A2). The test chemical had an LA transformation response in the first experiment, and an SP response in the

second experiment. Malaoxon was evaluated as active in the transformation assay, and its actual and estimated rank *t*-statistics were 8.14 and 11.4, respectively (Table A3).

1-Naphthylamine. 1-Naphthylamine is a level *F* noncarcinogen (Table A3). It had one difficult technical problem. It was reported to become oxidized upon exposure to air, and it was exposed to air during the treatment period (Table A1). The test chemical was cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 0.506 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 96 and 77/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 86 and 67/110, respectively (Table A2). In trials 1 and 2 the test chemical had SP transformation responses. 1-Naphthylamine was evaluated as active in the transformation assay, and its actual and estimated rank *t*-statistics were 2.82 and 4.18, respectively (Table A3).

N-(1-Naphthyl)ethylenediamine-2HCl. N-(1-Naphthyl)ethylenediamine-2HCl is a level *F* noncarcinogen (Table A3). It had no insurmountable technical problems (Table A1). The test chemical was cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 0.125 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 69 and 63/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 12 and 101/110, respectively (Table A2). In trials 1 and 2 the test chemical had LA transformation responses. *N*-(1-naphthyl)ethylenediamine-2HCl evaluated as having equivocal activity in the transformation assay, and its actual and estimated rank *t*-statistics were 1.64 and 1.83, respectively (Table A3).

1-Nitronaphthalene. 1-Nitronaphthalene is a level *F* noncarcinogen (Table A3). It had no insurmountable technical problems (Table A1). The test chemical was cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 0.464 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 49 and 63/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 24 and 101/110, respectively (Table A2). The test chemical had an SN transformation response in the first experiment, and an SP response in the second experiment. Because the mean *t*-statistic responses of the two transformation experiments were significantly different from one another, this chemical has to be tested in a third trial. In the absence of these data 1-nitronaphthalene was evaluated as having had an indeterminate activity in the transformation assay, and its actual and estimated rank *t*-statistics were 1.45 and 1.56, respectively (Table A3).

4-Nitro-o-Phenylenediamine. 4-Nitro-o-phenylenediamine is a level *F* noncarcinogen (Table A3). It had one difficult technical problem. It was reported to become oxidized upon exposure to air, and it was exposed to air during the treatment period (Table A1). The test chemical was cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 0.292 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 99 and 46/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 82 and 80/110, respectively (Table A2). The test chemical had an SP transformation response in the first experiment, and an LA response in the second experiment. 4-Nitro-*o*-phenylenediamine was evaluated as active in the transformation assay, and its actual and estimated rank *t*-statistics were 2.54 and 3.54, respectively (Table A3).

3-Nitropropionic Acid. 3-Nitropropionic acid is a level *E* noncarcinogen (Table A3). It had one difficult technical problem. It was reported to become oxidized upon exposure to air, and it was exposed to air during the treatment period (Table A1). The test chemical was moderately cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 1.23 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 82 and 67/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 18 and 78/110, respectively (Table A2). The test chemical had an SP transformation response in the first and second experiments. 3-Nitropropionic acid was evaluated as active in the transformation assay, and its actual and estimated rank *t*-statistics were 4.69 and 5.22, respectively (Table A3).

p-Phenylenediamine-2HCl. p-Phenylenediamine-2HCl is a level *F* noncarcinogen (Table A3). It had one difficult technical problem. It was reported to become oxidized upon exposure to air, and it was exposed to air during the treatment period (Table A1). The test chemical was very cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 0.0712 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 58 and 51/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 55 and 50/110, respectively (Table A2). In trials 1 and 2 the test chemical had SP transformation responses. p-Phenylenediamine-2HCl was evaluated as active in the transformation assay, and its actual and estimated rank *t*-statistics were both 3.04 (Table A3).

N-Phenyl-2-Naphthylamide. N-Phenyl-2-naphthylamide is a level *F* noncarcinogen (Table A3). It had one difficult technical problem. It was reported to become oxidized upon exposure to air, and it was exposed to air during the treatment period (Table A1). The test chemical was cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 0.195 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 93 and 63/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 100 and 101/110, respectively (Table A2). In trials 1 and 2 the test chemical had SP transformation responses. N-Phenyl-2-naphthylamide was evaluated as active in the transformation assay, and its actual and estimated rank *t*-statistics were 4.25 and 6.90, respectively (Table A3).

2,3,5,6-Tetrachloro-4-Nitroanisole. 2,3,5,6-Tetrachloro-4-nitroanisole is a level *F* noncarcinogen (Table A3). It had no insurmountable technical problems (Table A1). The test chemical was very cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 0.0437 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 68 and 43/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 40 and 9/110, respectively (Table A2). In trials 1 and 2 the test chemical had LA transformation responses. 2,3,5,6,-Tetrachloro-4-nitroanisole was evaluated as having equivocal activity in the transformation assay, and its actual and estimated rank *t*-statistics were both 2.08 (Table A3).

Tetraethylthiuram Disulfide. Tetraethylthiuram disulfide is a level *F* noncarcinogen (Table A3). It had one no insurmountable technical problems (Table A1). The test chemical was very cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 0.0000583 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 10 and 43/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 5 and 9/110, respectively (Table A2). In trials 1 and 2 the test chemical had SP transformation responses. Tetraethylthiuram disulfide was evaluated as active in the transformation assay, and its actual and estimated rank *t*-statistics were both 3.88 (Table A3).

2,6-Toluenediamine-2HCl. 2,6-Toluenediamine-2HCl is a level *F* noncarcinogen (Table A3). It had no insurmountable technical problems (Table A1). The test chemical was moderately cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 4.11 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 68 and 23/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 40 and 11/110, respectively (Table A2). In trials 1 and 2 the test chemical had SP transformation responses. 2,6-Toluenediamine-2HCl was evaluated as active in the transformation assay, and its actual and estimated rank *t*-statistics were both 6.95 (Table A3).

Cytotoxic, Nonmutagenic Carcinogens

Allyl Isothicyanate. Allyl isothiocyanate is a level *D* carcinogen (Table A3). It had one difficult technical problem because it was reported to react with water (Table A1). The test chemical was a very cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 0.00712 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 90 and 98/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 13 and 60/110, respectively (Table A2). The test chemical had an LA transformation response in the first experiment, and an SP response in the second experiment. Allyl isothiocyanate evaluated as active in the transformation assay, and its actual and estimated rank *t*-statistics were 2.86 and 3.39, respectively (Table A3).

Allyl Isovalerate. Allyl isovalerate is a level *A* carcinogen (Table A3). It had no insurmountable technical problems (Table A1). The test chemical was moderately cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 4.51 mM (Table A1). The statistical sensitivities of transformation assay trials 1–4 were 66, 78, 42, and 27/110, respectively; the detection sensitivities for BaP of trials 1–4 were 81, 58, 35, and 63/110, respectively (Table A2). The test chemical had an LN transformation response in the first two experiments, an SN response in the third experiment, and an LA response in the fourth experiment. Allyl isovalerate was evaluated as inactive in the transformation assay, and its actual and estimated rank *t*-statistics were both 1.65 (Table A3).

Chlorendic Acid. Chlorendic acid was a level *A* carcinogen (Table A3). This chemical was reported to bind metal salts (Table A1); thus, it could have affected the concentration of metal salts in FBS and culture medium. The test chemical was moderately cytotoxic chemical with an average LD_{50} of 4.07 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 9 and 57/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 37 and 88/110, respectively (Table A2). In a preliminary trial 1 the chemical had an SP transformation response. In trial 2 the chemical had an LA transformation response. Chlorendic acid was evaluated as active in the transformation assay. Its actual and estimated rank *t*-statistics were both 1.98 (Table A3).

3-Chloro-2-Methylpropene. 3-Chloro-2-methylpropene was a level *A* carcinogen (Table A3). It had many technical problems including its reported reaction (Table A1). The test chemical was a cytotoxic chemical with an average LD_{50} of 0.662 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 9 and 57/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 37 and 88/110, respectively (Table A2). In a preliminary trial 1 the chemical had an LA transformation responses. In trial 2 the chemical had an SP transformation response. 3-Chloro-2-methylpropene was evaluated as active in the transformation assay, and its actual and estimated rank *t*-statistics were both 2.14 (Table A3).

Chlorowax 40. Chlorowax 40 is a level *D* carcinogen (Table A3). It had no insurmountable technical problems (Table A1). The test chemical was moderately cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 1.43 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 12 and 26/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 98 and 93/110, respectively (Table A2). The test chemical had an LA transformation response in the first experiments and an SN response in the second experiment. Chlorowax 40 was evaluated as inactive in the transformation assay, and its actual and estimated rank *t*-statistics were 0.46 and 0.48, respectively (Table A3)

Chlorowax 500. Chlorowax 500 is a level *A* carcinogen (Table A3). It had no insurmountable technical problems (Table A1). The test chemical was moderately cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 1.58 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 32 and 15/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 108 and 21/110, respectively (Table A2). The test chemical had an SN transformation response in both experiments. Chlorowax 500 was evaluated as inactive in the transformation assay, and its actual and estimated rank *t*-statistics were both 0.06 (Table A3).

Cinnamyl Anthranilate. Cinnamyl anthranilate is a level *A* carcinogen (Table A3). It had one difficult technical problem. It was reported to become oxidized by air; thus, it could have reacted with air during the treatment period (Table A1). The test chemical was very cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 0.0947 mM (Table A1). The statistical sensitivities of transformation assay trials 1-3 were 53, 102 and 92/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 30, 95 and ND/110, respectively (Table A2). The test chemical had an SN transformation response in all three experiments. Cinnamyl anthranilate was evaluated as inactive in

the transformation assay, and its actual and estimated rank *t*-statistics were 0.004 and 0.005, respectively (Table A3).

Diethylstilbestrol. Diethylstilbestrol is a level *A* carcinogen (Table A3) with no technical problems (Table A1). It was very cytotoxic to the BALB/c-3T3 cells with an average LD_{50} of 0.0858 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 55 and 31/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 8 and 52/110, respectively (Table A2). In a preliminary trial 1 the chemical had an LA transformation response. In trial 2 the chemical had an SP transformation response. Diethylstilbestrol was evaluated as active in the transformation assay. Its actual and estimated rank *t*-statistics were both 2.91, respectively (Table A3).

Dimethylvinyl Chloride. Dimethylvinyl chloride is a level *A* carcinogen (Table A3). It had one serious technical problem because it was noted to be oxidized upon exposure to air (Table A1). It was moderately cytotoxic to the BALB/c-3T3 cells with an average LD_{50} of 4.74 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 12 and 27/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 98 and 63/110, respectively (Table A2). In a preliminary trial 1 the chemical had an LA transformation response. In trial 2 the chemical had an SP transformation response. Dimethylvinyl chloride was evaluated as active in the transformation assay. Its actual and estimated rank *t*-statistics were both 2.59 (Table A3).

Ethyl Acrylate. Ethyl acrylate is a level *A* carcinogen (Table A3). It has one difficult technical problem because it was reported to react with water (Table A1). It was very cytotoxic to the BALB/c-3T3 cells with an average LD_{50} of 0.0746 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 66 and 74/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 81 and 35/110, respectively (Table A2). In a preliminary trial 1 the chemical had an LA transformation response. In trial 2 the chemical had an SP transformation response. Ethyl acrylate was evaluated as active in the transformation assay. Its actual and estimated rank *t*-statistics were 4.51 and 5.25, respectively (Table A3).

Isophorone. Isophorone is a level *D* carcinogen (Table A3) with no insurmountable technical problems (Table A1). It was moderately cytotoxic to the BALB/c-3T3 cells with an average LD_{50} of 5.18 mM (Table A1). The statistical sensitivities of transformation assay trials 1-3 were 106, 74 and 26/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 51, 35 and 93/110, respectively (Table A2). In a preliminary trial 1 the chemical had an LN transformation response because the test chemical treatment doses were noncytotoxic to the cells. In trials 2 and 3 the chemical had an SP transformation response. Isophorone was evaluated as active in the transformation assay. Its actual and estimated rank *t*-statistics were 2.76 and 2.86, respectively (Table A3).

D-Limonene. D-Limonene is a level *D* carcinogen (Table A3). It had one difficult technical problem. It was reported to become oxidized by air; thus, it could have reacted with air during the treatment period (Table A1). The test chemical was cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 0.988 mM (Table A1). The statistical sensitivities of transformation assay trials 1-3 were 70, 12 and 57/110, respectively; the detection sensitivities for BaP of trials 1-3 were 41, 98 and 88/110, respectively (Table A2). The test chemical had an LN transformation response in the first experiments and an SN response in the second and third experiments. D-Limonene was evaluated as inactive in the transformation assay, and its actual and estimated rank *t*-statistics were 0.24 and 0.27, respectively (Table A3).

Malonaldehyde, Sodium Salt. Malonaldehyde, sodium salt, is a level *C* carcinogen (Table A3). It was had one serious technical problem because it is very temperature sensitive (Table 1). The test chemical was moderately cytotoxic to the BALB/c-3T3 cells with an average LD_{50} of 3.74 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 21 and 54/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 26 and 44/110, respectively (Table A2). In a preliminary trial 1 the chemical had an SP transformation response. In trial 2 the chemical had an LA transformation response. Malonaldehyde, sodium salt, was evaluated as active in the transformation assay. Its actual and estimated rank *t*-statistics were 1.87 and 1.87, respectively (Table A3).

Methapyrilene-HCl. Methapyrilene-HCl is a level *C* carcinogen (Table A3). It had no insurmountable technical problems (Table A1). The test chemical was cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 0.812 mM (Table A1). The statistical sensitivities of transformation assay trials 1-3 were 60, 91 and 82/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 27, 85 and ND/110, respectively (Table A2). The test chemical had an SN transformation response in all three experiments. Methapyrilene-HCL was evaluated as inactive in the transformation assay, and its actual and estimated rank *t*-statistics were 0.14 and 0.17, respectively (Table A3).

2-Mercaptobenzothiazole. 2-Mercaptobenzothiazole is a level *C* carcinogen (Table A3). It had one serious technical problem. It was reported to react with water; thus, its activity in the transformation assay could have been unavoidably affected by its exposure to an aqueous environment during the 48-hr treatment period (Table A1). The test chemical was cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 0.130 mM (Table A1). The statistical sensitivities of transformation assay trials 1-3 were 3,17 and 51/110, respectively, the detection sensitivities for BaP of trials 1-3 were 110, 6 and 50/110, respectively (Table A2). The test chemical had a LN transformation response in the first experiment, because the positive control did not induce significant transformation in this experiment. In the second and third experiments the test chemical had LA transformation responses. 2-Mercaptobenzothiazole was evaluated as having had equivocal activity in the transformation assay, and its actual and estimated rank *t*-statistics were both 1.23 (Table A3).

Nitrilotriacetic Acid, Trisodium Salt. Nitrilotriacetic acid, trisodium salt, is a level *A* carcinogen (Table A3).

Because this chemical was reported to binds metal salts, it could have affected the concentration of metal salts in FBS and culture medium (Table A1). It was moderately cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 5.98 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 80 and 33/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 89 and 14/110, respectively (Table A2). In trials 1 and 2 the chemical had an SP transformation response. Nitrilotriacetic acid, trisodium salt, was evaluated as active in the transformation assay. Its actual and estimated rank *t*-statistics were both 5.86, respectively (Table A3).

Polybrominated Biphenyl Mixture. Polybrominated biphenyl mixture is a level *A* carcinogen (Table A3). This test chemical was insoluble in culture medium at a portion of the treatment doses that were used to induce both cytotoxic and transforming activity (Table A1). It was cytotoxic to the BALB/c-3T3 cells with an average LD_{50} of 0.291 mM (Table A1). The statlstical sensitivities of transformation assay trials 1 and 2 were 81 and 39/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 22 and 68/110, respectively (Table A2). In trials 1 and 2 the chemical had an SP transformation responses for both type III and type I-III focus transformation responses. However, this test chemical was very unusual in that it induced proportionally a much higher response for the type I and II foci, than for the type III foci (refer to the Discussion). Polybrominated biphenyl mixture was evaluated as active in the transformation assay. Its actual and estimated rank *t*-statistics were both 1.93 for the type III focus response, and 5.81 for the type I and II focus response (Table A3).

Reserpine. Reserpine is a level *A* carcinogen (Table A3). It had one difficult technical problem. It was reported to become oxidized by air; thus, it could have reacted with air during the treatment period (Table A1). The test chemical was very cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 0.0133 mM (Table A1). The statistical sensitivities of transformation assay trials 1-3 were 25, 16 and 92/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 36 and 7/110, respectively (Table A2). The test chemical had an LA transformation response in the first experiment and an SN response in the second and third experiments. Reserpine was evaluated as inactive in the transformation assay, and its actual and estimated rank *t*-statistics were both 0.24, respectively (Table A3).

Tris(2-ethylhexyl)phosphate. Tris(2-ethylhexyl)phosphate is a level *D* carcinogen (Table A3). It had no insurmountable technical problems (Table A1). The test chemical was cytotoxic to the BALB/c3T3 cells and had an average LD_{50} of 0.338 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 57 and 59/110, respectively, the detection sensitivities for BaP of trials 1 and 2 were 88 and 17/110, respectively (Table A2). The test chemical had an SN transformation response in both experiments. Tris(2-ethylhexyl)-phosphate was evaluated as inactive in the transformation assay, and its actual and estimated rank *t*-statistics were both 0.00 (Table A3).

4-Vinylcyclohexene. 4-Vinylcyclohexene is a level *D* carcinogen (Table A3). It had one difficult technical problem. It was reported to become oxidized upon exposure to air; thus, it could have reacted with air during the treatment period (Table A1). The test chemical was moderately cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 3.88 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 32 and 38/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 108 and 16/110, respectively (Table A2). The test chemical had an SN transformation response in both experiments. Vinylcyclohexane was evaluated as inactive in the transformation assay, and its actual and estimated rank *t*-statistics were both 0.00 (Table A3).

Cytotoxic, Nonmutagenic Noncarcinogens

Anilazine. Anilazine is a level *F* noncarcinogen (Table A3). It had no insurmountable technical problems (Table 1). The test chemical was a very cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 0.0475 (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 68 and 67/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 40 and 78/110, respectively (Table A2). In trials 1 and 2 the test chemical had LA transformation responses. Aniline was evaluated as having equivocal activity in the transformation assay, and its actual and estimated rank *t*-statistics were 1.82 and 2.09, respectively (Table A3).

L-Ascorbic Acid. L-Ascorbic acid is a level *F* noncarcinogen (Table A3). It had two difficult technical problems. It was reported to become oxidized upon exposure to air; thus, it could have reacted with air during the treatment period. In addition, it was noted to bind metal salts; thus, it could have combined with metal salts in FBS and EMEM medium (Table A1). The test chemical was cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 0.363 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 50 and 101/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 59 and 104/110, respectively (Table A2). In trials 1 and 2 the test chemical had SN transformation responses. L-Ascorbic acid was evaluated as inactive in the transformation assay, and its actual and estimated rank *t*-statistics were 0.32 and 0.46, respectively (Table A3).

Bisphenol A. Bisphenol A is a level *E* noncarcinogen (Table A3). It had no insurmountable technical problems (Table A1). The test chemical was cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 0.147 mM (Table A1). The statistical sensitivities of transformation assay trials 1-4 were 53, 16, 99 and 105/110, respectively; the detection sensitivities for BaP of trials 1-4 were 30, 7, 37 and 39/110, respectively (Table A2). The test chemical had an SN transformation response in all four experiments. Bisphenol A was evaluated as inactive in the transformation assay, and its actual and estimated rank *t*-statistics were both 0.79 (Table A3).

Carbromal. Carbromal is a level *F* noncarcinogen (Table A3). It had one difficult technical problem. It was reported to become oxidized by air; thus, it could have reacted with

air during the treatment period (Table A1). The test chemical was moderately cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 3.60 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 14 and 23/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 72 and 11/110, respectively (Table A2). The test chemical had an SN transformation response in the first experiment, and an SP response in the second experiment. Although the test chemical had disparate transformation responses in two experiments, the mean *t*-statistics of the two responses were not significantly different from one another. Carbromal was evaluated as weakly active in the transformation assay, and its actual and estimated rank *t*-statistics were both 2.26 (Table A3).

Chlorpheniramine-Maleate. Chlorpheniramine maleate is a level *F* noncarcinogen (Table A3). It had no insurmountable technical problems (Table A1). The test chemical was cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 0.287 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 47 and 67/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 43 and 78/110, respectively (Table A2). The test chemical had an SN transformation response in the first experiment, and an SP response in the second experiment. Although the test chemical had disparate transformation responses, the mean *t*-statistics of the two responses were not significantly different from one another. Chlorpheniramine-maleate was evaluated as weakly active in the transformation assay, and its actual and estimated rank *t*-statistics were 1.18 and 1.26, respectively (Table A3).

C. I. Acid Red 14. C. I. Acid red 14 is a level *F* noncarcinogen (Table A3). It had no insurmountable technical problems (Table A1). The test chemical was moderately cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 3.38 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 40 and 35/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 54 and 57/110, respectively (Table A2). In trials 1 and 2 the test chemical had SP transformation responses. C. I. Acid red 14 was evaluated as active in the transformation assay, and its actual and estimated rank *t*-statistics were 60th 2.15 (Table A3).

C. I. Acid Yellow 73. C. I. Acid yellow 73 is a level *F(I)* noncarcinogen which has been reclassified as an incomplete bioassay study (Table A3). It had no insurmountable technical problems (Table A1). The test chemical was moderately cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 4.65 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 17 and 71/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 6 and 23/110, respectively (Table A2). In trials 1 and 2 the test chemical had SN transformation responses. C. I. Acid yellow 73 was evaluated as inactive in the transformation assay, and its actual and estimated rank *t*-statistics were both 0.03 (Table A3).

Ephedrine Sulfate. Ephedrine sulfate is a level *F* noncarcinogen (Table A3). It had no insurmountable technical problems (Table A1). The test chemical was moderately cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 1.53 mM (Table A1). The statistical sensitivities of transformation assay trials 1-3 were 24, 17 and 51/110, respectively; the detection sensitivities for BaP of trials 1-3 were 10, 6 and 50/110, respectively (Table A2). The test chemical had an LN transformation response in the firsl experiment because the test chemical did not have significant cytotoxic activity. The test chemical had an SN response in the second and third experiments. Ephedrine sulfate evaluated as inactive in the transformation assay, and its actual and estimated rank *t*-statistics were both 0.16 (Table A3).

Erythromycin Stearate. Erythromycin stearate is a level *F* noncarcinogen (Table A3). It had no insurmountable technical problems (Table A1). The test chemical was very cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 0.0746 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 24 and 51/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 10 and 50/110, respectively (Table A2). The test chemical had an SN transformation response in both the first and second experiments. Erythromycin stearate was evaluated as inactive in the transformation assay, and its actual and estimated rank *t*-statistics were both 0.08 (Table A3).

Ethoxylated Dodecyl Alcohol. Ethoxylated dodecyl alcohol is a level *F(I)* noncarcinogen which has been reclassified as an incomplete study (Table A3). It had no insurmountable technical problems (Table A1). The test chemical was very cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 0.0172 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 1 and 15/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 3 and 21/110, respectively (Table A2). The test chemical had an SN transformation response in both the first and second experiments. Ethoxylated dodecyl alcohol was evaluated as inactive in the transformation assay, and its actual and estimated rank *t*-statistics were both 0.29 (Table A3).

Ethylenediamine Tetraacetic Acid, Trisodium Salt. Ethylenediamine tetraacetic acid, trisodium salt, is a level *F* noncarcinogen (Table A3). It had one difficult technical problem. It was reported to bind with certain metal salts; thus, it could have reacted with metal salts in FBS and EMEM medium (Table A1). The test chemical was moderately cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 1.89 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 80 and 67/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 89 and 78/110, respectively (Table A2). The test chemical had an LA transformation response in the first experiment, and an SN response in the second experiment. Ethylenediamine tetraacetic acid, trisodium salt, was evaluated as inactive in the transformation assay, and its actual and estimated rank *t*-statistics were 1.41 and 2.01, respectively (Table A3).

Eugenol. Eugenol is a level *E* noncarcinogen (Table A3). It had one difficult technical problem. It was reported to become oxidized upon exposure to air; thus, it could have reacted with air during the treatment period (Table A1).

The test chemical was cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 0.875 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 32 and 18/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 108 and 31/110, respectively (Table A2). The test chemical had an LN transformation response in the first experiment, because the test chemical did not induce significant cytotoxic activity. In the second experiment the test chemical had an SN transformation response. Since the test chemical had an LN transformation response in the first experiment, it had to be tested in two additional trials. Therefore, eugenol was evaluated as having had an indeterminate activity in the transformation assay, and its actual and estimated rank *t*-statistics were both 1.07 (Table A3).

Geranyl Acetate. Geranyl acetate is a level *F* noncarcinogen (Table A3). It had no insurmountable technical problems (Table A1). The test chemical was cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 0.302 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 44 and 41/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 65 and 83/110, respectively (Table A2). The test chemical had an SN transformation response in the first and second experiments. Geranyl acetate was evaluated as inactive in the transformation assay, and its actual and estimated rank *t*-statistics were 0.35 and 0.37, respectively (Table A3).

4-Hexylresorcinol. 4-Hexylresorcinol is a level *E* noncarcinogen (Table A3). It had no insurmountable technical problems (Table A1). The test chemical was cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 0.103 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 13 and 67/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 66 and 78/110, respectively (Table A2). The test chemical had an SN transformation response in the first experiment, and an LA response in the second experiment. 4-Hexylresorcinol was evaluated as inactive in the transformation assay, and its actual and estimated rank *t*-statistics were 0.46 and 0.47, respectively (Table A3).

D,L-Menthol. D,L-Menthol is a level *F* noncarcinogen (Table A3). It had no insurmountable technical problems (Table A1). The test chemical was moderately cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 4.63 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 46 and 89/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 80 and 87/110, respectively (Table A2). The test chemical had an SN transformation response in the first experiment and second experiments. D,L-Menthol was evaluated as inactive in the transformation assay, and its actual and estimated rank *t*-statistics were 0.16 and 0.22, respectively (Table A3).

Methoxychlor. Methoxychlor is a level *F* noncarcinogen (Table A3). It had no insurmountable technical problems (Table A1). The test chemical was very cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 0.0978 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 58 and 51/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 55 and 50/110, respectively (Table A2). The test chemical had an SN transformation response in the first experiment, and an LA response in the second experiment. Methoxychlor was evaluated as inactive in the transformation assay, and its actual and estimated rank *t*-statistics were both 1.08 (Table A3).

Methyldopa Sesquihydrate. Methyldopa sesquihydrate is a level *E* noncarcinogen (Table A3). It had no insurmountable technical problems (Table A1). The test chemical was very cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 0.0810 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 21 and 56/110, respectively, the detection sensitivities for BaP of trials 1 and 2 were 26 and 109/110, respectively (Table A2). The test chemical had an LA transformation response in the first experiment, and an SN response in the second experiment. Methyldopa sesquihydrate was evaluated as inactive in the transformation assay, and its actual and estimated rank *t*-statistics were both 1.21, respectively (Table A3).

Methylphenidate-HCl. Methylphenidate is a level *I* noncarcinogen because it has not been evaluated in a complete rodent bioassay study (Table A3). It had no insurmountable technical problems (Table A1). The test chemical was moderately cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 5.63 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 64 and 86/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 84 and 74/110, respectively (Table A2). In trials 1 and 2 the test chemical had SN transformation responses. C. I. Acid red 14 was evaluated as inactive in the transformation assay, and its actual and estimated rank *t*-statistics were 1.05 and 1.47, respectively (Table A3).

Oxytetracycline-HCl. Oxytetracycline is a level *E* noncarcinogen (Table A3). It had one difficult technical problem. It was reported to hydrolyze in water; thus, it could have reacted with water during the treatment period (Table A1). The test chemical was cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 0.523 mM (Table A1). The statistical sensitivities of transformation assay trials 1-3 were 80, 22 and 5/110, respectively; the detection sensitivities for BaP of trials 1-3 were 89, 53 and 46/110, respectively (Table A2). The test chemical had an SN transformation response in all three experiments. The test chemical was tested in the third experiment, because the its cytotoxic activity in the first experiment was excessive. Oxytetracycline-HCI was evaluated as inactive in the transformation assay, and its actual and estimated rank *t*-statistics were both 0.00, respectively (Table A3).

Phenol. Phenol is a level *F* noncarcinogen (Table A3). It had two difficult technical problems. It was reported to become oxidized by air; thus, it could have reacted with air during the treatment period. In addition, it was reported to react with sulfate groups on chemicals; thus, it could have reacted with biochemicals in culture medium, as well as biochemicals in the target cells (Table A1). The test chemical was moderately cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 3.29 mM (Table A1). The

statistical sensitivities of transformation assay trials 1 and 2 were 12 and 15/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 26 and 21/110, respectively (Table A2). The test chemical had an SP transformation response in the first and second experiments. Phenol was evaluated as active in the transformation assay, and its actual and estimated rank *t*-statistics were both 7.60 (Table A3).

Phenylephrine-HCl. Phenylephrine-HCl is a level *F* noncaranogen (Table A3). It had one difficult technical problem. It was reported to become oxidized by air; thus, it could have reacted with air during the treatment period (Table A1). The test chemical was moderately cytotoxic to the BALB/c3T3 cells and had an average LD_{50} of 3.52 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 80 and 29/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 89 and $^{97}/_{110}$, respectively (Table A2). The test chemical had an SN transformation response in the first and second experiments. Phenylephrine-HCl was evaluated as inactive in the transformation assay, and its actual and estimated rank *t*-statistics were 0.31 and 0.42, respectively (Table A3).

Propyl Gallate. Propyl gallate is a level *E* noncarcinogen (Table A3). It had one difficult technical problem. It was reported to react with iron; thus, it could have reacted with the iron in FBS (Table A1). The test chemical was very cytotoxic to the BALB/c-3T3 cells and had an average LD50 of 0.0631 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 94 and 102/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 91 and 95/110, respectively (Table A2). The test chemical had an SP transformation response in the first experiment, and an LA response in the second experiment. Propyl gallate was evaluated as active in the transformation assay. Because the statistical sensitivity and detection sensitivity for BaP in both of the experiments were significantly low, and the test chemicals actual and estimated rank *t*-statistics were very different (i.e., 1.70 and 2.95, respectively (Table A3).

Rotenone. Rotenone is a level *E* noncarcinogen (Table A3). It had one difficult technical problem. It was reported to become oxidized by air; thus, it could have reacted with air during the treatment period (Table A1). The test chemical was very cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 0.000464 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 21 and 31/110, respectively, the detection sensitivities for BaP of trials 1 and 2 were 26 and 52/110, respectively (Table A2). The test chemical had an SN transformation response in the first and second experiments. Rotenone was evaluated as inactive in the transformation assay, and its actual and estimated rank *t*-statistics were both 0.26 (Table A3).

Sodium Diethyldithiocarbamate. Sodium diethyldithiocarbamate is a level *F* noncarcinogen (Table A3). It had no insurmountable technical problems (Table A1). The test chemical was very cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 0.000142 mM (Table A1). The statistical sensilivities of transformation assay trials 1 and 2 were 69 and 31/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 12 and 52/110, respectively (Table A2). The test chemical had an SP transformation response in the first and second experiments. Sodium diethyldithiocarbamate was evaluated as active in the transforrnation assay, and its actual and estimated rank t-statistics were both 2.94 (Table A3).

Stannous Chloride. Stannous chloride is a level *E* noncarcinogen (Table A3). It had two difficult technical problems. It was reported to react with both alcohols and amines; thus, it could have reacted with biochemicals with this groups in both FBS and EMEM medium (Table A1). The test chemical was very cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 0.0285 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 77 and 37/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 67 and 20/110, respectively (Table A2). The test chemical had an SN transformation response in the first and second experiments. Stannous chloride was evaluated as inactive in the transformation assay, and its actual and estimated rank *t*-statistics were both 0.78 (Table A3).

Tetracycline-HCl. Tetracycline-HCl is a level *F* noncarcinogen (Table A3). It had one difficult technical problem, because it was reported to become oxidized upon exposure to air; thus, it could have reacted with air during the treatment period (Table A1). The test chemical was moderately cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 3.24 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 24 and 51/110, respectively, the detection sensitivities for BaP of trials 1 and 2 were 10 and 50/110, respectively (Table A2). The test chemical had an SN transformation response in the first experiment and second experiments. Tetracycline-HCl was evaluated as inactive in the transformation assay, and its actual and estimated rank t-statistics were both 0.021 (Table A3).

Tetrakis(hydroxymethyl)phosphonium Chloride. Tetrakis(hydrosymethyl)-phosphonium chloride is a level *F* noncarcinogen (Table A3). It had no insurmountable technical problems (Table A1). The test chemical was very cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 0.00825 mM (Table A1). The slatistical sensitivities of transformation assay trials 1-3 were 70, 15 and 59/110, respectively; the detection sensitivities for BaP of trials 1-3 were 41, 21 and 17/110, respectively (Table A2). The test chemical had an SN transformation response in the first experiment, and an SP response in the second experiment. Because the mean *t*-statistics of the test chemical transformation responses in the first two experiments were significantly different from one another, the test chemical had to be tested in a third trial. The test chemical had a SN transformation response in the third experiment. Tetrakis(hydroxymethyl)phosphonium chloride was evaluated as having had equivocal activity in the transformation assay, and its actual and estimated rank t-statistics were both 1.27 (Table A3).

Tetrakis(hydroxymethyl)phosphonium Sulfate. Tetrakis(hydroxymethyl)phosphonium sulfate is a level *F* noncarcinogen (Table A3). It had no insurmountable technical problems (Table A1). The test chemical was very

cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 0.00438 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 70 and 44/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 41 and 65/110, respectively (Table A2). The test chemical had an SN transformation response in the first and second experiments. Tetrakis-(hydroxymethyl)phosphonium sulfate evaluated as inactive in the transformation assay, and its actual and estimated rank t-statistics were both 0.083 (Table A3).

Triphenyltin Hydroxide. Triphenyltin hydroxide is a level *F* noncarcinogen (Table A3). It had no insurmountable technical problems (Table A1). The test chemical was very cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 0.000134 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 82 and 43/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 18 and 9/110, respectively (Table A2). The test chemical had an SN transformation response in the first experiment, and an SP response in the second experiment. Since the mean *t*-statistics of the test chemical transformation responses in the first two experiments were significantly different from one another, it had to be tested in a third experiment. Therefore, triphenyltin hydroxide was evaluated as having had an indeterminate activity in the transformation assay, and its actual and estimated rank *t*-statistics were both 1.64 (Table A3).

Xylenes, (mixed). Xylenes (mixed) is a level *F* noncarcinogen (Table A3). It had no insurmountable technical problems (Table A1). The test chemical was moderately cytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 3.20 mM (Table A1). The statistical sensitivities of transformation assay trials 1 and 2 were 70 and 73/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 41 and 49/110, respectively (Table A2). The test chemical had an SN transformation response in the first and second experiments. Xylenes (mixed) was evaluated as inactive in the transformation assay, and its actual and estimated rank *t*-statistics were 0.47 and 0.50, respectively (Table A3).

Noncytotoxic, Mutagenic Carcinogens

DC Red No. 9. DC Red no. 9 is a level *B* carcinogen (Table A6). It had no insurmountable technical problems; however it had a solubility limit in culture medium of about 500 μg/ml. This improved to 2250 μg/ml when the medium was supplemented with the solvent vehicle pluronic F68 (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 3.82 mM and 6.52 mM, either with or without using pluronic F68 (Table A4). The statistical sensitivities of transformation assay trials 1-3 were 30, 91 and 107/110, respectively; the detection sensitivities for BaP of trials 1-3 were 4, 85 and 106/110, respectively (Table A5). The test chemical had an SP transformation response in two experiments, and an LA response in one experiment. Significant test chemical transforming activity was detected at doses both above and below its solubility limit in culture medium. DC Red No. 9 was evaluated as active in the transformation assay, and its actual and estimated rank *t*-statistics were 4.33 and 5.50, respectively (Table A6).

Diethanolnitrosamine. Diethanolnitrosamine is a level *I* carcinogen (Table A6). It had no insurmountable technical problems (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 61.1 mM (Table A4). The test chemical was only evaluated in one trial due to the limited availability during this investigation. The statistical sensitivity of transformation assay trial 1 was 52/110; the detection sensitivity for BaP of trial 1 was 79/110, respectively (Table A5). The test chemical had an SP transformation response in the only experiment conducted for this test chemical. Diethanolnitrosamine was evaluated as active in the transformation assay, and its actual and estimated rank *t*-statistics were 4.01 and 4.87, respectively (Table A6).

Diethylnitrosamine. Diethylnitrosamine is a level *A* carcinogen (Table A6). It had no insurmountable technical problems (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 46.0 mM (Table A4). The statistical sensitivities of transformation assay trials 1 and 2 were 4 and 27/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 1 and 63/110, respectively (Table A5). The test chemical had an SP transformation response in the two consecutive experiments. Diethylnitrosamine was evaluated as active in the transformation assay, and its actual and estimated rank *t*-statistics were both 4.69 (Table A6).

Table A4. Cytotoxic responses of 54 noncytotoxic chemicals.

Test Chemical[a]			Physicochemical Properties			Cytotoxic Responses[b] (millimolar LD_{50}) Co-culture
Name	CAS No.	M.W.	1	2	3	Assay
21 Carcinogens						
Group I. Very Non-Cytotoxic Chemicals						
3-amino-1,2,4-triazole	61-82-5	84.08	S	C	a, ach, m, o	109.
cyclamate, sodium salt	139-05-9	201.22	S	C	a, b, m, n, o	132.
dimethyl hydrogen phosphite	868-85-9	110.05	L	C	ac, b, o, v, W	130. (260 mOsM)
dimethyl methyl phosphonate	756-79-6	124.08	L	C	ai, alk, b, o, oh, v	172.
dimethylnitrosamine	62-75-9	74.08	L	C	ai, b, o, v	256.
methyl carbamate (uncoded)	598-55-0	75.07	S	C		225.
(coded)	"	"				195.
saccharin, sodium salt	81-07-2	205.2	S	C		76.5 (153 mOsM)
Group II. Non-Cytotoxic Chemicals						
11-aminoundecanoic acid	2432-99-7	201.35	S	FC	a, b, o	19.4
D & C red no. 9	5160-02-1	444.49	S	FC	o, sp	3.82 (6.52)
decabromodiphenyloxide	1163-19-5	959.22	S	FC	l, o, sp	>6.26
di(2-ethylhexyl)adipate	103-23-1	370.57	L	FC	o, sp, v	98.4
di(2-ethylhexyl)phthalate	117-81-7	390.54	L	FC	a, b, n, o, v	21.4
diethanolnitrosamine			L	C	a, l, v	61.1
diethylnitrosamine	55-18-5	102.14	L	C	l, o, r, v	46.0
dimethylmorpholino- (uncoded)	597-25-1	195.18	L	C	a, ai, v	17.1
phosphoramidate (coded)	"	"				24.4
ethylene thiourea	96-45-7	102.16	S	FC	o,	91.4
hexamethylphosphoramide	680-31-9	179.2	L	C	v	64.4
melamine	108-78-1	126.12	S	FC	a, o, sp	39.6
monuron	150-68-5	198.65	S	C	a, b, sp	5.54
phenobarbital, sodium salt	57-30-7	254.22	S	FC	a, ai, o	6.11
2,4- & 2,6-toluene diiso-thiocyanate	26471-62-5	174.16	L	FC	am, b, l, ts, v, W	7.93
26 Non-Carcinogens						
Group I. Very Non-Cytotoxic Chemicals						
D-mannitol	69-65-8	182.18	S	C		>329.
3-sulfolene	77-79-2	118.15	S	C	ts	117.
witch hazel	68916-39-2	46.07	L	C	o, v	540.
Group II. Non-Cytotoxic Chemicals						
aldicarb	116-06-3	190.27	S	FC	a, b, ts	10.7
ampicillin trihydrate	7177-48-2	403.50	S	FC	ts	23.8
o-anthranilic acid	118-92-3	137.14	S	FC	ai, l, ts	72.9
benzoin	119-53-9	212.25	S	FC	sp,	14.8
benzyl alcohol	100-51-6	108.13	L	FC	a, ach, ai, ts	17.9
caprolactam	105-60-2	113.16	S	C	ai, b, ch, o, ts	71.8
2-chloroethanol	107-07-3	80.52	L	C	b, o, ts, W	81.0
(2-chloroethyl)trimethyl-ammonium chloride	999-81-5	158.07	S	C	m, o	62.0
C. I. acid orange 10	1936-15-8	452.38	S	FC		26.5
dimethyl terephthalate	120-61-6	194.19	S	FC	a, ai, b, o, sp. ts, W	>15.4
diphenylhydantoin	57-41-0	252.27	S	FC	b, o, sp	5.02
FD & C yellow No. 6	2783-94-0	452.37	S	FC		67.7
methyl methacrylate	80-62-6	100.12	L	DFC	ai, am, b, l, ra, ts	10.8
molybdenum trioxide	1313-27-5	144.	S	FC	a&b, ts	9.38
4-nitroanthranilic acid	619-17-0	182.15	S	FC	a, ach, b, o	8.58
penicillin VK+	132-98-9	388.51	S	C		17.8
phthalamide	88-96-0	164.18	S	FC	a, sp	73.1
phthalic anhydride	85-44-9	148.12	S	FC	a, am, b, o, ra, ts, W,	13.2
roxarsone	121-19-7	~260.	S	FC	a, l, o, ts	43.8
sodium(2-ethylhexyl) alcohol sulfate	126-92-1	232.28	L	C		12.5
sulfisoxazole	127-69-5	267.32	S	FC	ai, l	18.7
tetrahydrofuran	109-99-9	72.11	L	FC	a, ai, b, o, ts	90.3
titanium dioxide	13463-67-7	79.90	S	FC	m, sp	12.1

(Continued on next page)

Table A4. *Continued.*

Test Chemical[a]			Physicochemical Properties			Cytotoxic Responses[b] (millimolar LD_{50}) Co-culture
Name	CAS No.	M.W.	1	2	3	Assay
7 Model Very Non-Cytotoxic Chemicals						
Group I. Non-Cytotoxic Chemicals						
acetone	67-64-1	58.08	L	C	a, l, o, oc, ts	257.
dimethyl sulfoxide	67-68-5	78.13	L	C	a, ach, am, r, o	507.
ethanol	64-17-5	46.07	L	C	a, ach, o, am	429.
glycerol	56-81-5	92.09	L	C	-	401.
sodium chloride	7647-14-5	58.44	S	C	-	144.
sucrose	57-50-1	342.30	S	C	a	240.
urea	57-13-6	60.07	S	C	-	254.

Abbreviations: CAS No., Chemical Abstract Service registry number; LD_{50}, lethal dose for 50% of the cells; M.W., molecular weight.

Abbreviations for Test Chemical Physicochemical Properties: Physicochemical considered in this study included: [1] physical state (S = solid, L = liquid); [2] solvent vehicle (D = dimethyl sulfoxide, C = culture medium, F = pluronic F68, A = acetone, E = ethanol) and [3] technical problems. The technical problems included test chemicals that were a = reacts with acids; ac = reacts with acid chlorides and acid anhydrides; ai = reacts with air, al = reacts with alcohols; alk = alkylating agent and reacts with labile hydrogen; b = reacts with bases; bc = reacts with biochemicals (amino, hydroxyl, and carboxyl groups); hc = reacts with halogenated chemicals; k = reacts with alpha keto acids; ls = light sensitive; m = binds metals; mel = reacts with hexachloro- and trichloromelamine; met = reacts with metals (aluminum, iron, magnesium, potassium, sodium, tin or zinc); mh = metal halides; msc = reacts with miscellaneous organic chemicals (i.e. alpha-aminoethanol, chlorosulfonic acid, ethylene imine, linseed oil, maleic anhydride, oleum, or K-tert-butyloxide); o = reacts with oxidizing agents; p = reacts with plastics; pi = polymerization initiators; r = reacts with reducing agents; ru = reacts with rubber; sp = solubility problem in culture medium; tc = reacts with thiocyanates; ts = temperature sensitive; vts = very temperature sensitive; v = volatile at 37,C; and w = reacts with water [refer to MATERIALS and METHODS].

[a]Test Chemical: Tables A1 and A3 contain 168 chemicals along with their individual CAS registry number and molecular weight. The chemicals were divided into groups of chemicals that correspond to the groups of chemicals that were compared in different text Tables 1–12. Thus, the chemicals were divided into two groups, including 114 cytotoxic test chemicals (LD_{50} < 5.0 mM) presented in Table A1, and 53 noncytotoxic chemicals (LD_{50} >5.0 mM presented in Table A4. The 114 cytotoxic chemicals in Table A1 were subdivided into groups of 43 mutagenic carcinogens, 21 mutagenic noncarcinogens, 20 nonmutagenic carcinogens, and 30 non-mutagenic noncarcinogens. The 53 noncytotoxic test chemicals in Table A4 were subdivided into groups of 21 carcinogens, 26 noncarcinogens and 7 model very noncytotoxic chemicals. In addition, all of the cytotoxic test chemicals were separated into three groups, including: Group I, moderately cytotoxic test chemicals [LD_{50} 1-5 mM]; Group II, cytotoxic chemicals [LD_{50} 0.1-1.0 mM]; and Group III, very cytotoxic chemicals [LD_{50}<0.1 mM]. In addition, this table presents important physicochemical properties that influenced the procedure used in testing the chemicals.

[b]Cytotoxic Response: The co-culture clonal sulvival assay design used to detect the cytotoxic response of the test chemical is described in Materials and Methods. The cytotoxic responses of chemicals in individual experiments are summarized in terms of the millimolar (mM) LD_{50} treatment dose that resulted in 50% survival of the chemically-treated cells relative to the survival of untreated or solvent control treated cell cultures. The LD_{50} cytotoxic response of each chemical in Tables A1 and A4 is an average of two or more experiments with the chemical. The molecular weight of each chemical is provided in order that treatment doses could be converted from mM to μg/ml. For example, based upon the molecular weight of 84.08, the LD_{50} detected for the first chemical in Table A4, 3-amino-1,2,4-triazole, was 109 mM or 9165 μg/ml.

Table A5. Transformation responses of 54 noncytotoxic chemicals.

Chemical[a]		Transformation Response[b]					
		Spontaneous[c]		Benzo(a)pyrene[d]		Test Chemical[e]	
Name	Exp. No.	Foci/Vessel	Rank Order	Call	Rank Order	Call	Mean *t*-statistic
21 Carcinogens							
Active Chemicals (false positives)							
dimethyl hydrogen phosphite	1 (84)	.511	44	SP	65	SP	6.02
	2 (104)	.878	26	SP	93***	SP	5.93
dimethyl methyl phosphonate	1 (84)	.511	44	SP	65	LA	2.17
	2 (102)	.697	27	SP	63	SP	3.63
Active Chemicals							
3-amino-1,2,4-triazole	1 (69)	.288	85	SP	102***	SP	3.54
	2 (99)	.586	33	SP	14***	SP	8.08
11-aminoundecanoic acid	1 (17)	.327	83	SP	70	LN	1.10
	2 (24)	.308	89	SP	87***	LN	.000
	3 (32)	1.99	19	SP	34	SP	1.86
	4 (67)	.085	107**	SP	106***	SP	2.37
cyclamate, sodium salt	1 (71)	1.06	24	SP	10***	SP	2.76
	2 (107)	2.95	5***	SP	46	SP	5.24
D & C red No. 9	1 (43)	1.05	30	SP	4***	SP	8.46
	2 (54)	.265	91	SP	85	LA	1.10
	3 (67)	.085	107***	SP	106***	SP	3.42
diethanolnitrosamine	1 (86)	.464	52	SP	79*	SP	4.01
	2 (NA)			NA		NA	
diethylnitrosamine	1 (79)	5.12	4**	SP	1***	SP	5.91
	2 (102)	.697	27	SP	63	SP	3.88
dimethylmorpholinophosphoramidate (uncoded)	1 (86)	.464	52	SP	79*	SP	3.70
	2 (106)	1.30	28	SP	15***	SP	2.68
dimethylmorpholinophosphoramidate (coded)	1 (86)	.464	52	SP	79*	SP	4.44
	2 (108)	1.17	20	SP	19**	SP	4.02
dimethylnitrosamine	1 (31)	.930	42	SP	25*	SP	3.64
	2 (100)	.268	73	SP	49	SP	5.66
hexamethylphosphoramide	1 (78)	3.28	9*	SP	37	SP	2.62
	2 (98)	.618	59	SP	17***	SP	1.53
melamine	1 (43)	1.05	30	SP	4***	SP	2.21
	2 (58)	.189	97	SP	94**	LA	1.99
21 Carcinogens Continued							
Active Chemicals Continued							
methyl carbamate	1 (42)	.861	55	SP	8***	SP	7.80
	2 (66)	.056	108**	SP	99***	LA	1.53
	3 (80)	3.02	10*	SP	5***	SP	5.08
methyl carbamate (315183-S)	1 (83)	.351	71	SP	23**	SP	2.38
	2 (99)	.586	33	SP	14**	SP	4.81
phenobarbital, sodium salt	1 (59)	.297	75	SP	76	LA	2.01
	2 (109)	2.55	6*	SP	92***	SP	3.52
saccharin, sodium salt	1 (75)	.882	21	SP	26*	SP	4.95
	2 (101)	.260	62	SP	48	SP	10.3

(Continued on next page)

Table A5. *Continued.*

Chemical[a]		Transformation Response[b]					
		Spontaneous[c]		Benzo(a)pyrene[d]		Test Chemical[e]	
Name	Exp. No.	Foci/Vessel	Rank Order	Call	Rank Order	Call	Mean *t*-statistic
2,4- & 2,6-toluene diisothiocyanate	1 (76)	1.79	12*	SP	98***	SP	2.61
	2 (106)	1.30	28	SP	15***	SP	3.54
Weakly Active Chemicals							
ethylene thiourea	1 (59)	.297	75	SP	76	LN	1.57
	2 (65)	.244	95	SP	61	SP	2.17
Inactive Chemicals							
di(2-ethylhexyl)adipate	1 (88)	.406	57	SP	88***	SN	.000
	2 (108)	1.17	20	SP	19**	SN	.000
di(2-ethylhexyl)phthalate	1 (36)	.424	74	SP	35	SN	.000
	2 (100)	.268	73	SP	49	SN	.000
monuron	1 (20)	.368	81	SP	22***	LA	1.57
	2 (28)	.818	39	SP	68	SN	.453
Inactive Chemical with an Indeterminate Activity							
decabromodiphenyloxide	1 (75)	.882	21	SP	26*	LN	.150
	2 (101)	.260	62	SP	48	LN	.430
26 Non-Carcinogens							
Active Chemicals							
benzyl alcohol	1 (81)	7.36	2***	SP	2***	LA	2.11
	2 (110)	.609	38	SP	16***	SP	1.79
2-chloroethanol	1 (78)	3.28	9*	SP	37	SP	3.78
	2 (100)	.268	73	SP	49	SP	2.66
(2-chloroethyl)trimethyl-ammonium chloride	1 (30)	.787	40	SP	54	SP	1.90
	2 (45)	.732	35	SP	57	LA	1.68
FD & C yellow No. 6	1 (34)	2.51	7*	SP	56	SP	8.18
	2 (65)	.244	95	SP	61	SP	4.85
	3 (103)	.874	22	SP	53	SP	9.90
penicillin VK+	1 (80)	3.02	10*	SP	5***	SP	6.26
	2 (101)	.260	62	SP	48	SP	5.76
3-sulfolene	1 (33)	1.04	49	SP	24**	SP	3.84
	2 (44)	1.52	23	SP	11***	LA	3.00
Weakly Active Chemicals							
methyl methacrylate	1 (79)	5.12	4**	SP	1***	SP	4.34
	2 (106)	1.30	28	SP	15***	SN	1,19
4-nitroanthranilic acid	1 (34)	2.51	7*	SP	56	SN	.015
	2 (103)	.874	22	SP	53	SP	2.16
Chemical with an Equivocal Activity							
ampicillin trihydrate	1 (83)	.351	71	SP	23***	LA	1.04
	2 (105)	.581	29	SP	97***	LA	1.52

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Table A5. *Continued.*

Chemical[a]		Transformation Response[b]					
		Spontaneous[c]		Benzo(a)pyrene[d]		Test Chemical[e]	
Name	Exp. No.	Foci/Vessel	Rank Order	Call	Rank Order	Call	Mean *t*-statistic
Inactive Chemicals							
benzoin	1 (4)	1.51	50	SP	59	SN	.000
	2 (10)	.053	109**	SP	103***	SN	1.48
caprolactam	1 (5)	.035	110***	SP	107***	SN	1.37
	2 (10)	.053	109**	SP	103***	SN	1.08
C. I. acid orange 10	1 (64)	.291	92	SP	71	SN	.825
	2 (103)	.874	22	SP	53	SN	.435
diphenylhydantoin	1 (56)	.260	84	SP	105***	SN	.00
	2 (65)	.244	95	SP	61	SN	.00
molybdenum trioxide	1 (47)	.579	61	SP	47	LA	.830
	2 (56)	.260	84	SP	105***	SN	.347
phthalic anhydride	1 (39)	.427	82	SP	18***	LA	1.60
	2 (107)	2.95	5**	SP	46	SN	.000
tetrahydrofuran	1 (82)	8.01	1***	SP	3***	SN	.393
	2 (106)	1.30	28	SP	15***	LA	1.36
Inactive Chemical with an Indeterminate Activity							
titanium dioxide	1 (38)	.496	82	SP	12***	SN	.000
	2 (109)	2.55	6*	SP	92***	SN	.000
Active Chemicals (false positive)							
D-mannitol	1 (18)	.663	46	SP	80*	LN	.728
	2 (45)	.732	35	SP	57	LA	1.23
	3 (110)	.609	38	SP	16***	SP	4.69
witch hazel	1 (81)	7.36	2***	SP	2***	LA	1.61
	2 (110)	.609	38	SP	16***	LA	2.17
Chemicals with an Indeterminate Activity							
aldicarb	1 (32)	1.99	19	SP	34	SN	.267
	2 (99)	.586	33	SP	14***	SP	3.17
o-anthranilic acid	1 (15)	.186	100	SP	42	SN	1.47
	2 (22)	.893	36	SP	28*	SP	2.99
dimethyl terephthalate	1 (103)	.874	22	SP	53	SP	1.71
	2 (107)	2.95	5***	SP	46	LA	1.86
phthalamide	1 (35)	1.97	14	SP	72	LN	.000
	2 (110)	.609	38	SP	16***	LA	2.02
roxarsone	1 (80)	3.02	10*	SP	5***	SP	2.94
	2 (109)	2.55	27	SP	92***	SN	.000
sodium(2-ethylhexyl) alcohol sulfate	1 (82)	8.01	1***	SP	3***	SN	.180
	2 (108)	1.17	6*	SP	19***	SP	3.42
sulfisoxazole	1 (19)	.357	77	SP	67	LN	.690
	2 (26)	.907	37	SP	20***	LA	1.26
	3 (NA)						

(Continued on next page)

Table A5. *Continued.*

Chemical[a]		Transformation Response[b]					
		Spontaneous[c]		Benzo(a)pyrene[d]		Test Chemical[e]	
Name	Exp. No.	Foci/Vessel	Rank Order	Call	Rank Order	Call	Mean *t*-statistic
7 Model Very Non-Cytotoxic Chemicals							
Active Chemicals (false positives)							
acetone	1 (82)	8.01	1***	SP	3***	LA	8.19
	2 (102)	.697	27	SP	63	SP	3.25
dimethyl sulfoxide	1 (41)	.274	90	SP	13***	LA	3.92
	2 (100)	.268	73	SP	49	SP	2.97
ethanol	1 (81)	7.36	2***	SP	2***	LA	3.03
	2 (108)	1.17	20	SP	19***	SP	2.23
glycerol	1 (82)	8.01	1***	SP	3***	SP	2.55
	2 (108)	1.17	20	SP	19***	SP	3.69
sodium chloride	1 (80)	3.02	10*	SP	5***	SP	12.2
	2 (109)	2.55	6*	SP	92***	SN	.855
	3 (R1)	.416	106*	NA		SP	4.35
sucrose	1 (101)	.260	62	SP	48	SP	10.4
	2 (107)	2.95	5**	SP	46	SP	2.24
urea	1 (109)	2.55	6*	SP	92***	SP	1.81
	2 (NA)						

General Abbreviations: Exp. No., experiment number; NA, not available.

Abbreviations for the Transformation Responses: SP, sufficient positive; LA, limited activity; SN, sufficient negative; LN, limited negative.

[a]Test Chemical: The 54 noncytotoxic chemicals in Table A5 are identical to those in Table A4, and they are subdivided into groups of 21 carcinogens, 26 noncarcinogens and 7 model very noncytotoxic chemicals.

[b]Transformation Response: This table presents a summary of the spontaneous, BaP, and test chemical transformation responses detected in two or more experiments per test chemical. The assay design and procedures used in the standard transformation assay are described in the Materials and Methods. The transforming activities of individual chemical treatment doses (i.e. focus data), as well as the individual transformation responses (i.e. type III foci/vessel), are provided in detail in the Appendices B-H. Appendices B, C, D, E, F, G and H contain the activities of the 43 cytotoxic, mutagenic carcinogens; 21 cytotoxic, mutagenic noncarcinogens; 20 cytotoxic, non-mutagenic carcinogens; 30 cytotoxic, nonmutagenic, noncarcinogens; 21 noncytotoxic carcinogens; 26 noncytotoxic noncarcinogens; and 7 very noncytotoxic model test chemicals.

[c]Spontaneous Transformation Response: The method used to calculate the spontaneous transformation response, as well as the positive control and test chemical responses, is explained in the Materials and Methods. The transformation responses are expressed as type III foci/vessel and were calculated using a $\log_{10}$ mathematical transformation procedure. The arithmetic value for foci/vessel in this table is the antilog of the $\log_{10}$ mean transformation response minus one.

The procedure for rank ordering the spontaneous responses from 110 experiments is based upon the different statistical sensitivities of transformation experirnents with different spontaneous responses is explained in the Statistical Sensitivity versus Spontaneous Transformation Response section of the Materials and Methods. Experiments with high spontaneous responses had a high statistical sensitivity and have relatively low rank-order numbers. For example, diethylnitrosamine had a high spontaneous response of 5.12 foci/vessel in exp. 79, which had a high statistical sensitivity and rank order number 4/110. Conversely, experiments with a low statistical sensitivity and have high rank-order numbers. For example, 11-aminoundecanoic acid had a low spontaneous response of .085 foci/vessel in exp. 67, which had a low statistical sensitivity with a high rank order number 107/110.

[d]Benzo(a)pyrene Transformation Response. The method used to call individual transformation experiments is described in detail in Materials and Methods. The method used to rank order the BaP transformation responses from the 110 experiments is based upon statistical comparison of the BaP transformation at the two treatment doses detected in an individual experiment with the median historical activity of the assay. This procedure is described in the Detection Sensitivity versus Benzo(a)pyrene Transformation Response section of the Materials and Methods. The rational for rank-ordering the experiments is analogous to that described for the spontaneous transformation responses (refer to footnote c above).

[e]Test Chemical Transformation Response: The method used to call individual experiments is described in detail in Materials and Methods, and the abbreviations for the calls are provided in footnote d above. The significance of the transformation responses of individual chemical treatment doses were calculated using SAS statistical software (*22*). The mean *t*-statistic represents the average of the *t*-statistics of the four test chemical treatment doses in the experiment. The *t*-statistics for individual chemical treatment doses which were used to calculate the mean *t*-statistic are provided in Appendices B-H.

*Significant spontaneous or BaP transformation response, $0.01 < p \leq 0.05$.

**Significant spontaneous or BaP transformation response, $0.001 < p \leq 0.01$.

***Significant spontaneous or BaP transformation response, $p \leq 0.001$.

Table A6. Rank-ordered potency of the transformation responses of 54 noncytotoxic chemicals compared to rodent bioassay activities.

Test Chemical[a] Name	Rodent Bioassay[b] Level of Activity			Transformation Response[c] Rank *t*-statistic	
	High	Low	None	Actual	Estimated[d]
6 Mutagenic Carcinogens					
Total Active Chemicals [100.%]					
Active Chemicals					
D & C red no. 9	B			4.33	5.50
diethanolnitrosamine		I		4.01	4.87
diethylnitrosamine	A			4.69	4.69
dimethylnitrosamine	A			4.45	4.45
phenobarbital, sodium salt	A			2.77	3.14
2,4- & 2,6-toluene diisothiocyanate	A			3.01	3.01
15 Non-Mutagenic Carcinogens					
Total Active Chemicals [73.3%]					
Active Chemicals (false positive)					
dimethyl hydrogen phosphite	B			5.97	6.19
dimethyl methyl phosphonate		D		2.90	2.90
Active Chemicals					
saccharin, sodium salt	A			7.99	7.99
3-amino-1,2,4-triazole	A			6.14	6.53
methyl carbamate (Average)		C		4.12	4.12
(uncoded)				(4.48)	(4.48)
(coded)				(3.76)	(3.76)
cyclamate, sodium salt	A			4.09	4.09
dimethyl morpholino- (average)		C		3.71	3.71
phosphoramidate (uncoded)				(3.19)	(3.19)
(coded)				(4.23)	(4.23)
11-aminoundecanoic acid	B			2.11	2.55
melamine		D		2.10	2.15
hexamethylphosphoramide		C		2.08	2.08
Weakly Active Chemical					
ethylene thiourea	A			2.84	3.96
.....					
Total Inactive Chemicals [26.7%]					
Inactive Chemicals					
monuron	B			1.01	1.01
di(2-ethylhexyl)adipate		C		.00	.00
di(2-ethylhexyl)phthalate	A			.00	.00
Inactive Chemical (Indeterminate Activity)					
decabromodiphenyloxide		C		.29	.29
3 Mutagenic Non-Carcinogens					
Total Active Chemicals [100.%]					
Active Chemicals					
2-chloroethanol			F	3.22	3.22
Weakly Active Chemicals					
methyl methacrylate			F	2.54	2.54
4-nitroanthranilic acid			F	1.09	1.09

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Table A6. *Continued.*

Test Chemical[a] Name	Rodent Bioassay[b] Level of Activity High	Low	None	Transformation Response[c] Rank *t*-statistic Actual	Estimated[d]
23 Non-Mutagenic Non-Carcinogens					
Total Active Chemicals [23.8%]					
Active Chemicals					
FD & C yellow No. 6		F		7.65	7.65
penicillin VK+		F		5.96	5.96
benzyl alcohol		F		1.95	1.95
(2-chloroethyl)trimethylammonium Cl		F		1.74	1.74
3-sulfolene		F		3.24	3.24
Total Inactive Chemicals [76.2%]					
Chemical with Equivocal Activity					
ampicillin trihydrate	E			1.32	1.32
Inactive Chemicals					
caprolactam		F		1.20	2.34
phthalic anhydride		F		.80	.80
benzoin	E			.74	1.08
C. I. acid orange 10		F		.63	.68
diphenylhydantoin			I	.00	.00
molybdenum trioxide			I	.64	.86
tetrahydrofuran			I	.72	.72
Inactive Chemical (Indeterminate Activity)					
titanium dioxide		F		.00	.00
23 Non-Mutagenic Non-Carcinogens Continued					
Total Inactive Chemicals [23.8%] Continued					
Chemicals with Indeterminate Activity					
aldicarb		F		1.93	1.93
o-anthranilic acid		F		2.19	2.19
dimethyl terephthalate	E			1.79	1.79
phthalamide		F		1.01	.64
roxarsone	E			1.67	1.67
sodium(2-ethylhexyl)alcohol sulfate		F(I)		2.04	2.04
sulfisoxazole		F		1.26	1.26
Active Chemicals (false positives)					
D-mannitol		F		3.00	1.99
witch hazel		F		1.89	1.89
7 Model Very Non-Cytotoxic Chemicals					
Total Active Chemicals [100%]					
False Positive Active Chemicals					
sodium chloride			I	6.53	6.53
sucrose			I	5.73	5.73
acetone			I	4.49	4.49
dimethyl sulfoxide			I	3.38	3.45
glycerol			I	3.09	3.09
ethanol			I	2.50	2.50
urea			I	1.81	1.81

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Table A6. *Continued.*

[a]Test Chemical: The 54 noncytotoxic chemicals in Table A6 are idenical to those in Table A4, and they are subdivided into groups of 21 carcinogens, 26 noncarcinogens and 7 model very noncytotoxic chemicals.

[b]Rodent Bioassay Level of Activity: The relative carcinogenic activity of chemicals in rodent bioassay has been described in terms of the chemical's level of effect (*1,3*). The highest level A corresponds to chemicals that cause cancer in both mice and rats at one or more sites, and level B refers to chemicals that cause cancer at multiple sites in one species of rodent. Level C includes chemicals carcinogenic at one site in both sexes of one species, and D includes chemicals carcinogenic at one site in only one sex of a single species. Level E includes chemicals that only equivocal evidence of carcinogenic activity. Finally, level F includes both noncarcinogens and chemicals that had inadequate carcinogenicity studies.

[c]Transformation Response Rank *t*-statistic: The method used to caIculate the significance of test chemical transformation responses employed SAS statistical software (*22*) and is described in detail in Materials and Methods. The correct *t*-statistics of each treatment dose of the test chemical in a single experiment are presented in the Appendices B-H, and these *t*-statistics were averaged to determine the mean t-statistic of the test chemical for the experiment (refer to Table A2). The mean *t*-statistics for two or experiments for each chemical was weighted according to the number of treatment doses evaluated and averaged to determine the actual rank *t*-statistic presented in this table. For example, the actual rank *t*-statistic of D&C red no. 9 in experiments 43, 54 and 67 is equal to 4.33 [i.e., 10.9 + 10.5 + 7.16 + 5.26 (exp. 43) + 2.96 + 1.39 + .04 + .00 (Exp. 54) + 5.47 + 3.37 + 3.07 + 1.78 (exp. 67); Appendix F].

[d]Estimated Rank *t*-statistic: The estimated rank *t*-statistic is used to estimate both the historical behavior of the test chemical in the transformation assay, as well as predicting the future behavior or the chemical. It is calculated by correcting the actual rank *t*-statistic. The data presented in Table A5 showed that individual experiments had very different rank-ordered sensitivities to detect chemical-induced transformation. Therefore, the estimated rank *t*-statistic modified the actual rank *t*-statistic to correct for differences in the sensitivities of individual experiments. The method uses the rank ordered sensitivity of individual experiments to detect spontaneous and BaP-induced transformation, and an example calculation is provided below.

The most active mutagenic carcinogen. D&C red no. 9, had statistical sensitivities for spontaneous transformation responses of 30, 91 and 107/110 for experiments 43, 54 and 67, respectively, and detection sensitivities for BaP of 4, 85 and 106/110 for the same experiments. The average rank order of the three experiments was 70.5 (i.e., 30 + 91 + 107 + 4 + 85 + 106/110 = 78.5). For a total of 110 experiments, the median experiment has an automatic average rank order of 55.0 (i.e. 110/2 = 55.0). Therefore, the correction factor for the experimental sensitivity to detect chemical-induced transformation was 70.5/55.0 or 1.28.

Thus, these two experiments had a combined statistical sensitivity and detection sensitivity that was above the median of 55.0. The actual rank *t*-statistic was multiplied by the correction factor to obtain the estimated rank *t*-statistic (i.e., 5.50). A justification for this correction factor has been reported (*18*), and it is explained in the Materials and Methods.

[e]Percentage (%) of Active Chemicals: Active chemicals included chemicals with active and weakly active transformation responses. In contrast, inactive chemicals included chemicals with equivocal and inactive transformation responses. Chemicals with an indeterminate activity have to be retested in an additional experiment in order to determine their activity in the standard transformation assay. Therefore, chemicals with indeterminate transformation responses were omitted from the computation of the percentage (%) of the total chemicals that were either active or inactive in the assay.

Dimethylnitrosamine. Dimethylnitrosamine is a level *A* carcinogen (Table A6). It had one difficult technical problem. It was reported to be oxidized upon exposure to air, and it was exposed to air during the standard treatment period (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 256 mM (Table A4). The statistical sensitivities of transformation assay trials 1 and 2 were 42 and 73/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 25 and 49/110, respectively (Table A5). The test chemical had an SP transformation response in the two consecutive experiments. Dimethylnitrosamine was evaluated as active in the transformation assay, and its actual and estimated rank *t*-statistics were both 4.45 (Table A6).

Phenobarbital, Sodium Salt. Phenobarbital, sodium salt, is a level *A* carcinogen (Table A6). It had one difficult technical problem. It was reported to be oxidized by air, and it was exposed to air during the standard treatment period (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 6.11 mM (Table A4). The statistical sensitivities of transformation assay trials 1 and 2 were 75 and 6/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 76 and 92/110, respectively (Table A5). The test chemical had an SP transformation response in the two consecutive experiments. Phenobarbital was evaluated as active in the transformation assay, and its actual and estimated rank *t*-statistics were 2.77 and 3.14, respectively (Table A6).

2,4- and 2,6-Toluene Diisothiocyanate. 2,4- and 2,6-Toluene diisothiocyanate is a level *A* carcinogen (Table A6). It had three difficult technical problems. It was reported to react with strong bases such as NaOH, and stock solutions were acidic and had to be neutralized before testing. It was also reported to react with water, and treatments were performed in an aqueous environment. Finally, it was noted to react with amines; thus, it could have reacted with the amine portion of biochemicals in culture medium, as well as in the target BALB/c-3T3 cells (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 7.93 mM (Table A4). The statistical sensitivities of transformation assay trials 1 and 2 were 12 and 28/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 98 and 15/110, respectively (Table A5). The test chemical had an SP transformation response in the two consecutive experiments. 2,4 and 2,6-Toluene diisothiocyanate was evaluated as active in the transformation assay, and its actual and estimated rank *t*-statistics were both 3.01 (Table A6).

Noncytotoxic, Nonmutagenic Carcinogens

3-Amino-1,2,4-Triazole. 3-Amino-1,2,4-triazole is a level *A* carcinogen (Table A6). It had one difficult technical problem. It was reported to bind metals; thus, it could have bound metals contained in FBS and EMEM medium (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 109 mM (Table A4). The statistical sensitivities of transformation assay trials 1 and 2 were 85 and 33/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 102 and 14/110, respectively (Table A5). The test chemical had an SP transformation response in the two consecutive experiments. 3-Amino-1,2,4-triazole was evaluated as active in the transformation assay, and its actual and estimated rank *t*-statistics were 6.14 and 6.53, respectively (Table A6).

11-Aminoundecanoic Acid. 11-Aminoundecanoic acid is a level *B* carcinogen (Table A6). It had no insurmountable technical problems; however, it had a limited solubility of 1500 μg/ml in culture medium supplemented with pluronic F68 (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 19.4 mM (Table A4). The statistical sensitivities of transformation assay trials 1-4 were 83, 89,19 and 107/110, respectively; the detection sensitivities for BaP of trials 1-4 were 70, 87, 34 and 106/110, respectively (Table A5). The test chemical had a LN transformation response in the first two experiments because it was not tested at cytotoxic treatment doses. In contrast, it had an SP transformation response in the last two experiments. Significant transforming activity was only detected at treatment doses that were slightly above the solubility limit of the test chemical in culture medium. 11-Aminoundecanoic acid was evaluated as active in the transformation assay, and its actual and estimated rank *t*-statistics were 2.11 and 2.55, respectively (Table A6).

Cyclamate, Sodium Salt. Cyclamate, sodium salt, is a level *A* carcinogen (Table A6). It had one difficult technical problem. It was reported to bind potassium salts; thus, it could have bound the potassium in culture medium (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 132 mM (Table A4). The statistical sensitivities of transformation assay trials 1 and 2 were 24 and 5/110, respectively, the detection sensitivities for BaP of trials 1 and 2 were 10 and 46/110, respectively (Table A5). The test chemical had an SP transformation response in the two consecutive experiments. Cyclamate was evaluated as active in the transformation assay, and its actual and estimated rank *t*-statistics were both 4.09 (Table A6).

Decabromodiphenyloxide. Decabromodiphenyloxide is a level *C* carcinogen (Table A6). It had one difficult technical problem. It had a limited solubility in culture medium of 250 μg/ml (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 6.26 mM (Table A4). Thus, the test chemical LD_{50} was about 24-fold higher than its solubility limit in culture medium. The statistical sensitivities of transformation assay trials 1 and 2 were 21 and 62/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 26 and 48/110, respectively (Table A5). The test chemical had an LN transformation response in the two consecutive experiments. It was tested at treatment doses that far exceeded its solubility limit in culture medium, but these doses were not cytotoxic to the target cells. Taken together, decabromodiphenyloxide was evaluated as both inactive and indeterminate in the transformation assay, and its actual and estimated rank *t*-statistics were both 0.29 (Table A6).

Di(2-ethylhexyl)adipate. Di(2-ethylhexyl)adipate is a level *C* carcinogen (Table A6). It had one difficult technical problem. Its solubility limit in culture medium supplemented with pluronic F68 was only 1000 nl/ml (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 98.4 mM (Table A4). Thus, this LD_{50} far exceeded the solubility limit of the test chemical. The statistical sensitivities of transformation assay trials 1 and 2 were 57 and 20/110 respectively; the detection sensitivities for BaP of trials 1 and 2 were 88 and 19/110, respectively (Table A5). The test chemical had an SN transformation response in the two consecutive experiments. The test chemical was tested using treatment doses that were both above and below its solubility limit. Di(2-ethylhexyl)adipate was evaluated as inactive in the transformation assay, and its actual and estimated rank *t*-statistics were both 0.00 (Table A6).

Di(2-ethylhexyl)phthalate. Di(2-elhylhexyl)phthalate is a level *A* carcinogen (Table A6). It had no insurmountable technical problems, and its solubility limit in culture medium supplemented with pluronic F68 was 12000 nl/ml (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 21.4 mM (Table A4). The statistical sensitivities of transformation assay trials 1 and 2 were 74 and 73/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 35 and 49/110, respectively (Table A5). The test chemical had an SN transformation response in the two consecutive experiments. Di(2-ethylhexy1)phthalate was evaluated as active in the transformation assay, and its actual and estimated rank *t*-statistics were both 0.00 (Table A6).

Dimethyl Hydrogen Phosphite. Dimethyl hydrogen phosphite is a level *B* carcinogen (Table A6). It had two difficult technical problems. It was a very acidic test chemical, and stock solutions had to be neutralized with NaOH. Unfortunately, the test chemical was reported to react with strong bases and with water; thus, it could have been altered during the treatment period (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 130 mM (Table A4). Since it required an equal molar concentration of NaOH to neutralize the test chemical, this LD_{50} was actually equal to 260 mOsM. The statistical sensitivities of transformation assay trials 1 and 2 were 44 and 26/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 65 and 93/110, respectively (Table A5). The test chemical had an SP transformation response in the two consecutive experiments; however, significant transforming activity was detected at treatment doses that exceeded the upper dose limit of the assay of 100 mOsM. Taken together, dimethyl hydrogen phosphite was evaluated as active and a false positive in the transformation assay. Its actual and estimated rank *t*-statistics were 5.97 and 6.19, respectively (Table A6).

Dimethyl Methyl Phosphonate. Dimethyl methyl phosphonate is a level *D* carcinogen (Table A6). It had two difficult technical problems. It was reported to react with air, and it was exposed to air during the treatment period. In addition, it was noted to be an alkylating agent and reacted with basic nitrogen compounds. Thus, this test chemical could have reacted not only with biochemicals in culture medium, but also with biochemicals in the target cells (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 172 mM (Table A4). The statistical sensitivities of transformation assay trials 1 and 2 were 44 and 27/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 65 and 63/110, respectively (Table A5). The test chemical had an LA transformation response in the first experiment, and an SP transformation response in the second experiment. Significant transforming activity for this test chemical was detected using treatment doses that exceeded the upper dose limit of the assay of 100 mOsM. Taken together, dimethyl methyl phosphonate was evaluated as active and a false positive in the transformation assay. Its actual and estimated rank *t*-statistics were both 2.90 (Table A6).

Dimethylmorpholinophosphoramidate. Dimethylmorpholinophosphoramidate is one of five chemicals that was tested as a coded and as a uncoded test chemical in this investigation. It is a level C carcinogen (Table A6), and it had no insurmountable technical problems (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells, and the uncoded and coded test chemicals had an average LD_{50} of 17.1 and 24.4 mM, respectively (Table A4). For the uncoded test chemical the statistical sensitivities of transformation assay trials 1 and 2 were 52 and 28/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 79 and 15/110, respectively (Table A5). For the coded test chemical the statistical sensitivities of transformation assay trials 1 and 2 were 52 and 20/110; the detection sensitivities for BaP of trials 1 and 2 were 79 and 19/110, respectively. Both the uncoded and coded test chemical had an SP transformation response in the two consecutive experiments. Dimethylmorpholinophosphoramidate was evaluated as active in the transformation assay. The actual and estimated rank *t*-statistics for the uncoded test chemical were both 3.19; the actual and estimated rank *t*-statistics for the coded test chemical were both 4.23 (Table A6). Taken together, the coded and uncoded test chemicals had virtually identical cytotoxic and transforming activities in the BALB/c-3T3 cell transformation assay.

Ethylene Thiourea. Ethylene thiourea is a level *A* carcinogen (Table A6). It had no insurmountable technical problems (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 91.4 mM (Table A4). The statistical sensitivities of transformation assay trials 1 and 2 were 75 and 95/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 76 and 61/110, respectively (Table A5). The test chemical had an LN transformation response in the first experiment and an SP transformation response in the second experiment. Ethylene thiourea was evaluated as weakly active in the transformation assay, and its actual and estimated rank *t*-statistics were 2.84 and 3.96, respectively (Table A6).

Hexamethylphosphoramide. Hexamethylphosphoramide is a level *C* carcinogen (Table A6). It had no insurmountable technical problems (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 64.4 mM CTable A4). The statisti-

cal sensitivities of transformation assay trials 1 and 2 were 9 and 59/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 37 and 17/110, respectively (Table A5). The test chemical had an SP transformation response in the two consecutive experiments. Hexamethylphosphoramide was evaluated as active in the transformation assay, and its actual and estimated rank *t*-statistics were both 2.08 (Table A6).

Melamine. Melamine is a level *D* carcinogen (Table A6). It had one difficult technical problem, because its solubility limit in culture medium supplemented with pluronic F68 was about 1000 μg/ml (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 39.6 mM (Table A4). Thus, this LD_{50} far exceeded the solubility limit of the test chemical in culture medium. The statistical sensitivities of transformation assay trials 1 and 2 were 30 and 97/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 4 and 94/110, respectively (Table A5). The test chemical had an SP transformation response in the first experiment, and an LA transformation response in the second experiment. Melamine was evaluated as active in the transformation assay, and its actual and estimated rank *t*-statistics were 2.10 and 2.15, respectively (Table A6).

Methyl Carbamate. Methyl carbamate is one of five chemicals that was tested as a coded and as a uncoded test chemical in this investigation. It is a level *C* carcinogen (Table A6). It had no insurmountable technical problems (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells, and the uncoded and coded test chemicals had an average LD_{50} of 225 and 195 mM, respectively (Table A4). For the uncoded test chemical the statistical sensitivities of transformation assay trials 1-3 were 55, 108 and 10/110, respectively; the detection sensitivities for BaP of trials 1-3 were 8, 99 and 5/110, respectively (Table A5). For the coded test chemical the statistical sensitivities of transformation assay trials 1 and 2 were 71 and 33/110; the detection sensitivities for BaP of trials 1 and 2 were 23 and 14/110, respectively. The uncoded test chemical had two SP and a LA transformation responses in three experiments. The coded test chemical had an SP transformation response in the two consecutive experiments. Methyl carbamate was evaluated as active in the transformation assay. The actual and estimated rank *t*-statistics for the uncoded test chemical were both 4.48; the actual and estimated rank *t*-statistics for the coded test chemical were 3.76 (Table A6). Taken together, the coded and uncoded test chemicals had virtually identical cytotoxic and transformation responses in the BALB/c-3T3 cell transformation assay.

Monuron. Monuron is a level *B* carcinogen (Table A6). It had one difficult technical problem. It had a limited solubility in culture medium supplemented with pluronic F68 of about 25 μg/ml (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 5.54 mM (Table A4). Thus, this LD_{50} far exceeded the solubility limit of the test chemical in culture medium. The statistical sensitivities of transformation assay trials 1 and 2 were 81 and 39/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 22 and 68/110, respectively (Table 2). The test chemical had an LA transformation response in the first experiment and an SN transformation response in the second experiment. Monuron was evaluated as inactive in the transformation assay, and its actual and estimated rank *t*-statistics were both 1.01 (Table A6).

Saccharin, Sodium Salt. Saccharin, sodium salt, is a level *A* caranogen (Table A6). It had no insurmountable technical problems (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 76.5 mM and 153 mOsM (Table A4). The statistical sensitivities of transformation assay lrials 1 and 2 were 21 and 62/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 26 and 48/110, respectively (Table A5). The test chemical had an SP transformation response in the two consecutive experiments. Significant transforming activity was detected at treatment doses that were below the upper dose limit of the assay of 100 mOsM. Saccharin was evaluated as active in the transformation assay, and its actual and estimated rank *t*-statistics were both 7.99 (Table A6).

Noncytotoxic, Mutagenic Noncarcinogens

2-Chloroethanol. 2-Chloroethanol is a level *F* noncarcinogen (Table A6). It had one difficult technical problem. It was reported to react with water; thus, it may have been altered by the aqueous environment during the treatment period (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 81.0 mM (Table A4). The statistical sensitivities of transformation assay trials 1 and 2 were 9 and 73/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 37 and 49/110, respectively (Table A5). The test chemical had an SP transformation response in the both the first and second experiments. 2-Chloroethanol was evaluated as active in the transformation assay, and its actual and estimated rank *t*-statistics were both 3.22 (Table A6).

Methyl Methacrylate. Methyl methacrylate is a level *F* noncarcinogen (Table A6). It had two difficult technical problems. It was reported to react with air; thus, it may have been altered by exposure to air during the treatment period. In addition, it was noted to react with amines; therefore, it may have reacted with amines on biochemicals in culture medium and in the target BALB/c-3T3 cells (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 10.8 mM (Table A4). The statistical sensitivities of transformation assay trials 1 and 2 were 4 and 28/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 1 and 15/110, respectively (Table A5). The test chemical had an SP transformation response in the first experiment and an SN transformation response in the second experiment. Since the mean *t*-statistics of the two transformation responses were not significantly different from one another, the test chemical did not have to be evaluated in a third trial. Methyl methacrylate was evaluated as having been weakly active in the transformation assay, and its actual and estimated rank *t*-statistics were both 2.54 (Table A6).

4-Nitroanthranilic Acid. 4-Nitroanthranilic acid is a level *F* noncarcinogen (Table A6). It had no insurmountable technical problems (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 8.58 mM (Table A4). The statistical sensitivities of transformation assay trials 1 and 2 were 7 and 22/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 56 and 53/110, respectively (Table A5). The test chemical had an SN transformation response in the first experiment and an SP transformation response in the second experiment. Because the mean *t*-statistics of the two transformation responses were not significantly different from one another, the test chemical was not evaluated in a third trial. 4-Nitroanthranilic acid was evaluated as having been weakly active in the transformation assay, and its actual and estimated rank *t*-statistics were both 1.09 (Table A6).

Noncytotoxic, Nonmutagenic Noncarcinogens

Aldicarb. Aldicarb is a level *F* noncarcinogen (Table A6). It had no insurmountable technical problems (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 10.7 mM (Table A4). The statistical sensitivities of transformation assay trials 1 and 2 were 19 and 33/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 34 and 14/110, respectively (Table A5). The test chemical had an SN transformation response in the first experiment, and an SP response in the second experiment. Since the mean *t*-statistics of the transformation responses in the two experiments were significantly different from one another, the test chemical has to be evaluated in a third trial. In the absence of the data from a third trial, aldicarb was evaluated as having an indeterminate activity in the transformation assay. Its actual and estimated rank *t*-statistics were both 1.93 (Table A6).

Ampicillin Trihydrate. Ampicillin trihydrate is a level *E* noncarcinogen (Table A6). It had no insurmountable technical problems (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 23.8 mM (Table A4). The statistical sensitivities of transformation assay trials 1 and 2 were 71 and 29/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 23 and 97/110, respectively (Table A5). The test chemical had an LA transformation response in the both the first and second experiments. Ampicillin trihydrate was evaluated as having an equivocal activity in the transformation assay, and its actual and estimated rank *t*-statistics were both 1.32 (Table A6).

o-Anthranilic Acid. *o*-Anthranilic acid is a level *F* noncarcinogen (Table A6). It had one difficult technical problem. It was reported to become oxidized by air; thus, it may have been altered by exposure to air during the treatment (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 72.9 mM (Table A4). The statistical sensitivities of transformation assay trials 1 and 2 were 100 and 36/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 42 and 28/110, respectively (Table A5). The test chemical had an SN transformation response in the first experiment and an SP transformation response in the second experiment. Since the mean *t*-statistics of the two transformation responses were significantly different from one another, the test chemical has to be evaluated in a third trial. In the absence of data from a third trial, *o*-anthranilic acid was evaluated as having an indeterminate activity in the transformation assay. Its actual and estimated rank *t*-statistics were both 2.19 (Table A6).

Benzoin. Benzoin is a level *E* noncaranogen (Table A6). It had one difficult technical problem. The solubility limit of this test chemical in culture medium supplemented with pluronic F68 was about 500 μg/ml (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 14.8 mM (Table A4). Thus, this test chemical had a LD_{50} that far exceeded its solubility in culture medium. The statistical sensitivities of transformation assay trials 1 and 2 were 50 and 109/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 59 and 103/110, respectively (Table A5). The test chemical had an SN transformation response in the both the first and second experiments. The test chemical was tested at treatment doses that far exceeded its solubility limit in culture medium. Benzoin was evaluated as inactive in the transformation assay, and its actual and estimated rank *t*-statistics were 0.74 and 1.08, respectively (Table A6).

Benzyl Alcohol. Benzyl alcohol is a level *F* noncarcinogen (Table A6). It had one difficult technical problem. It was reported to become oxidized by air; thus, it may have been altered by exposure to air during the treatment period (Table A4. The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 17.9 mM (Table A4). The statistical sensitivities of transformation assay trials 1 and 2 were 2 and 38/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 2 and 16/110, respectively (Table A5). The test chemical had an LA transformation response in the first experiment and an SP transformation response in the second experiment. Benyl alcohol was evaluated as active in the transformation assay, and its actual and estimated rank *t*-statistics were both 1.95 (Table A6).

Caprolactam. Caprolactam is a level *F* noncarcinogen (Table A6). It had no insurmountable technical problems (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 71.8 mM (Table A4). The statistical sensitivities of transformation assay trials 1 and 2 were 110 and 109/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 107 and 103/110, respectively (Table A5). Thus, both of these experiments had an unusually low statistical sensitivity and detection sensitivity for BaP. The test chemical had an SN transformation response in the both the first and second experiments. Caprolactam was evaluated as inactive in the transformation assay, and its actual and estimated rank *t*-statistics were 1.20 and 2.34, respectively (Table A6).

(2-Chloroethyl)trimethylammonium Chloride. (2-Chloroethyl)trimethylammonium chloride is a level F non-

carcinogen (Table A6). It had no insurmountable technical problems (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 62.0 mM (Table A4). The statistical sensitivities of transformation assay trials 1 and 2 were 40 and 35/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 54 and 57/110, respectively (Table A5). The test chemical had an SP transformation response in the first experiment and an LA transformation response in the second experiment. (2-Chloroethyl)trimethylammonium chloride was evaluated as active in the transformation assay, and its actual and estimated rank *t*-statistics were both 1.74 (Table A6).

C. I. Acid Orange 10. C. I. Acid orange 10 is a level *F* noncarcinogen (Table A6). It had no insurmountable technical problems (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 26.5 mM (Table A4). The statistical sensitivities of transformation assay trials 1 and 2 were 92 and 22/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 71 and 53/110, respectively (Table A5). The test chemical had an SN transformation response in the both the first and second experiments. C. I. Acid orange 10 was evaluated as inactive in the transformation assay, and its actual and estimated rank *t*-statistics were 0.63 and 0.68, respectively (Table A6)

Dimethyl Terephthalate. Dimethyl terephthalate is a level *E* noncarcinogen (Table A6). It two difficult technical problems. It was reported to be very temperature sensitive and react with water. Because the test chemical was insoluble in water, it had to be sonicated and warmed at 37°C for 30 minutes or more to become a fine particulate suspension in culture medium supplemented with pluronic F68. Its solubility in culture medium was about 125 μg/ml (Table A4). Thus, it is possible that the test chemical was altered during the procedure to solubilize the test chemical as well as the treatment period. The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 15.4 mM (Table A4). The statistical sensitivities of transformation assay trials 1 and 2 were 22 and 5/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 53 and 46/110, respectively (Table A5). The test chemical had an SP transformation response in the first experiment and an LA transformation response in the second experiment. The test chemical was tested at doses that far exceeded its solubility in culture medium. Taken together, dimethyl terephthalate was evaluated as having an indeterminate activity in the transformation assay, because it needs to be retested at treatment doses closer to its solubility limit in culture medium. Furthermore, it must be evaluated using a procedure less likely to alter it while it is being solubilized for testing. Its actual and estimated rank *t*-statistics were both 1.79 (Table A6).

Diphenylhydantoin. Diphenylhydantoin is a level *I* chemical, because its testing in rodent bioassay is incomplete (Table A6). It had no insurmountable technical problems (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 5.02 mM (Table A4). The solubility limit of this test chemical was about 500 μg/ml in culture medium supplemented with pluronic F68. The statistical sensitivities of transformation assay trials 1 and 2 were 84 and 95/I10, respectively; the detection sensitivities for BaP of trials 1 and 2 were 105 and 61/110, respectively (Table A5). The test chemical had an SN transformation response in two consecutive experiments, and it was tested at treatment doses above its solubility limit. Diphenylhydantoin was evaluated as inactive in the transformation assay, and its actual and estimated rank *t*-statistics were both 0.00 (Table A6).

FD and C Yellow No. 6. FD and C Yellow no. 6 is a level *F* noncarcinogen (Table A6). It had no insurmountable technical problems (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 67.7 mM (Table A4). The statistical sensitivities of transformation assay trials 1-3 were 7, 95 and 22/110, respectively; the detection sensitivities for BaP of trials 1-3 were 56, 61 and 53/110, respectively (Table A5). The test chemical had an SP transformation response in the all three experiments. FD and C Yellow No. 6 was evaluated as active in the transformation assay, and its actual and estimated rank *t*-statistics were both 7.65 (Table A6).

D-Mannitol. D-Mannitol is a level *F* noncarcinogen (Table A6). It had no insurmountable technical problems (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} estimated to be over 324 mM (Table A4). The statistical sensitivities of transformation assay trials 1-3 were 46, 35 and 38/110, respectively; the detection sensitivities for BaP of trials 1-3 were 80, 57 and 16/110, respectively (Table A5). The test chemical had an LN transformation response in the first experiment, an LA response in the second experiment, and an SP response in the third experiments. In all three experiments significant transforming activity was only detected at treatment doses that exceeded the upper dose limit of the assay of 100 mOsM. Taken together, D-mannitol was evaluated as active and a false positive in the transformation assay, and its actual and estimated rank *t*-statistics were 3.00 and 1.99, respectively (Table A6).

Molybdenum Trioxide. Molybdenum trioxide is a level *I* noncarcinogen (Table A6). It had one difficult technical problem. It was reported to form polymeric compounds when it was exposed to acids and bases. Since stocks of the test chemical were acidic and had to be neutralized with NaOH in order to tested, it is possible that the test chemical was altered during preparation of the dosing solutions (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 9.38 mM (Table A4). The statistical sensitivities of transformation assay trials 1 and 2 were 61 and 84/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 47 and 105/110, respectively (Table A5). The test chemical had an LA transforrnation response in the first experiment and an SN transformation response in the second experiment. Molybdenum trioxide was evaluated as inactive in the transformation assay, and ils actual and estimated rank *t*-statistics were 0.64 and 0.86, respectively (Table A6).

Penicillin VK+. Penicillin VK+ is a level *F* noncarcinogen (Table A6). It had no insurmountable technical problems (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 17.8 mM

(Table A4). The statistical sensitivities of transformation assay trials 1 and 2 were 10 and 62/110, respectively, the detection sensitivities for BaP of trials 1 and 2 were 5 and 48/110, respectively (Table A5). The test chemical had an SP transformation response in two consecutive experiments. Penicillin VK+ was evaluated as active in the transforrnation assay, and its actual and estimated rank *t*-statistics were both 5.96 (Table A6).

Phthalamide. Phthalamide is a level *F* noncarcinogen (Table A6). It had no insurmountable technical problems (Table A4). The lest chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 73.1 mM (Table A4). This LD_{50} was far above the solubility limit of the test chemical of 1000 μg/ml in culture medium supplemented with pluronic F68. The statistical sensitivities of transformation assay trials 1 and 2 were 14 and 38/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 72 and 16/110, respectively (Table A5). The test chemical had an LN transformation response in the first experiment and an LA transformation response in the second experiment. Phthalamide was evaluated as having an indeterminate activity in the transformation assay, and its actual and estimated rank *t*-statistics were 1.01 and 0.64, respectively (Table A6).

Phthalic Anhydride. Phthalic anhydride is a level *F* noncarcinogen (Table A6). It had three difficult technical problems. It was reported to react with water and strong bases. Because the test chemical stock solutions were very acidic, they had to be neutralized with NaOH; thus, the test chemical may have been altered during the preparation of dosing solutions. In addition, the test chemical was noted to react with amine groups, thus it may have reacted with amine groups on biochemicals in culture medium, as well as biochemicals in the target cells (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 13.2 mM (Table A4). The statlstical sensitivities of transformation assay trials 1 and 2 were 82 and 5/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 18 and 46/110, respectively (Table A5). The test chemical had an LA transformation response in the first experiment and an SN transformation response in the second experiment. Phthalic anhydride was evaluated as inactive in the transformation assay, and its actual and estimated rank *t*-statistics were both 0.80 (Table A6).

Roxarsone. Roxarsone is a level *E* noncarcinogen (Table A6). It had no insurmountable technical problems (Table A4). The test chemical was noncytotoxic to the BALB/c-3I3 cells and had an average LD_{50} of 43.8 mM (Table A4). The statistical sensitivities of transformation assay trials 1 and 2 were 10 and 27/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 5 and 92/110, respectively (Table A5). The test chemical had an SP transformation response in the first experiment and an SN transformation response in the second experiment. Since the mean *t*-statistics of the two test chemical transformation responses were significantly different from one another, it has to be evaluated in a third trial. In the absence of data from a third trial, roxarsone was evaluated as having an indeterminate activity in the transformation assay, and its actual and estimated rank *t*-statistics were both 1.67 (Table A6).

Sodium(2-ethylhexyl) Alcohol Sulfate. Sodium(2-ethylhexyl) alcohol sulfate is a level *F(I)* noncarcinogen (Table A6). It had no insurmountable technical problems (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 12.5 mM (Table A4). The statistical sensitivities of transformation assay trials 1 and 2 were 1 and 6/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 3 and 19/110, respectively (Table A5). The test chemical had an SN transformation response in the first experiment and an SP transformation response in the second experiment. Since the mean *t*-statistics of the two test chemical transformation responses were significantly different from one another, the chemical has to be evaluated in a third trial. In the absence of data from a third experiment, sodium(2-ethylhexyl alcohol sulfate was evaluated as having an indeterminute activity in the transformation assay, and its actual and estimated rank *t*-statistics were both 2.04 (Table A6).

Sulfisoxazole. Sulfisoxazole is a level *F* noncarcinogen (Table A6). It had one difficult technical problem. It was reported to become oxidized upon exposure to air; thus, it may have been altered by exposure to air during the treatment period (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} estimated to be 18.7 mM (Table A4). The statistical sensitivities of transformation assay trials 1 and 2 were 1 and 6/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 3 and 19/110, respectively (Table A5). The test chemical had an LN transformation response in the first experiment and an SP transformation response in the second experiment. Since the LN transformation response did not qualify as one of the two required trials, the test chemical has to be tested in a third experiment. Taken together, sulfisoxazole was evaluated as having an indeterminate activity in the transformation assay, and its actual and estimated rank *t*-statistics were both 1.26 (Table A6).

3-Sulfolene. 3-Sulfolene is a level *F* noncarcinogen (Table A6). It had no insurmountable technical problems (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 117 mM (Table A4). The statistical sensitivities of transformation assay trials 1 and 2 were 49 and 23/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 24 and 11/110, respectively (Table A5). The test chemical had an SP transformation response in two consecutive experiments. 3-Sulfolene was evaluated as having been weakly active in the transformation assay, and its actual and estimated rank *t*-statistics were both 3.24 (Table A6).

Tetrahydrofuran. Tetrahydrofuran is a level *I* chemical because its testing in rodent bioassay is incomplete (Table A6). It had one difficult technical problem because it was reported to react with water (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 90.3 mM (Table A4). The statistical sensitivities of transformation assay trials 1 and 2 were 1 and 28/110, respectively; the detection sensitivities for BaP of

trials 1 and 2 were 3 and 15/110, respectively (Table A5). The test chemical had an SN transformation response in two consecutive experiments. Tetrahydrofuran was evaluated as inactive in the transformation assay, and its actual and estimated rank *t*-statistics were both 0.72 (Table A6).

Titanium Dioxide. Titanium dioxide is a level *F* noncarcinogen (Table A6). It had one difficult technical problem. It was reported to be reduced by metals such as calcium, magnesium, potassium and sodium; thus, it could have been altered by these metals in culture medium. In addition, this test chemical was very insoluble in culture medium supplemented with pluronic F68, and had a solubility limit of about 125 μg/ml (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 12.1 mM (Table A4). Thus, this LD_{50}, far exceeded the solubility limit of the test chemical in culture medium. The statistical sensitivities of transformation assay trials 1 and 2 were 82 and 6/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 12 and 92/110, respectively (Table A5). The test chemical had an LN transformation response in two consecutive experiments, and it was tested at treatment doses that far exceeded its solubility limit. Titanium dioxide was evaluated as an inactive with an indeterminate activity in the transfonnation assay. Its actual and estimated rank *t*-statistics were both 0.00 (Table A6).

Witch Hazel. Witch hazel is a mixture of chemicals that include 15% v/v ethanol, 85% v/v water and some inert ingredients. It has been shown to be a level *F* noncarcinogen (Table A6). It had no insurmountable technical problems (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} estimated to be 540 mOsM (Table A4). The statistical sensitivities of transformation assay trials 1 and 2 were 2 and 38/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 2 and 16/110, respectively (Table A5). The test chemical had an LA transformation response in two consecutive experiments. This test chemical only induced significant transforming activity at treatment doses that exceeded the upper dose limit of the assay of 100 mOsM. Taken together, witch hazel was evaluated as having an equivocal activity and a false positive in the transformation assay. Its actual and estimated rank *t*-statistics were both 1.95 (Table A6).

Very Noncytotoxic Chemicals

Acetone. Acetone has not been evaluated in rodent bioassay, therefore, its level of carcinogenicity is classified as a level *I* chemical (Table A6). It had no insurmountable technical problems (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of about 257 mOsM (Table A4). The statistical sensitivities of transformation assay trials 1 and 2 were 1 and 27/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 3 and 63/110, respectively (Table A5). The test chemical had an LA transformation response in the first experiment, and an SP response in the second experiment. This test chemical induced significant transforming activity only at treatment doses above the upper dose limit of the assay of 100 mOsM. Taken together, acetone was evaluated as active and a false positive in the transfonnation assay. Its actual and estimated rank *t*-statistics were both 4.49 (Table A6).

Dimethyl Sulfoxide. Dimethyl sulfoxide has not been evaluated in rodent bioassay; therefore, its level of carcinogenicity is classified as a level *I* chemical (Table A6). It had no insurmountable technical problems (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 507 mM (Table A4). The statistical sensitivities of transformation assay trials 1 and 2 were 90 and 73/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 13 and 49/110, respectively (Table A5). The test chemical had an LA transformation response in the first experiment, and an SP response in the second experiment. This test chemical induced significant transforming activity only at treatment doses above the upper dose limit of the assay of 100 mOsM. Taken together, dimethyl sulfoxide was evaluated as active and a false positive in the transformation assay. Its actual and estimated rank *t*-statistics were 3.38 and 3.45, respectively (Table A6).

Ethanol. Ethanol has not been evaluated in rodent bioassay; therefore, its level of carcinogenicity is classified as a level *I* chemical (Table A6). It had no insurmountable technical problems (Table A4). The test chemical was noncytotoxic to the BALBtc-3T3 cells and had an average LD_{50} of 429 mM (Table A4). The statistical sensitivities of transformation assay trials 1 and 2 were 2 and 20/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 2 and 19/110, respectively (Table A5). The test chemical had an LA transformation response in the first experiment, and an SP response in the second experiment. This test chemical induced significant transforming activity only at treatment doses above the upper dose limit of the assay of 100 mOsM. Taken together, ethanol was evaluated as active and a false positive in the transformation assay. Its actual and estimated rank *t*-statistics were both 2.50 (Table A6).

Glycerol. Glycerol has not been evaluated in rodent bioassay; therefore, its level of carcinogenicity is classified as a level *I* chemical (Table A6). It had no insurmountable technical problems (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 401 mM (Table A4). The statistical sensitivities of transformation assay trials 1 and 2 were 1 and 20/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 3 and 19/110, respectively (Table A5). The test chemical had an SP transformation response in both the first and second experiments. This test chemical induced significant transforming activity only at treatment doses above the upper dose limit of the assay of 100 mOsM. Taken together, glycerol was evaluated as active and a false positive in the transformation assay. Its actual and estimated rank *t*-statistics were both 3.09 (Table A6).

Sodium Chloride. Sodium chloride has not been evaluated in rodent bioassay; therefore, its level of carcinogenicity is classified as a level *I* chemical (Table A6). It had no insurmountable technical problems (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells

and had an average LD_{50} of about 144 mM (288 mOsM) (Table A4). The statistical sensitivities of transformation assay trials 1-3 were 10, 6 and 106/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 5, 92 and ND/110, respectively (Table A5). The test chemical had an SP transformation response in the first and third experiments, and an SN response in the second experiment. There was no apparent explanation for the absence of test chemical transforming activity in the second experiment. This test chemical induced significant transforming activity only at treatment doses above the upper dose limit of the assay of 100 mOsM. Taken together, sodium chloride was evaluated as active and a false positive in the transformation assay. Its actual and estimated rank *t*-statistics were both 6.53 (Table A6).

Sucrose. Sucrose has not been evaluated in rodent bioassay, therefore, its level of carcinogenicity is classified as a level *I* chemical (Table A6). It had no insurmountable technical problems (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of 240 mM (Table A4). The statistical sensitivities of transformation assay trials 1 and 2 were 62 and 5/110, respectively; the detection sensitivities for BaP of trials 1 and 2 were 48 and 46/110, respectively (Table A5). The test chemical had an SP transformation response in both the first and second experiments. This test chemical induced significant transforming activity only at treatment doses above the upper dose limit of the assay of 100 mOsM. Taken together, sucrose was evaluated as active and a false positive in the transformation assay. Its actual and estimated rank *t*-statistics were both 5.73 (Table A6).

Urea. Urea has not been evaluated in rodent bioassay; therefore, its level of carcinogenicity is classified as a level *I* chemical (Table A6). It had no insurmountable technical problems (Table A4). The test chemical was noncytotoxic to the BALB/c-3T3 cells and had an average LD_{50} of about 254 mM (Table A4). The statistical sensitivity of transfonnation assay trial 1 was 6/110; the detection sensitivity for BaP of trial 1 was 92/110 (Table A5). The test chemical had an SP transformation response in the first experiment. This test chemical induced significant transforming activity only at treatment doses above the upper dose limit of the assay of 100 mOsM. Taken together, urea was evaluated as active and a false positive in the transformation assay. Its actual and estimated rank *t*-statistics were both 1.81 (Table A6).

Appendix B.

Summary of the transformation responses of 43 cytotoxic, mutagenic carcinogens.

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
				Focus Data Type Vessels		Foci/Vessel Focus Type	
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	*t*-statistic
2-Acetylaminofluorene [2AAF, M.W. =223.3]							
Trial 1 [17]							
B(a)P	.000791	.000	52.9	94	(20)	4.43 ***	+ 13.5
B(a)P	.000250	3.54	79.1	86	(20)	3.91 ***	+ 11.6
2AAF	.358	.442	47.4	36	(20)	1.54 ***	+ 5.56
2AAF	.179	3.54	55.6	62	(20)	2.34 ***	+ 5.81
2AAF	.0900	19.5	102.	30	(20)	1.25 ***	+ 4.39
2AAF	.0450	66.8	113.	14	(20)	.473	+ .85
NC-1	Control	100.	100.	18	(40)	.327	Control
							Mean t = 4.73
Trial 2 [24]							
B(a)P	.000791	.000	18.7	83	(20)	3.65 ***	+ 10.7
B(a)P	.000250	5.66	70.0	86	(20)	3.73 ***	+ 10.7
2AAF	.358	.000	34.8	21	(20)	.781*	+ 2.19
2AAF	.179	7.55	47.1	31	(20)	1.18 ***	+ 4.06
2AAF	.0900	23.3	83.3	18	(20)	.686*	+ 2.08
2AAF	.0450	59.7	108.	2	(20)	.072	.00 (-1.01)
NC-1	Control	100.	100.	18	(40)	.308	Control
							Mean t = 2.08
Acrylonitrile [ACRL, M.W. = 53.06, Density = 0.806 g/ml]							
Trial 1 [86]							
B(a)P	.000791	18.7	63.0	64	(18)	3.32***	+ 9.07
B(a)P	.000250	47.9	87.6	42	(18)	2.02***	+ 5.74
ACRL	.608	.000	.000	4	(15,18)	.203	+ 0.84
ACRL	.304	1.17	71.7	79	(18)	3.90***	+ 12.5
ACRL	.152	49.0	83.9	13	(18)	.503	+ 2.28
ACRL	.0760	84.4	94.0	8	(18)	.297	+ 1.31
NC-1	Control	100.	100.	47	(72)	.464	Control
							Mean t = 5.36
Trial 2 [92]							
B(a)P	.00250	.394	45.3	69	(18)	3.54***	+ 8.31
B(a)P	.000791	18.2	72.2	44	(18)	2.14***	+ 5.20
B(a)P	.000250	19.4	78.2	32	(17)	1.52***	+ 3.32
ACRL	.608	.000	.000	0	(15,18)	.000	.00 (-7.93)
ACRL	.456	.000	.000	11	(18)	.479	.00 (- .41)
ACRL	.304	.396	39.3	57	(18)	2.57***	+ 6.43
ACRL	.152	28.5	80.5	10	(18)	.423	.00 (- .90
NC-1	Control	100.	100.	62	(71)	.579	Control
							Mean t = 2.14
2-Amino-4-nitrophenol [847910-S, M.W. = 154.13]							
Trial 1 [63]							
B(a)P	.000791	4.00	80.5	141	(20)	6.13***	+ 6.33
B(a)P	.000250	24.8	93.1	93	(20)	3.20*	+ 2.02
847910-S	2.00	.000	.201	2	(12)	.122	.00 (-7.26)
847910-S	1.00	.000	78.5	2	(9)	.167	.00 (-6.04)
847910-S	.500	9.45	90.6	8	(13)	.696	.00 (-3.61)
847910-S	.250	57.5	93.4	33	(18)	1.63	.00 (- .85)
NC-1	Control	100.	100.	84	(39)	1.92	Control
							Mean t = .00

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Appendix B. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c] Focus Data Type Vessels		Transformation Response[d] Foci/Vessel Focus Type	Significance[e]
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	*t*-statistic
Trial 2 [91]							
B(a)P	.000791	28.9	73.6	60	(20)	2.00***	+ 7.77
B(a)P	.000250	58.1	89.9	14	(20)	.503	+ 2.63
847910-S	1.67	.000	.000	0	(0,20)	.000	.00 (-3.67)
847910-S	1.25	.000	.000	0	(0,20)	.000	.00 (-7.36)
847910-S	.833	2.36	.000	0	(0,20)	.000	.00 (-4.20)
847910-S	.417	54.2	92.5	7	(20)	.256	.00 (-1.48)
NC-1A+1B	Control	100.	100.	31	(75)	.322	Control Mean t = .00
2-Amino-5-Nitrophenol [738717-S, M.W. =154.13]							
Trial 1 [62]							
B(a)P	.000791	5.24	72.5	188	(20)	8.32*	+ 2.50
B(a)P	.000250	18.6	85.1	105	(18)	5.59 NS	.00 (- .62)
738717-S	2.00	.000	32.1	13	(20)	.473	.00 (- 13.6)
738717-S	.632	1.43	26.2	5	(19)	.182	.00 (- 17.0)
738717-S	.200	10.5	78.6	30	(20)	1.26	.00 (- 9.93)
738717-S	.0632	46.2	95.0	96	(20)	4.31	.00 (- 2.54)
NC-1	Control	100.	100.	261	(40)	6.02	Control Mean t = .00
Trial 2 [83]							
B(a)P	.000791	2.86	73.0	141	(20)	6.14***	+ 13.5
B(a)P	.000250	13.8	78.9	64	(20)	2.93***	+ 9.12
738717-S	2.00	.000	20.1	10	(19)	.419	+ .41
738717-S	.632	3.33	42.7	15	(16)	.738	+ 1.89
738717-S	.200	23.3	71.7	5	(14)	.281	.00 (- .39)
738717-S	.0632	31.9	85.1	6	(15)	.294	.00 (- .32)
NC-1A+1B	Control	100.	100.	48	(80)	.351	Control Mean t = .575
Trial 3 [93]							
B(a)P	.000791	2.65	46.7	138	(20)	6.48***	+ 16.8
B(a)P	.000250	7.96	79.9	115	(20)	4.80***	+ 12.0
738717-S	1.33	.000	7.92	38	(20)	1.44***	+ 3.66
738717-S	.667	.000	11.2	50	(20)	2.28***	+ 8.22
738717-S	.333	5.31	16.6	43	(20)	1.73***	+ 4.66
738717-S	.167	8.85	31.7	36	(20)	1.47***	+ 5.10
NC-1A+1B	Control	100.	100.	43	(79)	.416	Control Mean t= 5.41
Trial 4 [103]							
B(a)P	.000791	8.60	76.9	95	(20)	4.59***	+ 13.7
B(a)P	.000250	23.6	91.5	75	(20)	2.86***	+ 5.37
738717-S	1.67	.000	30.3	90	(20)	4.24***	+ 11.3
738717-S	.833	6.45	38.3	115	(20)	4.97***	+ 9.09
738717-S	.417	24.5	32.7	140	(20)	6.79***	+ 18.4
738717-S	.208	38.3	41.5	122	(20)	5.02***	+ 8.57
NC-1A+1B	Control	100.	100.	89	(79)	.874	Control Mean t = 11.8
5-Azacytidine [5AZA, M.W. = 244.2]							
Trial 1 [6]							
B(a)P	.000791	1.71	26.9	141	(20)	6.80***	+ 21.9
B(a)P	.000250	13.7	74.0	69	(20)	3.14***	+ 18.5

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Appendix B. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
Drug	Conc., mM	S.A	CC.A.	Focus Data Type Vessels III	(N)	Foci/Vessel Focus Type III	*t*-statistic
5AZA	.0164	.000	11.1	62	(20)	2.59***	+ 8.36
5AZA	.0123	.000	18.2	70	(20)	2.92***	+ 9.11
5AZA	.00615	4.79	41.0	134	(20)	6.16***	+ 15.3
5AZA	.00308	15.4	ND	316	(20)	15.3***	+ 33.9
NC-1	Control	100.	100.	24	(74)	.431	Control
						Mean t = 14.5	
Trial 2 [11]							
B(a)P	.000791	1.37	34.4	128	(20)	4.94***	+ 10.5
B(a)P	.000250	7.22	36.0	37	(20)	1.62***	+ 5.38
5AZA	.0061	.000	25.8	182	(20)	8.10***	+ 14.7
5AZA	.0031	4.12	73.1	248	(20)	12.0***	+ 16.5
5AZA	.0015	20.9	71.0	52	(20)	1.77***	+ 4.21
5AZA	.0008	27.8	77.4	11	(20)	.423	+ .70
NC-1	Control	100.	100.	21	(40)	.301	Control
						Mean t = 9.03	
Benzidine-2HCl [BENZD, M.W. = 257.18]							
Trial 1 [43]							
B(a)P	.000791	1.00	53.0	382	(20)	18.9***	+ 26.3
B(a)P	.000250	4.80	77.5	270	(20)	13.0***	+ 19.0
BENZD	.194	.000	3.94	42	(18)	2.00**	+ 2.71
BENZD	.146	.000	26.3	64	(17)	3.57***	+ 6.41
BENZD	.0972	2.38	62.1	82	(20)	3.74***	+ 6.79
BENZD	.0486	42.9	70.9	40	(18)	1.85*	+ 2.26
NC-1	Control	100.	100.	44	(35)	1.05	Control
						Mean t = 4.54	
Trial 2 [52]							
B(a)P	.000791	4.05	39.1	150	(20)	6.63***	+ 9.35
B(a)P	.000250	11.1	73.8	126	(20)	5.61***	+ 8.18
BENZD*	.1944	96.3	94.0	37	(20)	1.66	+ 1.73
BENZD*	.1458	98.3	96.3	49	(20)	2.20**	+ 3.04
BENZD*	.0972	98.7	103.	46	(20)	1.95*	+ 2.43
BENZD*	.0486	99.7	110.	36	(20)	1.63	+ 1.66
NC-1	Control	100.	100.	55	(38)	1.09	Control
						Mean t = 2.22	
2-Biphenylamine [2BPA, M.W. =169.22]							
Trial 1 [33]							
B(a)P	.000791	3.44	2.40	214	(20)	10.0***	+ 13.6
B(a)P	.000250	5.73	51.4	130	(20)	5.86***	+ 7.74
2-BPA	.591	.000	7.46	12	(18)	.562	.00 (- 1.97)
2-BPA	.433	3.05	28.5	49	(20)	2.26***	+ 3.66
2-BPA	.296	4.58	53.8	77	(20)	3.33***	+ 4.74
2-BPA	.148	40.1	106.	44	(19)	1.91*	+ 2.10
NC-1	Control	100.	100.	54	(37)	1.04	Control
						Mean t = 2.63	
Trial 2 [52]							
B(a)P	.000791	4.04	39.8	150	(20)	6.63***	+ 9.35
B(a)P	.000250	11.1	75.4	126	(20)	5.61***	+ 8.18
2BPA	.296	8.08	59.0	33	(20)	1.43	+ 1.03
2BPA	.222	36.0	80.3	63	(20)	2.85***	+ 4.40
2BPA	.148	59.6	84.9	66	(20)	3.06***	+ 5.54
2BPA	.074	83.8	95.9	93	(20)	3.21***	+ 4.20
NC-1	Control	100.	100.	55	(38)	1.09	Control
						Mean t = 3.79	

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Appendix B. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
				Focus Data Type Vessels		Foci/Vessel Focus Type	
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	*t*-statistic
4-Biphenylamine [4BPA, M.W. =169.22]							
Trial 1 [35]							
B(a)P	.000791	4.51	66.1	103	(20)	4.91***	+ 6.41
B(a)P	.000250	12.3	87.5	133	(20)	6.01***	+ 6.34
4BPA	.591	.000	23.7	15	(18)	.671	.00 (- 4.00)
4BPA	.443	7.79	70.3	54	(20)	2.51	+ 1.47
4BPA	.295	18.9	86.7	92	(20)	4.05***	+ 3.81
4BPA	.148	40.6	111.	61	(20)	2.63	+ 1.36
NC-1	Control	100.	100.	94	(40)	1.97	Control
						Mean t = 2.21	
Trial 2 [53]							
B(a)P	.000791	4.78	58.9	182	(20)	8.30***	+ 7.68
B(a)P	.000250	7.97	76.8	195	(20)	8.74***	+ 7.74
4BPA	.473	3.59	67.1	96	(20)	4.25**	+ 2.70
4BPA	.355	14.7	72.3	99	(20)	4.59**	+ 3.39
4BPA	.236	42.2	80.0	100	(20)	4.53**	+ 3.25
4BPA	.118	74.9	91.0	68	(20)	3.10	+ .70
NC-1	Control	100.	100.	128	(20)	2.78	Control
						Mean t = 2.51	
3-(Chloromethyl)pyridine [3CMP, M.W. = 164.04]							
Trial 1 [14]							
B(a)P	.000791	1.75	36.1	177	(20)	6.73***	+ 13.3
B(a)P	.000250	5.26	74.7	61	(20)	2.45***	+ 7.12
3CMP	.0732	.000	52.9	10	(20)	.374	+ 1.22
3CMP	.0488	8.33	89.5	3	(20)	.110	.00 (- 1.02
3CMP	.0244	55.7	101.	15	(20)	.611**	+ 2.76
3CMP	.0122	74.1	102.	25	(20)	.542	+ 1.42
NC-1	Control	100.	100.	12	(20)	.213	Control
						Mean t = 1.35	
Trial 2 [22]							
B(a)P	.000791	1.86	53.4	187	(20)	8.86***	+ 16.0
B(a)P	.000250	6.52	81.4	116	(20)	5.25***	+ 9.33
3CMP	.0488	12.4	99.7	52	(20)	2.07**	+ 3.43
3CMP	.0244	59.6	94.3	68	(20)	2.59***	+ 4.41
3CMP	.0122	68.6	84.7	37	(20)	1.65*	+ 2.65
3CMP	.00610	89.8	88.9	31	(20)	1.07	+ .60
NC-1	Control	100.	100.	45	(40)	.893	Control
						Mean t = 2.77	
4-Chloro-*o*-Phenylenediamine [4CPD, NM.W. = 142.59]							
Trial 1 [13]							
B(a)P	.000791	.823	30.6	95	(20)	4.38***	+ 14.2
B(a)P	.000250	2.06	75.4	66	(20)	3.14***	+ 15.7
4CPD	.0245	4.12	85.1	23	(20)	.871**	+ 3.37
4CPD	.0123	25.6	97.9	6	(20)	.214	+ .11
4CPD	.00614	63.3	100.	1	(20)	.035	.00 (- 2.39)
4CPD	.00307	88.5	99.4	2	(20)	.056	.00 (- 1.55)
NC-1	Control	100.	100.	11	(40)	.201	Control
						Mean t = .870	
Trial 2 [20]							
B(a)P	.000791	.000	39.4	268	(20)	13.0***	+ 26.3
B(a)P	.000250	2.34	77.3	69	(20)	4.00***	+ 9.22

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Appendix B. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
				Focus Data Type Vessels		Foci/Vessel Focus Type	
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	t-statistic
4CPD	.0351	3.91	58.2	59	(20)	2.61***	+ 8.16
4CPD	.0245	5.47	82.4	28	(20)	1.03**	+ 2.89
4CPD	.0140	4.69	96.6	9	(20)	.308	.00 (- .37)
4CPD	.00701	21.1	112.	4	(20)	.132	.00 (- 1.71)
NC-1	Control	100.	100.	21	(40)	.368	Control
							Mean t = 2.76
Trial 3 [101]							
B(a)P	.000791	ND	64.8	108		4.63***	+ 12.7
B(a)P	.000250	ND	83.6	48		2.11***	+ 9.73
4CPD	.0526	25.6	35.7	107		5.19***	+ 19.4
4CPD	.0394	27.0	47.1	55		2.37***	+ 8.26
4CPD	.0263	43.3	76.4	38		1.59***	+ 5.83
4CPD	.0131	76.8	90.9	18		.652*	+ 2.19
NC-1	Control	100.	100.	27		.260	Control
							Mean t = 8.92
4-Chloro-*o*-Toluidine-HCl [M.W. = 178.07]							
Trial 1 [81]							
B(a)P	.000791	10.2	63.9	378	(18)	20.8***	+ 15.5
B(a)P	.000250	28.0	67.8	280	(18)	15.3***	+ 10.5
4CT	.842	.000	.516	88	(16,18)	4.05	.00 (- 2.44)
4CT	.632	7.34	92.3	349	(18)	19.2***	+ 14.6
4CT	.421	28.3	102.	209	(18)	11.4***	+ 5.93
4CT	.211	55.9	106.	150	(18)	8.04	+ 1.00
NC-1	Control	100.	100.	583	(72)	7.36	Control
							Mean t = 7.18
Trial 2 [92]							
B(a)P	.00250	.394	45.3	69	(18)	3.54***	+ 8.31
B(a)P	.000791	18.2	72.2	44	(18)	2.14***	+ 5.20
B(a)P	.000250	19.4	78.2	32	(17)	1.52**	+ 3.32
4CT	.786	6.73	33.3	35	(17,18)	1.59**	+ 3.39
4CT	.590	11.9	81.0	47	(18)	2.28***	+ 5.54
4CT	.393	61.4	95.8	31	(18)	1.52***	+ 3.56
4CT	.197	108.	95.5	30	(18)	1.56***	+ 5.28
NC-1	Control	100.	100.	62	(71)	.597	Control
							Mean t = 4.44
5-Chloro-*o*-Toluidine [M.W = 141.61]							
Trial 1 [81]							
B(a)P	.000791	10.2	63.9	378	(18)	20.8***	+ 15.5
B(a)P	.000250	28.0	67.8	280	(18)	15.3***	+ 10.5
5CT	2.26	.000	.344	12	(9,18)	1.03	.00 (- 7.06)
5CT	1.69	14.3	62.8	221	(18)	11.4***	+ 3.71
5CT	1.13	38.5	93.3	288	(18)	15.4***	+ 6.51
5CT	.565	72.7	106.	158	(18)	7.88	+ .54
NC-1	Control	100.	100.	583	(72)	7.36	Control
							Mean t = 3.57
Trial 2 [92]							
B(a)P	.00250	.394	45.3	69	(18)	3.54***	+ 8.31
B(a)P	.000791	18.2	72.2	44	(18)	2.14***	+ 5.20
B(a)P	.000250	19.4	78.2	32	(17)	1.52**	+ 3.32
5CT	2.26	1.19	19.9	19	(17,18)	.682	+ .58
5CT	1.69	7.92	49.5	48	(17)	2.36***	+ 5.43
5CT	1.13	22.2	92.8	78	(17)	3.75***	+ 7.67
5CT	.565	49.9	87.2	53	(18)	1.80**	+ 3.03
NC-1	Control	100.	100.	62	(71)	.597	Control
							Mean t = 5.38

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Appendix B. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
				Focus Data Type Vessels		Foci/Vessel Focus Type	
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	*t*-statistic
C. I. Acid Orange 3 [038399-S, M.W. = 453.51]							
Trial 1 [70]							
B(a)P	.000791	1.24	48.9	11	(3)	3.38***	+ 4.23
B(a)P	.000250	11.8	81.0	19	(5)	3.62***	+ 5.73
038399-S	.222	.000	.000	0	(0,20)	.000	ND
038399-S	.111	.000	38.9	34	(16)	1.69***	+ 4.44
038399-S	.0555	1.86	90.7	13	(18)	.562	+ .20
038399-S	.0278	61.5	97.4	5	(15)	.260	.00 (-1.62)
NC-1A+1B	Control	100.	100.	36	(54)	.526	Control
							Mean t = 1.55
Trial 2 [87]							
B(a)P	.000791	25.1	77.0	59	(20)	2.26***	+ 5.84
B(a)P	.000250	42.2	80.2	34	(20)	1.45***	+ 5.24
038399-S	.178	.000	.000	0	(0,20)	.000	ND
038399-S	.1334	.000	35.9	44	(20)	1.81***	+ 6.11
038399-S	.0890	.000	67.0	37	(20)	1.54***	+ 5.45
038399-S	.0445	13.8	109.	14	(20)	.534	+ 1.13
NC-1	Control	100.	100.	43	(80)	.346	Control
							Mean t = 4.23
C. I. Basic Red 9-HCl [CIBR9, M.W. = 323.83]							
Trial 1 [48]							
B(a)P	.000791	2.28	47.9	148	(20)	7.06***	+ 16.5
B(a)P	.000250	10.1	90.8	63	(20)	2.68***	+ 6.53
CIBR9	.00679	.000	.000	4	(20)	.149	.00 (- 2.98)
CIBR9	.00509	.000	.252	5	(20)	.189	.00 (- 2.53)
CIBR9	.00340	2.61	29.0	5	(20)	.189	.00 (- 2.53)
CIBR9	.00170	32.6	67.3	23	(20)	.861	+ 1.41
NC-1	Control	100.	100.	29	(40)	.537	Control
							Mean t = .353
Trial 2 [66]							
B(a)P	.000791	2.33	52.0	90	(20)	3.92***	+ 14.1
B(a)P	.000250	6.08	98.4	24	(20)	.795**	+ 3.69
CIBR9	.00617	.000	.188	2	(18,20)	.080	+ .39
CIBR9	.00309	44.9	29.7	4	(20)	.149	+ 1.19
CIBR9	.00154	62.8	97.8	1	(20)	.035	.00 (- .40)
CIBR9	.00077	74.3	109.	3	(19)	.116	+ .91
NC-1	Control	100.	100.	3	(38)	.056	Control
							Mean t = .700
Trial 3 [DRI4]							
B(a)P	.000791	ND	ND	ND		ND	
B(a)P	.000250	ND	ND	ND		ND	
CIBR9	.00463	ND	10.6	5	(20)	.189	.00 (- 2.51)
CIBR9	.00309	ND	79.5	12	(20)	.443	.00 (- .94)
CIBR9	.00154	ND	103.	8	(20)	.301	.00 (- 1.73)
NC-1	Control	ND	100.	18	(20)	.668	Control
							Mean t = .000
C. I. Basic Red 9-HCl [947733-S, m.w. = 323.83]							
Trial 1 [73]							
B(a)P	.000791	2.21	71.9	61	(19)	2.60***	+ 8.09
B(a)P	.000250	7.18	88.8	48	(20)	1.58***	+ 4.37

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Appendix B. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
Drug	Conc., mM	S.A	CC.A.	Focus Data Type Vessels III	(N)	Foci/Vessel Focus Type III	*t*-statistic
947733-S	.00375	.000	3.3	12	(20)	.338	+ .39
947733-S	.00250	.000	33.6	7	(20)	.238	.00 (- .25)
947733-S	.00125	30.4	88.6	3	(20)	.110	.00 (- 1.24)
947733-S	.000625	43.6	96.9	7	(20)	.203	.00 (- .50)
NC-1A+1B	Control	100.	100.	45	(79)	.274	Control Mean t = .098
Trial 2 [95]							
B(a)P	.000791	16.0	ND	152	(20)	7.35***	+ 9.48
B(a)P	.000250	33.3	ND	115	(20)	5.27***	+ 3.82
947733-S	.00281	.000	ND	18	(20)	.620	.00 (- 6.44)
947733-S	.00211	.000	ND	38	(19)	1.82	.00 (- 2.39)
947733-S	.00140	15.1	ND	26	(20)	1.07	.00 (- 4.70)
947733-S	.000700	65.2	ND	37	(19)	1.69	.00 (- 2.68)
NC-1A+1B	Control	100.	ND	263	(77)	2.84	Control Mean t = .000
C. I. Disperse Blue 1 [933178-S, M.W. = 268.3]							
Trial 1 [73]							
B(a)P	.000791	2.21	71.9	61	(19)	2.60***	+ 8.09
B(a)P	.000250	7.18	88.8	48	(20)	1.58***	+ 4.37
933178-S	3.70	.000	17.1	20	(20)	.572	+ 1.66
933178-S	1.17	4.42	59.3	18	(20)	.787**	+ 2.93
933178-S	.370	33.1	72.3	36	(20)	1.56***	+ 5.85
933178-S	.117	68.0	100.	22	(20)	.955***	+ 3.67
NC-1A+1B	Control	100.	100.	45	(79)	.274	Control Mean t = 3.53
Trial 2 [97]							
B(a)P	.000791	4.74	78.0	118	(20)	5.02***	+ 12.5
B(a)P	.000250	17.4	104.	52	(20)	2.26***	+ 7.26
933178-S	1.48	3.16	27.9	6	(18)	.260	.00 (- 1.01)
933178-S	.741	9.49	32.6	9	(20)	.282	.00 (- .86)
933178-S	.370	13.4	45.2	13	(18)	.513	+ .55
933178-S	.185	20.2	41.9	14	(18)	.608	+ 1.07
NC-1A+1B	Control	100.	100.	47	(80)	.414	Control Mean t = .405
Trial 3 [107]							
B(a)P	.000791	5.81	47.3	131	(20)	6.09***	+ 5.74
B(a)P	.000250	21.2	75.8	122	(20)	5.53***	+ 4.05
933178-S	1.85	19.1	6.98	11	(20)	.443	.00 (- 7.86)
933178-S	.926	22.2	16.5	22	(20)	.792	.00 (- 6.00)
933178-S	.463	36.6	23.8	5	(20)	.189	.00 (-12.9)
933178-S	.231	44.1	28.2	6	(19)	.226	.00 (- 9.38)
NC-1A+1B	Control	100.	100.	274	(80)	2.95	Control Mean t = .000
C. I. Disperse Blue 1 [933178-S, M.W. = 268.3]							
Trial 1 [73]							
B(a)P	.000791	2.21	71.9	132	(19)	6.38***	+ 13.1
B(a)P	.000250	7.18	88.8	90	(20)	3.20***	+ 5.92
933178-S	3.70	.000	17.1	104	(20)	3.91 ***	+ 7.00
933178-S	1.17	4.42	59.3	86	(20)	3.97 ***	+ 10.4
933178-S	.370	33.1	72.3	122	(20)	5.49 ***	+ 11.8
933178-S	.117	68.0	100.	77	(20)	3.25 ***	+ 6.22
NC-1A+1B	Control	100.	100.	99	(79)	.608	Control Mean t = 8.86

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Appendix B. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
				Focus Data Type Vessels		Foci/Vessel Focus Type	
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	*t*-statistic
Trial 2 [97]							
B(a)P	.000791	4.74	78.0	248	(20)	9.95***	+ 11.3
B(a)P	.000250	17.4	104.	126	(20)	5.14***	+ 7.49
933178-S	1.48	3.16	27.9	46	(18)	2.05 *	+ 2.51
933178-S	.741	9.49	32.6	51	(20)	2.05 *	+ 2.64
933178-S	.370	13.4	45.2	67	(18)	3.51 ***	+ 7.80
933178-S	.185	20.2	41.9	78	(18)	3.45 ***	+ 5.03
NC-1A+1B	Control	100.	100.	118	(80)	1.07	Control
							Mean t = 4.50
Trial 3 [107]							
B(a)P	.000791	5.81	47.3	402	(20)	19.5 ***	+ 8.11
B(a)P	.000250	21.2	75.8	330	(20)	16.0 ***	+ 6.00
933178-S	1.85	19.1	6.98	594	(20)	27.8 ***	+ 15.3
933178-S	.926	22.2	16.5	650	(20)	32.0 ***	+ 16.2
933178-S	.463	36.6	23.8	160	(20)	7.07	.00 (- 1.63)
933178-S	.231	44.1	28.2	143	(19)	7.31	.00 (- 3.00)
NC-1A+1B	Control	100.	100.	929	(80)	9.56	Control
							Mean t = 7.88
C. I. Disperse Yellow 3 [DY3, M.W. = 269.31]							
Trial 1 [71]							
B(a)P	.000791	4.48	50.7	251	(20)	11.0***	+ 12.1
B(a)P	.000250	18.1	68.7	77	(20)	3.50***	+ 7.12
DY3	1.423	.000	41.8	19	(20)	.772	.00 (- 1.030
DY3	.450	.000	92.4	12	(20)	.423	.00 (- 2.53)
DY3	.142	.000	89.3	15	(17)	.340	.00 (- 2.60)
DY3	.045	14.3	104.	3	(20)	.094	.00 (- 6.70)
NC-1	Control	100.	100.	110	(75)	1.06	Control
							Mean t = .000
Trial 2 [91]							
B(a)P	.000791	28.9	73.6	60		2.00***	+ 5.11
B(a)P	.000250	58.1	89.9	14		.503	+ 1.31
DY3	1.577	21.7	79.3	8		.301	.00 (- .17)
DY3	.788	29.2	87.4	11		.402	+ .62
DY3	.394	41.4	89.1	1		.035	.00 (- 4.52)
DY3	.197	66.7	93.4	2		.072	.00 (- 3.31)
NC-1	Control	100.	100.	31		.322	Control
							Mean t = .155
Cytembena [CYTB, M.W. = 307.09]							
Trial 1 [70]							
B(a)P	.00079	1.24	48.9	11	(3)	3.38***	+ 4.23
B(a)P	.00025	11.8	81.0	19	(5)	3.62***	+ 5.73
CYTB	.325	.000	.000	0	(9,16)	.000	.00 (- 7.39)
CYTB	.243	.000	2.25	7	(15)	.356	.00 (- .98)
CYTB	.162	4.97	29.9	88	(18)	4.48***	+ 11.2
CYTB	.081	19.9	89.7	42	(20)	1.11	+ 1.83
NC-1	Control	100.	100.	36	(54)	.526	Control
							Mean t = 4.34
Trial 2 [83]							
B(a)P	.00079	2.86	73.0	141	(20)	6.14***	+ 13.5
B(a)P	.00025	13.8	78.9	64	(20)	2.93***	+ 9.12
CYTB	.243	.000	8.74	21	(16)	.979**	+ 2.76
CYTB	.183	.000	39.1	92	(19)	4.35***	+ 11.2
CYTB	.122	5.71	73.3	119	(20)	5.18***	+ 12.1
CYTB	.061	20.5	94.1	20	(20)	.808*	+ 2.41
NC-1	Control	100.	100.	48	(80)	.351	Control
							Mean t = 7.12

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Appendix B. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
Drug	Conc., mM	S.A	CC.A.	Focus Data Type Vessels III	(N)	Foci/Vessel Focus Type III	*t*-statistic
C. I. Solvent Yellow 14 [CISY14, M.W. = 248.30]							
Trial 1 [7]							
B(a)P	.000791	1.52	48.4	212	(20)	10.0***	+ 26.6
B(a)P	.000250	3.41	56.9	41	(19)	1.80***	+ 7.07
CIS	.242	6.06	70.4	13	(19)	.535*	+ 2.69
CIS	.121	13.6	66.5	16	(19)	.739***	+ 4.72
CIS	.0605	19.7	66.2	16	(19)	.713***	+ 4.33
CIS	.0303	20.1	75.8	12	(19)	.480*	+ 2.34
NC-1	Control	100.	100.	7	(36)	.135	Control
						Mean t = 3.52	
Trial 2 [67]							
B(a)P	.000791	5.87	34.4	48	(20)	2.07***	+ 8.71
B(a)P	.000250	20.8	63.9	39	(20)	.969**	+ 3.32
CISY14	.242	34.9	73.9	16	(20)	.634***	+ 3.79
CISY14	.161	52.5	80.7	26	(20)	.927***	+ 4.18
CISY14	.0805	52.8	79.5	28	(20)	1.09***	+ 5.23
CISY14	.0403	56.5	83.4	21	(20)	.888***	+ 5.34
NC-1*	Control	100.	100.	5	(39)	.085	Control
						Mean t = 4.64	
Trial 3 [IP17]							
B(a)P	.000791	.000	ND	100	(15)	5.28***	+ 8.30
B(a)P	.000250	3.1	ND	84	(15)	5.27***	+ 11.4
CISY14	.201	.000	ND	15	(15)	.838	+ 1.63
CISY14	.101	.5	ND	21	(15)	1.12*	+ 2.36
CISY14	.0503	.5	ND	19	(14)	1.16*	+ 2.50
NC-1	Control	100.	ND	20	(29)	.411	Control
						Mean t = 2.16	
Trial 4 [IP18]							
B(a)P	.000791	1.33	3.77	110	(12)	8.86***	+ 20.5
B(a)P	.000250	2.67	22.1	82	(12)	6.31***	+ 10.7
CISY14	.242	1.71	10.6	6	(12)	.348	+ .98
CISY14	.161	5.15	14.7	12	(12)	.861***	+ 3.68
CISY14	.0805	11.2	35.7	25	(12)	1.73***	+ 4.82
CISY14	.0403	22.3	58.1	19	(12)	1.32***	+ 4.23
NC-1**	Control	100.	100.	6	(24)	.189	Control
						Mean t = 3.43	
1,3-Dibromo-3-Chloropropane [DBCP, M.W. = 236.35, Density = 2.093 g/ml]							
Trial 1 [23]							
B(a)P	.000791	.000	61.0	157	(18)	6.71***	+ 9.25
B(a)P	.000250	4.84	100.	57	(18)	3.04***	+ 8.07
DBCP	.213	.000	98.2	14	(18)	.572	.00 (- .37)
DBCP	.159	2.42	85.6	19	(18)	.937	+ 1.17
DBCP	.106	6.06	93.1	16	(18)	.698	+ .16
DBCP	.0531	39.4	95.4	20	(18)	.590	.00 (- .26)
NC-1	Control	100.	100.	23	(27)	.661	Control
						Mean t = .333	
Trial 2 [27]							
B(a)P	.000791	4.04	33.7	170	(18)	8.90***	+ 14.8
B(a)P	.000250	13.0	47.4	73	(18)	3.18***	+ 5.71
DBCP	.531	.000	36.9	163	(18)	8.21***	+ 12.0
DBCP	.398	1.24	67.0	111	(18)	5.51***	+ 9.35
DBCP	.266	9.94	81.1	36	(18)	1.64***	+ 3.26
DBCP	.133	35.7	70.2	17	(18)	.765	+ .83
NC-1	Control	100.	100.	31	(36)	.555	Control
						Mean t = 6.36	

[e] Trial was conducted in 100mm culture dishes.

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Appendix B. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c] Focus Data Type Vessels		Transformation Response[d] Foci/Vessel Focus Type	Significance[e]
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	*t*-statistic
Trial 3 [102]							
B(a)P	.000791	9.64	69.9	99	(18)	4.65***	+ 9.48
B(a)P	.000250	19.3	93.1	45	(18)	2.22***	+ 5.22
DBCP	.708	.000	1.33	9	(18)	.392	.00 (- 1.65)
DBCP	.531	.000	7.73	51	(18)	2.27***	+ 4.97
DBCP	.354	1.68	34.7	109	(18)	5.09***	+ 9.56
DBCP	.177	13.4	70.1	47	(18)	2.14***	+ 4.77
NC-1	Control	100.	100.	64	(72)	.697	Control
							Mean t = 4.83
2,6-Dichloro-*p*-Phenylenediamine [26DCPD, M.W. = 177.]							
Trial 1 [32]							
B(a)P	.000791	10.1	51.7	197	(19)	10.1***	+ 12.6
B(a)P	.000250	6.37	77.4	115	(20)	5.46***	+ 6.94
26DCPD	1.130	.000	39.5	68	(20)	2.96	+ 1.91
26DCPD	.847	1.87	62.7	86	(20)	4.07***	+ 4.63
26DCPD	.565	4.12	76.0	101	(20)	4.05**	+ 3.41
26DCPD	.282	41.6	86.3	38	(20)	1.55	+ .00 (- 1.08)
NC-1	Control	100.	100.	91	(38)	1.99	Control
							Mean t = 2.50
Trial 2 [54]							
B(a)P	.000791	1.47	17.6	105	(20)	4.75***	+ 13.8
B(a)P	.000250	.490	61.1	100	(20)	4.52***	+ 13.5
26DCPD	1.13	.000	35.8	17	(20)	.677*	+ 2.51
26DCPD	.847	2.94	55.7	21	(20)	.845**	+ 3.27
26DCPD	.565	4.41	90.5	11	(20)	.443	+ 1.25
26DCPD	.282	34.9	89.5	11	(20)	.423	+ 1.08
NC-1	Control	100.	100.	15	(40)	.265	Control
							Mean t = 2.03
1,3-Dichloropropene [13DCP, M.W. = 110.98, Density = 1.217 g/ml]							
Trial 1 [79]							
B(a)P	.000791	22.7	79.6	279	(13)	20.8***	+ 13.9
B(a)P	.000250	39.3	94.2	241	(18)	12.9***	+ 9.34
13DCP	.308	.000	59.0	263	(18)	13.9***	+ 8.91
13DCP	.231	.000	91.8	194	(18)	9.08***	+ 3.53
13DCP	.154	.413	96.6	93	(18)	4.74	.00 (- .48)
13DCP	.0770	40.1	108.	80	(18)	4.01	.00 (- 1.49)
NC-1	Control	100.	100.	430	(72)	5.12	Control
							Mean t = 3.11
Trial 2 [94]							
B(a)P	.000791	.000	75.7	122	(18)	5.92***	+ 6.26
B(a)P	.000250	17.4	114.	81	(18)	3.88***	+ 4.08
13DCP	.330	.000	.000	11	(11,18)	.753	.00 (- 1.80)
13DCP	.247	.000	63.2	33	(18)	1.41	.00 (- .26)
13DCP	.165	.000	132.	37	(18)	1.47	.00 (- .12)
13DCP	.0824	.000	155.	21	(18)	.634	.00 (- 2.58)
NC-1	Control	100.	100.	150	(71)	1.52	Control
							Mean t = .000
Trial 3 [104]							
B(a)P	.000791	25.0	64.5	62	(18)	2.79***	+ 4.87
B(a)P	.000250	50.6	89.6	63	(18)	2.47***	+ 4.10

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Appendix B. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c] Focus Data Type Vessels		Transformation Response[d] Foci/Vessel Focus Type	Significance[e]
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	*t*-statistic
13DCP	.384	.000	23.1	16	(14,18)	.669	.00 (- .73)
13DCP	.288	.000	98.2	51	(17)	2.36***	+ 3.84
13DCP	.192	.000	96.4	26	(18)	1.27	+ 1.41
13DCP	.0960	20.6	86.4	17	(18)	.655	.00 (- .88)
NC-1	Control	100.	100.	83	(71)	.878	Control
							Mean t = 1.75
Diglycidyl Resorcinol Ether [DIG, M.W. = 222.26, Density = 1.21 g/ml]							
Trial 1 [6]							
B(a)P	.000791	1.71	30.6	175	(18)	9.16***	+ 21.9
B(a)P	.000250	13.7	85.9	101	(18)	5.42***	+ 18.5
DIG	.00953	2.02	.000	0	(2,18)	.000	.00 (- 7.42
DIG	.00626	3.63	.588	31	(7,18)	1.51	+ 1.25
DIG	.00408	5.24	19.4	230	(18)	11.8***	+ 20.1
DIG	.00218	17.7	80.0	71	(18)	3.49***	+ 10.3
NC-1	Control	100.	100.	18	(74)	.348	Control
							Mean t = 15.2
Trial 2 [12]							
B(a)P[@]	.000791	90.7	99.0	61	(19)	2.90***	+ 13.1
B(a)P	.000250	77.6	114.	81	(17)	3.94***	+ 10.5
DIG	.00544	.000	2.20	63	(17)	3.45***	+ 15.0
DIG	.00408	.000	18.9	46	(11)	3.45***	+ 6.71
DIG	.00272	.000	70.4	17	(14)	.842*	+ 2.79
DIG	.00136	41.1	104.	9	(18)	.392*	+ 2.14
NC-1	Control	100.	100.	8	(40)	.160	Control
							Mean t = 6.66
Dichlorvos [DCV, M.W. = 220.98, Density = 1.415 g/ml]							
Trial 1 [68]							
B(a)P	.000791	3.60	51.9	91	(18)	4.63***	+ 3.24
B(a)P	.000250	4.50	77.2	37	(18)	1.73***	+ 15.0
DCV	.181	.000	.000	4	(13,18)	.211	.00 (- .11)
DCV	.0905	16.7	83.2	11	(18)	.433	+ 1.38
DCV	.0452	50.0	101.	2	(18)	.080	.00 (- 1.43)
DCV	.0226	73.9	101.	4	(18)	.167	.00 (- .53)
NC-1	Control	100.	100.	11	(36)	.226	Control
							Mean t = .460
Trial 2 [98]							
B(a)P	.000791	8.38	79.6	132	(18)	6.82***	+ 11.8
B(a)P	.000250	29.3	91.3	75	(18)	3.38***	+ 6.81
DCV	.181	.000	.000	0	(1,18)	.000	.00 (- .92)
DCV	.136	.000	.186	7	(16,18)	.330	.00 (- 1.38)
DCV	.0905	9.42	52.1	9	(18)	.370	.00 (- 1.21)
DCV	.0452	67.0	83.5	9	(18)	.326	.00 (- 1.43)
NC-1	Control	100.	100.	39	(45)	.618	Control
							Mean t = .000
Trial 3 [DRI5]							
B(a)P	.000791	NA	NA				
B(a)P	.000250	NA	NA				
DCV	.136	NA	16.6	187	(18)	9.84**	+ 8.82
DCV	.0905	NA	79.0	47	(18)	2.20	+ 1.71
DCV	.0452	NA	98.3	6	(18)	.29	.00 (- 2.88)
NC-1	Control	NA	100.	46	(18)	1.27	Control
							Mean t = 3.51

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Appendix B. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
				Focus Data Type Vessels		Foci/Vessel Focus Type	
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	*t*-statistic
Dichlorvos [676384-L, M.W. = 220.98, Density = 1.415 g/ml]							
Trial 1 [78]							
B(a)P	.000791	8.09	60.6	116	(18)	6.11***	+ 4.93
B(a)P	.000250	14.9	84.2	184	(18)	9.84***	+ 9.74
676384-L	.182	.000	2.49	17	(6,18)	1.98	.00 (- 1.40)
676384-L	.136	.426	26.6	96	(18)	4.47	.00 (- 1.57)
676384-L	.0910	8.94	72.2	97	(18)	4.83*	+ 2.09
676384-L	.0450	37.4	88.4	41	(18)	2.01	.00 (- 2.36)
NC-1	Control	100.	100.	296	(72)	3.28	Control Mean t = .697
Trial 2 [90]							
B(a)P	.000791	28.5	76.0	157	(18)	7.60***	+ 5.89
B(a)P	.000250	51.8	95.8	110	(18)	4.91***	+ 3.71
676384-L	.159	.000	1.95	27	(14,17)	1.51	.00 (- .78)
676384-L	.119	.000	24.7	103	(18)	4.85***	+ 3.76
676384-L	.080	52.5	83.6	104	(18)	4.10**	+ 2.82
676384-L	.040	107.	114.	85	(18)	2.91	+ 1.41
NC-1	Control	100.	100.	219	(71)	1.95	Control Mean t = 2.66
2,4-Dinitrotoluene [24DNT, M.W. = 182.14]							
Trial 1 [46]							
B(a)P	.00791	5.52	35.7	127	(20)	6.14***	+ 12.4
B(a)P	.00250	22.4	81.1	75	(20)	3.32***	+ 8.63
24DNT	2.333	.000	.000	0	(5,20)	.000	.00 (- 4.15)
24DNT	1.167	11.0	55.1	38	(20)	1.61***	+ 4.70
24DNT	.583	56.6	85.8	20	(20)	.527	+ .66
24DNT	.292	79.7	93.3	7	(20)	.275	.00 (- .67)
NC-1	Control	100.	100.	24	(40)	.384	Control Mean t = 1.57
Trial 2 [55]							
B(a)P	.000791	2.26	23.9	133	(20)	5.49***	+ 15.2
B(a)P	.000250	4.07	62.4	48	(20)	1.88***	+ 7.12
24DNT	1.333	.452	13.3	8	(20)	.301	+ 1.68
24DNT	1.000	3.17	39.6	1	(20)	.035	.00 (- 1.59)
24DNT	.667	22.7	69.8	4	(20)	.149	+ .23
24DNT	.333	57.0	84.7	0	(20)	.000	.00 (- 1.87)
NC-1	Control	100.	100.	7	(40)	.129	Control Mean t = .478
Trail 3 [DRI2]							
MNNG	.00850	60.8	NA	242	(20)	11.7 ***	+ 19.0
24DNT	1.373	23.0	NA	2	(20)	.072	.00 (-3.10)
24DNT	.686	41.6	NA	6	(20)	.196	.00 (-1.76)
24DNT	.343	79.0	NA	12	(20)	.357	.00 (- .68)
NC-1	Control	100.	100.	13	(20)	.503	Control Mean *t* = 0.00
Epichlorohydrin [EPCH, M.W. = 92.53, Density = 1.181 g/ml]							
Trial 1 [68]							
B(a)P	.000791	3.60	51.9	91	(18)	4.63**	+ 3.24
B(a)P	.000250	4.50	77.2	37	(18)	1.73***	+ 15.0

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Appendix B. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
Drug	Conc., mM	S.A	CC.A.	Focus Type III	Data Vessels (N)	Foci/Vessel Focus Type III	t-Statistic
EPCH	.670	.000	.000	0	(0,17)	.000	ND
EPCH	.447	.000	.531	0	(3,18)	.000	+ .00 (- 3.60)
EPCH	.223	.450	73.6	39	(18)	1.57***	+ 4.51
EPCH	.112	33.8	105.	8	(18)	.260	+ .24
NC-1	Control	100.	100.	11	(36)	.226	Control
							Mean t = 2.38
Trial 2 [DRI5]							
B(a)P	.000791	ND	ND				
B(a)P	.000250	ND	ND				
EPCH	.383	ND	ND	98	(18)	4.58**	+ 4.20
EPCH	.255	ND	ND	115	(18)	6.02**	+ 6.22
EPCH	.128	ND	ND	27	(18)	1.27	.00 (- .01)
NC-1	Control	ND	100.	46	(18)	1.27	Control
							Mean t = 3.47
1,2-Epoxybutane [835701-L, M.W. = 72.11, Density = 0.8297 g/ml]							
Trial 1 [79]							
B(a)P	.000791	22.7	79.6	279	(18)	20.8***	+ 13.9
B(a)P	.000250	39.3	94.2	241	(18)	12.9***	+ 9.34
835701-L	22.9	.000	.000	0	(0,18)	.000	NA
835701-L	17.1	.000	.000	0	(0,18)	.000	NA
835701-L	11.4	.000	.000	0	(0,18)	.000	NA
835701-L	5.71	.000	.000	0	(0,18)	.000	NA
NC-1A+1B	Control	100.	100.	430	(62)	5.12	Control
							Mean t = ND
Trial 2 [104]							
B(a)P	.000791	25.0	64.5	62	(18)	2.79***	+ 4.87
B(a)P	.000250	50.6	89.6	63	(18)	2.47***	+ 4.10
835701-L	4.29	.000	.000	0	(0,18)	.000	NA
835701-L	2.14	.000	28.1	57	(18)	2.48***	+ 4.16
835701-L	1.07	5.06	92.3	66	(18)	3.31***	+ 6.07
835701-L	.536	37.3	108.	15	(5)	1.61	+ .69
NC-1A+1B	Control	100.	100.	83	(71)	.878	Control
							Mean t = 3.64
Trial 3 [108]							
B(a)P	.000791	15.5	31.3	139	(18)	7.38***	+ 13.9
B(a)P	.000250	30.0	65.7	72	(13)	4.77***	+ 5.96
835701-L	2.86	.000	2.52	10	(11)	.603	.00 (- 1.71
835701-L	2.14	.000	30.8	60	(16)	3.55***	+ 7.18
835701-L	1.43	7.42	92.0	18	(4)	3.82**	+ 2.83
835701-L	.714	82.5	98.7	20	(18)	.777	.00 (- 1.38)
NC-1A+1B	Control	100.	100.	108	(70)	1.17	Control
							Mean t = 2.50
1,2-Epoxypropane [12EP, M.W. = 58.08, Density = NA g/ml]							
Trial 1 [72]							
B(a)P	.000791	2.75	56.8	85	(18)	4.11***	+ 12.7
B(a)P	.000250	6.61	82.1	84	(18)	3.04***	+ 6.20
12EP	1.38	.000	90.9	34	(18)	1.62***	+ 6.64
12EP	1.03	.000	78.5	37	(18)	1.61***	+ 4.81
12EP	.689	21.5	63.1	23	(18)	.994**	+ 3.17
12EP	.344	51.8	97.4	16	(18)	.603	+ 1.53
NC-1	Control	100.	100.	29	(72)	.289	Control
							Mean t = 4.04

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Appendix B. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
				Focus Data Type Vessels		Foci/Vessel Focus Type	
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	*t*-statistic
Trial 2 [88]							
B(a)P	.000791	15.8	73.9	54	(15)	3.36***	+ 9.58
B(a)P	.000250	35.4	82.6	24	(15)	1.50***	+ 4.90
12EP	1.38	.000	74.0	39	(18)	1.90***	+ 6.22
12EP	1.03	5.92	82.2	60	(17)	2.73***	+ 6.05
12EP	.689	28.4	87.4	29	(14)	1.81***	+ 5.41
12EP	.344	83.6	93.8	7	(6)	1.04*	+ 2.04
NC-1	Control	100.	100.	37	(67)	.406	Control
							Mean t = 4.93
Ethylene Dibromide [EDB, M.W. = 187.88, Density = 2.177 g/ml]							
Trial 1 [74]							
B(a)P	.000791	3.00	69.2	43	(18)	2.02***	+ 4.51
B(a)P	.000250	9.58	78.4	27	(18)	1.29*	+ 2.49
EDB	.852	.000	48.5	137	(18)	7.33***	+ 18.7
EDB	.639	35.9	97.2	89	(18)	3.86***	+ 7.81
EDB	.426	62.3	104.	50	(18)	2.46***	+ 5.66
EDB	.213	105.	93.8	59	(18)	1.51	+ 1.93
NC-1	Control	100.	100.	65	(71)	.657	Control
							Mean t = 8.53
Trial 2 [92]							
B(a)P	.00250	.394	45.3	69	(18)	3.54***	+ 8.31
B(a)P	.000791	18.2	72.2	44	(18)	2.14***	+ 5.20
B(a)P	.000250	19.4	78.2	32	(17)	1.52**	+ 3.32
EDB	.958	.396	54.1	33	(16)	1.68***	+ 3.68
EDB	.719	2.77	80.5	38	(18)	1.90***	+ 4.71
EDB	.479	28.1	94.6	26	(18)	1.20*	+ 2.45
EDB	.240	72.8	89.5	16	(18)	.698	+ .47
NC-1	Control	100.	100.	62	(71)	.597	Control
							Mean t = 2.82
HC Blue 1 [HCB1, M.W. = 256.31]							
Trial 1 [15]							
B(a)P	.000791	.851	12.4	209	(20)	8.95***	+ 15.8
B(a)P	.000250	3.83	56.7	72	(20)	3.40***	+ 14.9
HCB1	2.35	1.28	32.5	11	(17)	.488	+ 2.13
HCB1	1.76	2.55	83.7	11	(20)	.423	+ 1.86
HCB1	1.18	16.2	101.	9	(19)	.291	+ .71
HCB1	.588	87.7	121.	11	(19)	.397	+ 1.35
NC-1	Control	100.	100.	10	(39)	.186	Control
							Mean t = 1.51
Trial 2 [21]							
B(a)P	.000791	.441	46.1	133	(18)	6.92***	+ 15.8
B(a)P	.000250	1.32	75.8	63	(19)	3.11***	+ 10.5
HCB1	2.45	.000	.000	6	(7,19)	.524	+ .72
HCB1	1.75	.000	39.8	17	(18)	.671	+ 1.65
HCB1	1.05	1.32	97.9	4	(17)	.177	.00 (- 1.22)
HCB1	.526	54.6	103.	15	(18)	.576	+ 1.25
NC-1	Control	100.	100.	19	(40)	.347	Control
							Mean t = .725
Trial 3 [DRI2]							
MNNG	.00850	60.8	NA	242	(20)	11.7***	+ 19.0
HCB1	2.45	49.8	NA	26	(20)	1.07*	+ 2.18
HCB1	1.23	51.9	NA	51	(19)	2.08***	+ 4.12
HCB1	.613	92.0	NA	46	(19)	1.65**	+ 2.98
NC-1	Control	100.	100.	13	(20)	.503	Control
							Mean t = 3.09

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Appendix B. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
Drug	Conc., mM	S.A	CC.A.	Focus Data Type Vessels III	(N)	Foci/Vessel Focus Type III	*t*-statistic
Iodinated Glycerol [513502-L, M.W. = 258.07, Density = 1.797 g/ml]							
Trial 1 [74]							
B(a)P	.000791	3.00	69.2	43	(17)	2.02***	+ 4.51
B(a)P	.000250	9.58	78.4	27	(17)	1.29*	+ 2.49
513502-L	2.69	.000	.000	9	(13,18)	.498	.00 (- .67)
513502-L	2.02	.000	50.6	89	(18)	4.22***	+ 8.41
513502-L	1.35	.000	86.4	99	(17,18)	4.29***	+ 6.11
513502-L	.673	10.2	102.	37	(18)	1.68***	+ 3.61
NC-1	Control	100.	100.	65	(71)	.657	Control
						Mean t = 6.04	
Trial 2 [106]							
B(a)P	.000791	24.8	56.9	134	(18)	6.88***	+ 8.00
B(a)P	.000250	40.7	77.9	91	(18)	4.53***	+ 5.56
513502-L	2.69	.000	1.31	6	(8,18)	.622	.00 (- 1.58)
513502-L	2.02	.000	24.7	24	(18)	.923	.00 (- 1.06)
513502-L	1.35	.000	68.5	42	(18)	2.13	+ 1.99
513502-L	.673	16.8	95.4	42	(18)	2.11	+ 1.95
NC-1	Control	100.	100.	74	(43)	1.30	Control
						Mean t = 1.31	
Melphalan [MELP, M.W. = 305.23]							
Trial 1 [80]							
B(a)P	.000791	16.9	65.2	261	(20)	12.9***	+ 13.0
B(a)P	.000250	35.6	87.2	185	(20)	8.53***	+ 7.65
MELP	.00721	.890	3.40	0	(0,12)	.000	NA
MELP	.00361	2.67	6.16	0	(0,20)	.000	NA
MELP	.00180	8.01	30.3	48	(20)	1.72	.00 (- 2.44
MELP	.00090	46.3	75.9	113	(20)	5.10**	+ 2.73
NC-1	Control	100.	100.	317	(80)	3.02	Control
						Mean t = 1.37	
Trial 2 [96]							
B(a)P	.000791	11.0	43.5	86	(20)	4.03***	+ 9.40
B(a)P	.000250	38.9	75.4	62	(20)	2.88***	+ 7.12
MELP	.00262	5.11	6.66	13	(20)	.443	.00 (- 1.11)
MELP	.00197	14.9	26.2	37	(20)	1.70***	+ 5.21
MELP	.00131	32.6	47.0	62	(20)	2.92***	+ 9.30
MELP	.000655	76.6	104.	43	(20)	1.92***	+ 4.71
NC-1	Control	100.	100.	62	(70)	.660	Control
						Mean t = 4.81	
N-Methyl-*o*-Acrylamide [027516-S, M.W. = 101.11]							
Trial 1 [70]							
B(a)P	.000791	1.24	48.9	11	(3)	3.38***	+ 4.23
B(a)P	.000250	11.8	81.0	19	(5)	3.62***	+ 5.73
027516-S	5.00	.000	.000	0	(0,19)	.000	NA
027516-S	2.50	.000	2.89	7	(12,14)	.381	.00 (- .72)
027516-S	1.25	.000	85.2	27	(16)	1.27**	+ 2.97
027516-S	.625	15.5	107.	12	(16)	.542	+ .08
NC-1A+1B	Control	100.	100.	36	(54)	.526	Control
						Mean t = 1.53	
Trial 2 [85]							
B(a)P	.000791	18.8	55.6	133	(20)	3.43***	+ 5.10
B(a)P	.000250	28.7	91.8	66	(19)	2.10***	+ 4.56

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Appendix B. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c] Focus Data Type Vessels		Transformation Response[d] Foci/Vessel Focus Type	Significance[e]
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	*t*-statistic
027516-S	2.00	.000	20.2	76	(19)	3.41***	+ 10.5
027516-S	1.50	.000	69.8	32	(20)	1.17***	+ 4.28
027516-S	1.00	10.4	100.	9	(20)	.327	+ .10
027516-S	.500	34.7	101.	18	(20)	.445	+ .65
NC-1	Control	100.	100.	38	(80)	.313	Control
							Mean t = 3.88
4,4-Methylenedianiline [44MD, M.W. = 271.21]							
Trial 1 [40]							
B(a)P	.000791	1.06	39.7	182	(19)	8.71***	+ 14.9
B(a)P	.000250	8.48	79.2	101	(18)	4.86***	+ 9.93
44MD	2.21	.000	.000	5	(11,20)	.287	.00 (- 1.14)
44MD	1.66	.000	30.9	18	(20)	.668	+ .65
44MD	1.11	.353	76.0	36	(20)	1.60***	+ 4.54
44MD	.554	16.3	96.5	25	(20)	1.08*	+ 2.51
NC-1	Control	100.	100.	28	(40)	.533	Control
							Mean t = 1.93
Trial 2 [55]							
B(a)P	.000791	2.26	23.9	133	(20)	5.49***	+ 15.2
B(a)P	.000250	4.07	62.4	48	(20)	1.88***	+ 7.12
44MD	1.85	.000	45.1	4	(20)	.132	+ .04
44MD	1.38	.000	73.3	26	(20)	1.11***	+ 5.74
44MD	.923	2.71	65.9	12	(20)	.330	+ 1.26
44MD	.461	29.0	82.4	4	(20)	.149	+ .23
NC-1	Control	100.	100.	7	(40)	.129	Control
							Mean t = 1.82
N-Methyl-N'-Nitro-N-Nitrosoguanidine [MNNG, M.W. = 147.1]							
Trial 1 [93]							
B(a)P	.000791	2.65	46.7	138	(20)	6.48***	+ 16.8
B(a)P	.000250	7.96	79.9	115	(20)	4.80***	+ 12.0
MNNG	.0204	.000	1.58	19	(20)	.634	+ 1.02
MNNG	.0153	.000	14.2	102	(19)	4.54***	+ 9.02
MNNG	.0102	8.85	46.7	169	(20)	8.01***	+ 18.8
MNNG	.00510	21.2	90.2	80	(19)	3.83***	+ 11.7
NC-1	Control	100.	100.	43	(79)	.416	Control
							Mean t = 10.1
Trial 2 [IP2]							
B(a)P	.000791	ND	ND				
B(a)P	.000250	ND	ND				
MNNG	.0170	30.0	ND	228	(20)	10.9***	+ 13.2
MNNG	.00850	81.0	ND	219	(20)	6.51***	+ 5.64
MNNG	.00425	79.6	ND	101	(20)	3.47***	+ 3.72
NC-1	Control	100.	ND	71	(40)	1.13	Control
							Mean t = 7.52
2-Naphthylamine [2NAP, M.W. = 143.18]							
Trial 1 [13]							
B(a)P	.000791	.823	30.6	95	(20)	4.38***	+ 14.2
B(a)P	.000250	2.06	75.4	66	(20)	3.14***	+ 15.7
2NAP	1.40	.000	34.8	9	(20)	.347	+ 1.20
2NAP	.698	4.12	73.1	21	(20)	.850***	+ 4.14
2NAP	.348	14.0	99.2	25	(20)	1.05***	+ 5.21
2NAP	.175	51.9	95.7	14	(18)	.608**	+ 2.75
NC-1	Control	100.	100.	11	(40)	.201	Control
							Mean t = 3.33

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Appendix B. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c] Focus Data Type Vessels		Transformation Response[d] Foci/Vessel Focus Type	Significance[e]
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	*t*-statistic
Trial 2 [26]							
B(a)P	.000791	.382	13.5	204	(20)	9.88***	+ 17.8
B(a)P	.000250	1.15	56.2	144	(20)	6.58***	+ 10.5
2NAP	1.05	.763	101.	112	(20)	4.57***	+ 7.41
2NAP	.698	11.1	93.4	49	(20)	2.09***	+ 3.55
2NAP	.348	46.6	94.5	42	(20)	1.86**	+ 3.08
2NAP	.175	71.0	97.8	38	(20)	1.50	+ 1.92
NC-1	Control	100.	100.	46	(40)	.907	Control
							Mean t = 3.99
Nitrofurantoin [291535-S, M.W. = 238.16]							
Trial 1 [61]							
B(a)P	.000791	.377	32.6	94	(20)	4.28***	+ 14.3
B(a)P	.000250	9.81	69.9	43	(20)	1.92***	+ 8.76
291535-S	.167	.000	7.90	9	(20)	.327	+ .83
291535-S	.125	6.04	48.4	10	(15)	.557*	+ 2.28
291535-S	.0833	48.3	93.8	3	(20)	.110	.00 (- 1.13)
291535-S	.0417	100.	98.5	4	(20)	.149	.00 (- .71)
NC-1	Control	100.	100.	12	(40)	.222	Control
							Mean t = .778
Trial 2 [93]							
B(a)P	.000791	2.65	46.7	138	(20)	6.48***	+ 16.8
B(a)P	.000250	7.96	79.9	115	(20)	4.80***	+ 12.0
291535-S	.167	.000	.000	3	(20)	.110	.00 (- 3.34)
291535-S	.125	.000	3.17	31	(20)	1.17**	+ 2.99
291535-S	.0833	21.2	44.3	59	(20)	2.59**	+ 8.73
201535-S	.0417	97.3	105.	15	(20)	.578	+ 1.03
NC-1	Control	100.	100.	43	(79)	.416	Control
							Mean t = 4.25
Nitrofurazone [196993-S, M.W. = 198.14]							
Trial 1 [71]							
B(a)P	.000791	4.48	50.7	251	(20)	11.0***	+ 12.1
B(a)P	.000250	18.1	68.7	77	(20)	3.50***	+ 7.12
196993-S	1.00	.000	.000	0	(0,19)	.000	NA
196993-S	.750	.000	.000	0	(0,20)	.000	NA
196993-S	.500	.000	.000	0	(0,19)	.000	NA
196993-S	.250	.000	.000	6	(20)	.196	.00 (- 4.94)
NC-1	Control	100.	100.	110	(75)	1.06	Control
							Mean t = .000
Trial 2 [77]							
B(a)P	.000791	5.66	69.5	179	(20)	8.33***	+ 13.6
B(a)P	.000250	16.5	78.7	114	(19)	5.53***	+ 10.0
196993-S	.100	.000	1.47	18	(20)	.737	.00 (- 1.07)
196993-S	.0750	.000	8.25	44	(20)	1.77**	+ 2.72
196993-S	.0500	.000	39.8	77	(20)	3.13***	+ 5.85
196993-S	.0250	2.83	92.1	55	(20)	2.38***	+ 4.46
NC-1	Control	100.	100.	94	(78)	.972	Control
							Mean t = 3.26
Trial 3 [87]							
B(a)P	.000791	25.1	77.0	59	(20)	2.26***	+ 5.84
B(a)P	.000250	42.2	80.2	34	(20)	1.45***	+ 5.24
196993-S	.0750	.000	16.6	57	(20)	2.33***	+ 7.56
196993-S	.0563	1.09	35.0	64	(20)	2.69***	+ 8.33
196993-S	.0375	3.27	72.1	29	(20)	1.14***	+ 3.89
196993-S	.0188	45.1	90.0	10	(20)	.374	+ .18
NC-1	Control	100.	100.	43	(80)	.346	Control
							Mean t = 4.99

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Appendix B. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
				Focus Data Type Vessels		Foci/Vessel Focus Type	
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	*t*-statistic
4,4-Oxydianiline [OXY, M.W. = 200.24]							
Trial 1 [1]							
B(a)P	.000791	9.75	56.3	171	(20)	8.16***	+ 12.1
B(a)P	.000250	15.5	91.0	114	(20)	5.18***	+ 6.68
OXY	.674	.000	10.4	17	(20)	.644	.00 (- 2.68)
OXY	.549	.000	32.6	36	(20)	1.41	.00 (- .06)
OXY	.370	1.08	73.6	51	(20)	2.34*	+ 2.31
OXY	.165	26.0	117.	70	(20)	3.28***	+ 4.92
NC-1	Control	100.	100.	73	(40)	1.44	Control
							Mean t = 1.81
Trial 2 [8]							
B(a)P	.000791	1.45	5.83	244	(19)	10.7***	+ 8.19
B(a)P	.000250	4.35	52.4	254	(20)	11.8***	+ 11.2
OXY	.499	.000	.000	32	(20)	1.25	.00 (- 2.24)
OXY	.375	.000	.000	68	(20)	2.93	+ 1.38
OXY	.250	2.90	25.2	134	(20)	5.88**	+ 5.12
OXY	.125	22.2	89.3	163	(20)	7.70***	+ 8.36
NC-1	Control	100.	100.	110	(40)	2.19	Control
							Mean t = 3.72
2-Nitro-*p*-Phenylenediamine [2NPD, M.W. = 153.16]							
Trial 1 [15]							
B(a)P	.000791	.851	12.4	209	(20)	8.95***	+ 15.8
B(a)P	.000250	3.83	56.7	72	(20)	3.40***	+ 14.9
2NPD	6.53	.426	.000	0	(8,20)	.000	.00 (- 3.31)
2NPD	3.27	.851	9.64	22	(19)	.965**	+ 4.84
2NPD	1.63	2.31	11.6	27	(20)	.977**	+ 3.55
2NPD	.816	4.26	54.0	50	(20)	2.16***	+ 8.23
NC-1	Control	100.	100.	10	(39)	.186	Control
							Mean t = 4.21
Trial 2 [21]							
B(a)P	.000791	.441	46.1	133	(18)	6.92***	+ 15.8
B(a)P	.000250	1.32	75.8	63	(19)	3.11***	+ 10.5
2NPD	1.63	.000	21.4	21	(20)	.726	+ 1.92
2NPD	.816	.000	63.9	16	(19)	.652	+ 1.69
2NPD	.408	17.2	90.8	17	(20)	.551	+ 1.09
2NPD	.204	45.8	97.4	12	(20)	.473	+ .80
NC-1	Control	100.	100.	19	(40)	.347	Control
							Mean t = 1.38
Trial 3 [96]							
B(a)P	.000791	11.0	43.5	86	(20)	4.03***	+ 9.40
B(a)P	.000250	38.9	75.4	62	(20)	2.88***	+ 7.12
2NPD	1.63	3.54	61.7	35	(19)	1.57**	+ 3.39
2NPD	1.22	7.47	62.1	29	(18)	1.17	+ 1.93
2NPD	.816	19.3	60.4	46	(19)	2.28***	+ 7.57
2NPD	.408	64.8	82.5	43	(19)	1.98***	+ 4.64
NC-1	Control	100.	100.	62	(70)	.660	Control
							Mean t = 4.38
Quinoline [QUIN, M.W. = 129.16, Density = 1.095 g/ml]							
Trial 1 [16]							
B(a)P	.000791	.881	47.5	139	(17)	7.96***	+ 17.5
B(a)P	.000250	4.85	76.6	58	(17)	2.77***	+ 7.77

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Appendix B. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
				Focus Data Type Vessels		Foci/Vessel Focus Type	
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	*t*-statistic
QUIN	1.70	8.81	94.3	6	(18)	.260	.00 (- .570
QUIN	1.10	27.8	96.8	7	(18)	.289	.00 (- .36)
QUIN	.551	53.3	94.6	2	(18)	.080	.00 (- 2.51)
QUIN	.276	99.1	109.	4	(18)	.148	.00 (- 1.40)
NC-1	Control	100.	100.	17	(36)	.344	Control
							Mean t = .000
Trial 2 [27]							
B(a)P	.000791	4.04	33.7	170	(18)	8.90***	+ 14.8
B(a)P	.000250	13.0	47.4	73	(18)	3.18***	+ 5.71
QUIN	2.54	17.4	77.4	16	(18)	.737	+ .73
QUIN	1.70	23.6	95.2	18	(18)	.765	+ .81
QUIN	.848	52.8	81.1	14	(18)	.537	.00 (- .07)
QUIN	.424	91.3	44.2	5	(18)	.193	.00 (- 2.12)
NC-1	Control	100.	100.	31	(36)	.555	Control
							Mean t = .385
Trial 3 [31]							
B(a)P	.000791	1.87	69.1	136	(15)	8.63***	+ 11.1
B(a)P	.000250	5.14	99.9	126	(18)	6.06***	+ 8.51
QUIN	6.32	.000	.485	2	(4,18)	.316	.00 (- 1.330
QUIN	4.24	.467	29.1	39	(17)	1.80*	+ 2.29
QUIN	3.18	9.35	64.0	33	(18)	1.59*	+ 2.05
QUIN	2.12	12.6	93.1	28	(18)	1.36	+ 1.44
NC-1	Control	100.	100.	43	(36)	.930	Control
							Mean t = 1.93
Trial 4 [104]							
B(a)P	.000791	25.0	64.5	62	(18)	2.79***	+ 4.87
B(a)P	.000250	50.6	89.6	63	(18)	2.47***	+ 4.10
QUIN		.000	22.5	21	(14,18)	.950	+ .22
QUIN		21.8	72.8	51	(18)	2.37***	+ 4.11
QUIN		46.5	101.	66	(18)	3.22***	+ 5.83
QUIN		65.8	96.2	32	(18)	1.47	+ 1.94
NC-1	Control	100.	100.	83	(71)	.878	Control
							Mean t = 3.96
Selenium Sulfide [SESU, M.W. = 111.02]							
Trial 1 [7]							
B(a)P	.000791	1.52	48.4	212	(19)	10.0***	+ 26.6
B(a)P	.000250	3.41	56.9	41	(20)	1.80***	+ 7.07
SESU	.180	.758	21.3	26	(19)	1.06***	+ 4.34
SESU	.108	14.4	48.7	18	(18)	.793**	+ 3.69
SESU	.0721	22.0	45.6	13	(20)	.503*	+ 2.56
SESU	.0360	57.2	76.2	11	(19)	.471**	+ 2.78
NC-1	Control	100.	100.	7	(36)	.135	Control
							Mean t = 3.34
Trial 2 [11]							
B(a)P	.000791	1.37	34.4	128	(20)	4.94**	+ 10.5
B(a)P	.000250	7.22	36.0	37	(20)	1.62**	+ 5.38
SESU	.180	6.19	49.5	7	(18,20)	.309	+ .06
SESU	.108	14.4	103.	16	(20)	.410	+ .56
SESU	.0721	25.1	125.	6	(20)	.214	.00 (- .56)
SESU	.0360	32.6	151.	15	(20)	.322	+ .11
NC-1	Control	100.	100.	21	(40)	.301	Control
							Mean t = .223
Trial 3 [97]							
B(a)P	.000791	4.74	78.0	118	(20)	5.02***	+ 12.5
B(a)P	.000250	17.4	104.	52	(20)	2.26***	+ 7.26

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Appendix B. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
Drug	Conc., mM	S.A	CC.A.	Focus Data Type Vessels III	(N)	Foci/Vessel Focus Type III	*t*-statistic
SESU	.225	79.8	84.6	22	(16)	.956*	+ 2.45
SESU	.169	92.5	85.4	22	(19)	1.03**	+ 3.17
SESU	.113	111.	84.6	18	(18)	.765	+ 1.81
SESU	.0563	113.	92.0	44	(19)	.916	+ 1.51
NC-1	Control	100.	100.	47	(80)	.414	Control
							Mean t = 2.24
o-Toluidine [OTOL, M.W. = 107.16, Density = 1.008 g/ml]							
Trial 1 [16]							
B(a)P	.000791	.881	47.5	139	(17)	7.96***	+ 17.5
B(a)P	.000250	4.85	76.6	58	(17)	2.77***	+ 7.77
OTOL	4.70	1.32	88.3	22	(18)	.950**	+ 2.81
OTOL	3.06	5.73	90.5	11	(18)	.409	+ .37
OTOL	1.41	26.4	100.	10	(18)	.414	+ .42
OTOL	.705	44.1	109.	6	(18)	.212	.00 (- .87)
NC-1	Control	100.	100.	17	(36)	.344	Control
							Mean t = .900
Trial 2 [25]							
B(a)P	.000791	NA	21.4	192	(18)	9.74***	+ 19.9
B(a)P	.000250	NA	73.4	59	(18)	2.55***	+ 7.52
OTOL	9.41	NA	6.77	25	(17,18)	1.25**	+ 5.90
OTOL	7.05	NA	12.5	30	(18)	1.47***	+ 7.50
OTOL	4.70	NA	26.6	39	(18)	2.01***	+ 12.6
OTOL	2.35	NA	25.0	57	(18)	2.80***	+ 11.1
NC-1	Control	NA	100.	5	(36)	.101	Control
							Mean t = 9.28
Trial 3 [98]							
B(a)P	.000791	8.38	79.6	132	(18)	6.82***	+ 11.8
B(a)P	.000250	29.3	91.3	75	(18)	3.38***	+ 6.81
OTOL	4.70	6.28	80.4	33	(18)	1.62***	+ 3.48
OTOL	3.53	7.33	79.4	60	(18)	2.78***	+ 5.73
OTOL	2.35	12.6	81.1	60	(18)	2.38***	+ 4.87
OTOL	1.18	23.0	86.8	38	(18)	1.63**	+ 3.17
NC-1	Control	100.	100.	39	(45)	.618	Control
							Mean t = 4.31
Ziram [ZIRAM, M.W. = 305.81]							
Trial 1 [77]							
B(a)P	.000791	5.66	69.5	179	(20)	8.33***	+ 13.6
B(a)P	.000250	16.5	78.7	114	(19)	5.53***	+ 10.0
ZIRAM	.000164	.000	1.65	22	(19)	.825	.00 (- .63)
ZIRAM	.0000818	.000	3.30	36	(19)	1.62*	+ 2.33
ZIRAM	.0000409	4.72	27.8	34	(18)	1.58*	+ 2.12
ZIRAM	.0000204	12.7	74.8	26	(16)	1.06	+ .24
NC-1	Control	100.	100.	94	(78)	.972	Control
							Mean t = 1.17
Trial 2 [99]							
B(a)P	.000791	28.9	73.6	60	(20)	2.00***	+ 5.11
B(a)P	.000250	58.1	89.9	14	(20)	.503	+ 1.31
ZIRAM	.000114	.000	2.12	40	(20)	1.62***	+ 5.21
ZIRAM	.0000572	12.0	8.91	54	(20)	2.54***	+ 11.2
ZIRAM	.0000286	56.4	78.5	8	(20)	.301	.00 (- .17)
ZIRAM	.0000143	91.3	101.	27	(20)	.303	.00 (- .08)
NC-1	Control	100.	100.	31	(75)	.322	Control
							Mean t = 4.10

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Appendix B. ***Continued.***

Abbreviations: BaP, benzo(a)pyrene; CC.A., co-culture clonal survival assay; Conc., concentration; mM, millimole; M.W., molecular weight; N, number of culture vessels, NC, negative control; ND; not determined; %RCE, percent relative cloning efficiency; S.A., standard clonal survival assay.

[a]Treatment condition: The experimental design for the transformation assay is described in detail in the Materials and Methods. The concentration of the positive control and test chemical treatment are presented in mM, but they can be converted to μg/ml using the molecular weight that is provided with each chemical. The solvent vehicles used for the individual test chemicals were listed in Appendix Tables A1 and A3, and the concentrations of the solvent vehicles are presented in the Materials and Methods.

[b]Cytotoxic activity: The experimental design for the standard survival assay (SA) and the co-culture clonal survival assay (CCA) were described in the Materials and Methods. The test chemical cytotoxic response was expressed as % RCE and was calculated as described in the Materials and Methods.

[c]The criteria used to evaluate the transformed foci of BALB/c-3T3 cells is described in the Materials and Methods. The number of type III foci > 2-mm in diameter per culture vessel scored are recorded in this table.

[d]Transformation response: The transformation responses are expressed as type III foci/vessel and were calculated using a $\log_{10}$ mathematical transformation procedure (refer to Materials and Methods). The arithmetic value or foci/vessel represents the antilog of the $\log_{10}$ mean transformation response minus one.

[e]Significance: The significance of test chemical transformation responses was calculated by a computer using the SAS statistical software (*22*), and the method is described in detail in Materials and Methods. The correct *t*-statistic according to the F-test is presented in this table. The *t*-statistics of each treatment dose of the test chemical in a single experiment were averaged to determine the mean *t*-statistic of the test chemical for the experiment (refer to Appendix Tables A2 and A5). The mean *t*-statistics for two or experiments for each chemical was weighted to the number of treatment doses evaluated and averaged to determine the rank *t*-statistic which was used to rank-order the test chemical transformation responses in Appendix Tables A3 and A6. Arbitrarily, transformation responses with negative (−) *t*-statistics were given a value of zero (0).

*Significant BaP or test chemical transformation response, $0.01 < p \leq 0.05$.

**Significant BaP or test chemical transformation response, $0.001 < p \leq 0.01$.

***Significant or BaP or test chemical transformation response, $p \leq 0.001$.

Appendix C.

Summary of the transformation responses of 21 cytotoxic, mutagenic noncarcinogens.

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
				Focus Data Type Vessels		Foci/Vessel Focus Type	
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	t-statistic
4-Acetylaminofluorene [4AAF, M.W. = 223.29]							
Trial 1 [12]							
B(a)P*	.000791	90.7	99.0	61	(19)	2.90 ***	+ 13.1
B(a)P	.000250	77.6	114.	81	(17)	3.94 ***	+ 10.5
4AAF	1.79	47.3	123.	11	(18)	.432	+ 1.92
4AAF	1.31	63.5	129.	5	(19)	.200	+ .61
4AAF	.896	77.1	129.	5	(17)	.206	+ .61
4AAF	.448	71.9	128.	5	(18)	.212	+ .71
NC-1	Control	100.	100.	8	(40)	.160	Control
							Mean t = .963
*NOTE: B(a)P was accidentally not dosed in the standard and the co-culture clonal survival assays.							
Trial 2 [17]							
B(a)P	.000791	.000	52.9	94	(20)	4.43***	+ 13.5
B(a)P	.000250	3.54	79.1	86	(20)	3.91***	+ 11.6
4AAF	1.79	55.8	93.0	29	(20)	.997*	+ 2.62
4AAF	.896	55.2	99.0	30	(20)	.724	+ 1.44
4AAF	.448	51.8	104.	5	(20)	.189	.00 (-1.07)
4AAF	.224	65.9	106.	23	(20)	.821*	+ 2.50
NC-1	Control	100.	100.	18	(40)	.327	Control
							Mean t = 1.64
4'-(Chloroacetyl)acetanilide [4CAA, M.W. = 211.66]							
Trial 1 [37]							
B(a)P	.000791	1.60	46.0	101	(20)	4.59***	+ 9.94
B(a)P	.000250	6.80	77.2	113	(20)	5.38***	+ 13.5
4CAA	.00378	.000	57.1	55	(20)	2.37***	+ 5.49
4CAA	.00283	.000	74.9	21	(20)	.702	+ .31
4CAA	.00189	1.60	90.9	21	(20)	.839	+ .92
4CAA	.00094	58.0	86.2	22	(20)	.787	+ .66
NC-1	Control	100.	100.	32	(39)	.631	Control
							Mean t = 1.85
Trial 2 [95]							
B(a)P	.000791	16.0	ND	152	(20)	7.35***	+ 9.48
B(a)P	.000250	33.3	ND	115	(20)	5.27***	+ 3.82
4CAA	.00472	.000	ND	127	(15)	6.71***	+ 4.54
4CAA	.00354	.000	ND	24	(8)	2.60	.00 (- .33)
4CAA	.00236	.000	ND	27	(14)	1.82	.00 (-3.13)
4CAA	.00118	46.1	ND	70	(18)	3.56	+ 1.29
NC-1	Control	100.	ND	263	(77)	2.84	Control
							Mean t = 1.46
2-(Chloromethyl)pyridine-HCl [2CMP, M.W. = 164.04]							
Trial 1 [14]							
B(a)P	.000791	1.75	36.1	177	(20)	6.73***	+ 13.3
B(a)P	.000250	5.26	74.7	61	(20)	2.45***	+ 7.12
2CMP	.152	.000	8.89	23	(20)	.938***	+ 4.28
2CMP	.107	4.82	73.0	12	(19)	.526*	+ 2.21
2CMP	.0533	36.4	103.	18	(20)	.550	+ 1.66
2CMP	.0267	93.0	108.	15	(20)	.452	+ 1.29
NC-1	Control	100.	100.	12	(40)	.213	Control
							Mean t = 2.36
Trial 2 [22]							
B(a)P	.000791	1.86	53.4	187	(20)	8.86***	+ 16.0
B(a)P	.000250	6.52	81.4	116	(20)	5.25***	+ 9.33

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Appendix C. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
Drug	Conc., mM	S.A	CC.A.	Focus Data Type Vessels III	(N)	Foci/Vessel Focus Type III	*t*-statistic
2CMP	.213	.000	99.7	63	(20)	2.94***	+ 6.03
2CMP	.152	39.1	105.	54	(20)	2.14***	+ 3.59
2CMP	.0914	72.0	107.	35	(20)	1.33	+ 1.45
2CMP	.0457	84.5	94.0	23	(20)	.877	.00 (- .06)
NC-1	Control	100.	100.	45	(40)	.893	Control
							Mean t = 2.77
3-Chloro-*p*-Toluidine [3CT, M.W. = 141.60]							
Trial 1 [81]							
B(a)P	.000791	10.2	63.9	378	(18)	20.8***	+ 15.5
B(a)P	.000250	28.0	67.8	280	(18)	15.3***	+ 10.5
3CT	1.41	.000	42.2	215	(18)	11.2***	+ 3.55
3CT	1.06	7.34	91.4	225	(18)	11.9***	+ 4.13
3CT	.706	13.6	94.3	211	(18)	10.3*	+ 2.70
3CT	.353	50.3	107.	105	(18)	5.45	.00 (-2.45)
NC-1	Control	100.	100.	583	(72)	7.36	Control
							Mean t = 2.60
Trial 2 [92]							
B(a)P	.00250	.394	45.3	69	(18)	3.54***	+ 8.31
B(a)P	.000791	18.2	72.2	44	(18)	2.14***	+ 5.20
B(a)P	.000250	19.4	78.2	32	(17)	1.52**	+ 3.32
3CT	1.55	11.9	92.3	37	(18)	1.78***	+ 4.29
3CT	1.17	44.8	87.4	49	(18)	2.44***	+ 5.95
3CT	.777	45.5	89.3	33	(18)	1.50**	+ 3.40
3CT	.388	75.2	94.8	22	(18)	1.07*	+ 2.05
NC-1	Control	100.	100.	62	(71)	.597	Control
							Mean t = 3.92
Coumaphos [COU, M.W. = 362.78]							
Trial 1 [30]							
B(a)P	.000791	1.32	60.3	158	(20)	7.23***	+ 12.3
B(a)P	.000250	2.63	101.	98	(19)	4.24***	+ 8.08
COU	.276	.000	.510	2	(18,20)	.080	.00(-4.29)
COU	.138	5.70	4.08	8	(20)	.301	.00(-2.61)
COU	.0689	18.4	54.0	17	(20)	.751	.00(- .17)
COU	.0345	31.6	94.8	13	(20)	.459	.00(-1.56)
NC-1	Control	100.	100.	40	(40)	.787	Control
							Mean t = .000
Trial 2 [95]							
B(a)P	.000791	16.0	ND	152	(20)	7.35***	+ 9.48
B(a)P	.000250	33.3	ND	115	(20)	5.27***	+ 3.82
COU	.221	66.5	ND	125	(18)	6.11***	+ 4.54
COU	.110	77.1	ND	93	(18)	4.89***	+ 4.37
COU	.0551	81.1	ND	71	(12)	5.47**	+ 3.25
COU	.0276	86.0	ND	88	(17)	4.68**	+ 2.83
NC-1	Control	100.	100.	263	(77)	2.84	Control
							Mean t = 3.75
Trial 3 [99]							
B(a)P	.000791	18.9	68.4	160	(20)	6.67***	+ 12.4
B(a)P	.000250	32.1	85.0	94	(20)	3.59***	+ 7.95
COU	.331	44.2	56.9	83	(20)	3.20***	+ 7.18
COU	.165	50.2	62.0	100	(20)	4.53***	+ 10.3
COU	.0827	52.5	91.0	89	(20)	3.83***	+ 8.84
COU	.0413	60.4	87.9	39	(20)	1.64***	+ 4.06
NC-1	Control	100.	100.	65	(80)	.586	Control
							Mean t = 7.60

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Appendix C. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
				Focus Data Type Vessels		Foci/Vessel Focus Type	
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	*t*-statistic
Dimethoate [CYGON, M.W. = 229.27, Density = 1.277 g/ml]							
Trial 1 [41]							
B(a)P	.000791	1.29	33.6	189	(18)	10.2***	+
B(a)P	.000250	6.45	78.2	123	(18)	6.37***	+
CYGON	.668	1.29	50.1	16	(18)	.655*	+
CYGON	.334	14.8	78.2	16	(18)	.698*	+
CYGON	.167	85.8	89.2	8	(18)	.309	+
CYGON	.0835	91.3	103.	6	(18)	.260	+
NC-1	Control	100.	100.	13	(36)	.274	Control Mean t =
Trial 2 [94]							
B(a)P	.000791	.000	75.7	122	(18)	5.92***	+
B(a)P	.000250	17.4	114.	81	(18)	3.88***	+
CYGON	.835	2.05	55.9	117	(18)	6.33***	+
CYGON	.627	2.05	53.5	129	(18)	6.26***	+
CYGON	.418	14.4	64.6	69	(18)	3.39***	+
CYGON	.209	40.0	123.	30	(18)	.935	+
NC-1	Control	100.	100.	150	(71)	1.52	Control Mean t =
2,4-Dimethoxyaniline-HCl [DMAN, M.W. = 189.66]							
Trial 1 [34]							
B(a)P	.000791	6.91	58.7	167	(20)	8.23***	+ 14.5
B(a)P	.000250	19.3	84.5	138	(20)	6.39***	+ 7.66
DMAN	1.32	.000	55.6	91	(20)	4.20***	+ 4.09
DMAN	.923	.000	78.9	108	(20)	5.18***	+ 6.27
DMAN	.527	.000	86.5	124	(20)	5.76***	+ 6.81
DMAN	.264	9.09	69.5	127	(20)	5.85***	+ 6.63
NC-1	Control	100.	100.	108	(40)	2.51	Control Mean t = 5.95
Trial 2 [87]							
B(a)P	.000791	25.1	77.0	59	(20)	2.26***	+ 5.84
B(a)P	.000250	42.2	80.2	34	(20)	1.45***	+ 5.24
DMAN	1.58	.000	2.69	1	(18,20)	.039	.00(-4.02)
DMAN	1.19	.000	58.7	18	(20)	.662	+ 1.79
DMAN	.791	.000	77.3	17	(20)	.601	+ 1.46
DMAN	.395	44.7	88.3	13	(20)	.460	+ .70
NC-1	Control	100.	100.	43	(80)	.346	Control Mean t = 1.32
Trial 3 [97]							
B(a)P	.000791	4.74	78.0	118	(20)	5.02***	+ 12.5
B(a)P	.000250	17.4	104.	52	(20)	2.26***	+ 7.26
DMAN	1.48	.000	.000	3	(16,20)	.139	.00(-2.50)
DMAN	1.11	.000	10.9	7	(20)	.238	.00(-1.19)
DMAN	.738	.000	60.2	16	(20)	.644	+ 1.32
DMAN	.369	9.48	93.9	15	(17)	.711	+ 1.56
NC-1	Control	100.	100.	47	(80)	.414	Control Mean t = .960
HC Blue 2 [HCB2, M.W. = 285.34]							
Trial 1 [5]							
B(a)P	.000791	7.76	9.41	84	(19)	3.97***	+ 15.5
B(a)P	.000250	12.5	12.5	57	(20)	2.58***	+ 12.5

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Appendix C. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
				Focus Data Type Vessels		Foci/Vessel Focus Type	
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	*t*-statistic
HCB2	7.01	.000	.000	0	(0,20)	.000	ND
HCB2	5.84	.000	14.1	0	(0,20)	.000	ND
HCB2	4.21	.431	11.8	2	(8,20)	.072	+ .65
HCB2	3.50	.000	127.	11	(20)	.423	+ 3.30
NC-1	Control	100.	100.	2	(40)	.035	Control
							Mean t = 3.30
Trial 2 [10]							
B(a)P	.000791	1.89	30.6	105	(20)	4.79***	+ 18.3
B(a)P	.000250	8.49	91.7	34	(20)	1.37***	+ 6.23
HCB2	4.21	.000	94.4	12	(20)	.264	+ 1.42
HCB2	3.50	.000	168.	3	(20)	.110	+ .90
HCB2	2.80	.000	135.	7	(20)	.256	+ 2.02
HCB2	2.10	.000	214.	5	(19)	.182	+ 1.38
NC-1	Control	100.	100.	3	(40)	.053	Control
							Mean t = 1.43
HC Red 3 [HCR3, M.W. = 197.22]							
Trial 1 [40]							
B(a)P	.000791	1.06	39.7	182	(19)	8.71***	+ 14.9
B(a)P	.000250	8.48	79.2	101	(18)	4.86***	+ 9.93
HCR3	6.09	.000	34.3	25	(19)	1.16**	+ 2.86
HCR3	3.04	.353	35.1	27	(19)	1.07*	+ 2.19
HCR3	1.52	11.0	72.1	44	(20)	1.29*	+ 2.24
HCR3	.761	78.8	91.1	25	(20)	1.11**	+ 2.71
NC-1	Control	100.	100.	28	(40)	.533	Control
							Mean t = 2.50
Trial 2 [57]							
B(a)P	.000791	3.55	30.1	162	(20)	7.55***	+ 18.7
B(a)P	.000250	5.32	69.9	37	(20)	1.63***	+ 6.91
HCR3	6.09	.000	9.42	29	(20)	1.18***	+ 4.69
HCR3	3.04	28.0	88.0	31	(20)	1.22***	+ 3.99
HCR3	1.52	80.5	88.8	14	(20)	.534	+ 1.66
HCR3	.761	81.6	84.0	10	(17)	.395	+ .74
NC-1	Control	100.	100.	15	(40)	.278	Control
							Mean t = 2.77
HC Red 3 [HCR3, 260886-S, M.W. = 197.22]							
Trial 1 [61]							
B(a)P	.000791	.377	32.6	95	(20)	4.28***	+ 14.3
B(a)P	.000250	9.81	69.9	43	(20)	1.92***	+ 8.76
260886-S	6.00	.000	46.1	11	(18)	.503*	+ 2.07
260886-S	3.00	2.26	67.1	12	(20)	.494*	+ 2.08
260886-S	1.50	13.6	86.4	17	(20)	.568	+ 1.89
260886-S	.750	44.9	86.4	7	(19)	.272	+ .41
NC-1	Control	100.	100.	12	(40)	.222	Control
							Mean t = 1.61
Trial 2 [99]							
B(a)P	.000791	18.9	68.4	160	(20)	6.67***	+ 12.4
B(a)P	.000250	32.1	85.0	94	(20)	3.59***	+ 7.95
260886-S	7.89	3.40	38.5	135	(20)	5.94***	+ 11.7
260886-S	3.95	9.43	38.7	81	(20)	3.38***	+ 7.92
260886-S	1.97	17.7	74.9	94	(20)	4.07***	+ 9.28
260886-S	.986	67.2	81.8	89	(20)	4.01***	+ 9.41
NC-1	Control	100.	100.	65	(80)	.586	Control
							Mean t = 9.58

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Appendix C. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c] Focus Data Type Vessels		Transformation Response[d] Foci/Vessel Focus Type	Significance[e]
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	*t*-statistic
8-Hydroxyquinoline [8HYQ, M.W. = 145.16]							
Trial 1 [3]							
B(a)P	.000791	10.7	47.1	127	(20)	5.84***	+ 14.8
B(a)P	.000250	13.4	81.6	39	(20)	1.61***	+ 5.63
8HYD	.00379	.000	2.30	6	(19,20)	.245	.00(- .30)
8HYD	.00331	.000	13.8	21	(20)	.787*	+ 2.61
8HYD	.00276	.000	19.5	20	(20)	.850**	+ 3.19
8HYD	.00186	.000	46.0	14	(20)	.547	+ 1.59
NC-1	Control	100.	100.	17	(40)	.285	Control
							Mean t = 2.46
Trial 2 [9]							
B(a)P	.000791	3.14	3.83	108	(20)	4.93***	+ 16.2
B(a)P	.000250	8.52	87.2	47	(20)	1.92***	+ 7.04
8HYD	.00344	.000	1.03	4	(20)	.149	.00
8HYD	.00276	.000	3.08	3	(20)	.110	.00(- .46)
8HYD	.00207	.000	41.0	4	(20)	.149	.00
8HYD	.00138	2.24	89.2	17	(20)	.270	+ .68
NC-1	Control	100.	100.	8	(40)	.149	Control
							Mean t = .170
Trial 3 [28]							
B(a)P	.000791	2.84	28.6	189	(20)	9.02***	+ 16.9
B(a)P	.000250	6.74	68.0	62	(20)	2.78***	+ 5.73
8HYD	.00413	.000	48.9	22	(20)	.937	+ .51
8HYD	.00276	.000	66.0	21	(20)	.756	.00(- .25)
8HYD	.00207	5.32	70.0	25	(20)	.341	.00(-1.72)
8HYD	.00138	84.8	75.2	19	(20)	.726	.00(- .39)
NC-1	Control	100.	100.	41	(40)	.818	Control
							Mean t = .128
Malaoxon [MALX, M.W. = 314.32; Density = NA g/ml]							
Trial 1 [16]							
B(a)P	.000791	.881	47.5	139	(17)	7.96***	+ 17.5
B(a)P	.000250	4.85	76.6	58	(17)	2.77***	+ 7.77
MALX	2.36	.000	.000	0	(0,18)	.000	ND
MALX	1.57	.000	.000	0	(0,18)	.000	ND
MALX	.786	.000	.000	0	(0,18)	.000	ND
MALX	.393	.000	78.8	44	(18)	1.81***	+ 4.17
NC-1	Control	100.	100.	17	(36)	.344	Control
							Mean t = 4.17
Trial 2 [25]							
B(a)P	.000791	ND	21.4	192	(18)	9.74***	+ 19.9
B(a)P	.000250	ND	73.4	59	(18)	2.55***	+ 7.52
MALX	.589	ND	9.90	0	(0,18)	.000	ND
MALX	.393	ND	61.9	87	(18)	4.64***	+ 22.7
MALX	.255	ND	84.4	29	(18)	1.15***	+ 4.24
MALX	.118	ND	85.9	9	(18)	.309	+ 1.43
NC-1	Control	ND	100.	5	(36)	.101	Control
							Mean t = 9.46
1-Naphthylamine [1NAP, M.W. = 143.18]							
Trial 1 [13]							
B(a)P	.000791	.823	30.6	95	(20)	4.38***	+ 14.2
B(a)P	.000250	2.06	75.4	66	(20)	3.14***	+ 15.7

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Appendix C. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
Drug	Conc., mM	S.A	CC.A.	Focus Data Type III	Vessels (N)	Foci/Vessel Focus Type III	t-statistic
1NAP	.210	.823	84.3	22	(20)	.775**	+ 2.85
1NAP	.140	4.94	82.0	21	(20)	.899***	+ 4.65
1NAP	.0698	14.4	96.3	16	(20)	.634**	+ 3.02
1NAP	.0349	50.6	113.	6	(20)	.214	+ .11
NC-1	Control	100.	100.	11	(40)	.201	Control
							Mean t = 2.66
Trial 2 [19]							
B(a)P	.000791	.000	62.5	99	(20)	4.61***	+ 13.4
B(a)P	.000250	1.67	89.0	100	(20)	4.02***	+ 10.6
1NAP	.279	5.83	62.9	26	(19)	1.14***	+ 3.80
1NAP	.140	15.4	72.8	28	(20)	1.09***	+ 3.46
1NAP	.0698	25.0	86.2	32	(20)	1.00*	+ 2.35
1NAP	.0349	52.5	97.6	24	(20)	.888*	+ 2.28
NC-1	Control	100.	100.	18	(38)	.357	Control
							Mean t = 2.97
N-(1-Naphthyl)ethylenediamine-2HCl [NED, M.W. = 259.18]							
Trial 1 [38]							
B(a)P	.000791	2.10	28.4	174	(20)	7.75***	+ 15.6
B(a)P	.000250	9.44	74.7	162	(20)	8.17***	+ 18.0
NED	.193	.000	.000	4	(12,19)	.230	.00(-1.33)
NED	.145	.000	64.4	9	(19)	.368	.00(- .73)
NED	.0965	33.9	78.5	16	(20)	.494	.00)- .01
NED	.0482	71.7	75.5	31	(20)	1.21**	+ 2.87
NC-1	Control	100.	100.	27	(40)	.496	Control
							Mean t = .957
Trial 2 [87]							
B(a)P	.000791	25.1	77.0	59	(20)	2.26***	+ 5.84
B(a)P	.000250	42.2	80.2	34	(20)	1.45***	+ 5.24
NED	.193	.000	.000	0	(1,20)	.000	.00(- .64)
NED	.145	.000	39.9	19	(19)	.826*	+ 2.60
NED	.0965	22.2	72.4	11	(19)	.419	+ .45
NED	.0482	105.	71.6	23	(19)	1.09***	+ 3.89
NC-1	Control	100.	100.	43	(80)	.346	Control
							Mean t = 2.31
1-Nitronaphthalene [1NNAP, M.W. = 173.17]							
Trial 1 [33]							
B(a)P	.000791	3.44	2.40	214	(20)	10.0***	+ 13.6
B(a)P	.000250	5.73	51.4	130	(20)	5.86***	+ 7.74
1NNAP	.866	.000	.000	1	(3,20)	.260	.00(-1.32)
1NNAP	.577	1.53	10.7	20	(19)	.727	.00(- .98)
1NNAP	.289	29.4	93.2	10	(20)	.394	.00(-2.85)
1NNAP	.144	49.6	98.0	9	(20)	.327	.00(-2.79)
NC-1	Control	100.	100.	54	(37)	1.04	Control
							Mean t = .000
Trial 2 [87]							
B(a)P	.000791	25.1	77.0	59	(20)	2.26***	+ 5.84
B(a)P	.000250	42.2	80.2	34	(20)	1.45***	+ 5.24
1NNAP	.577	16.4	54.5	20	(19)	.826*	+ 2.54
1NNAP	.433	25.8	68.7	24	(16)	1.11**	+ 3.37
1NNAP	.289	33.8	73.6	17	(14)	.950*	+ 2.72
1NNAP	.144	59.6	75.8	11	(14)	.641	+ 1.50
NC-1	Control	100.	100.	43	(80)	.346	Control
							Mean t = 2.53

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Appendix C. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
				Focus Data Type Vessels		Foci/Vessel Focus Type	
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	*t*-statistic
4-Nitro-*o*-Phenylenediamine [4NPD, M.W. = 153.14]							
Trial 1 [14]							
B(a)P	.000791	1.75	36.1	177	(20)	6.73***	+ 13.3
B(a)P	.000250	5.26	74.7	61	(20)	2.45***	+ 7.12
4NPD	.490	1.32	46.8	23	(20)	.955***	+ 4.48
4NPD	.245	3.95	52.2	22	(20)	.860***	+ 3.85
4NPD	.123	7.46	70.9	41	(20)	1.58***	+ 5.14
4NPD	.0306	22.8	102.	13	(20)	.481	+ 1.86
NC-1	Control	100.	100.	12	(40)	.213	Control
							Mean t = 3.83
Trial 2 [18]							
B(a)P	.000791	2.24	45.8	125	(20)	5.91***	+ 13.0
B(a)P	.000250	8.52	78.5	86	(20)	3.36***	+ 7.28
4NPD	.261	.000	81.1	25	(20)	1.10*	+ 1.99
4NPD	.131	.448	88.6	34	(20)	1.32*	+ 2.46
4NPD	.0653	8.97	86.1	14	(17)	.671	+ .04
4NPD	.0326	23.8	90.2	17	(19)	.777	+ .57
NC-1	Control	100.	100.	33	(40)	.663	Control
							Mean t = 1.27
3-Nitropropionic Acid [3NPA, M.W. = 119.08]							
Trial 1 [39]							
B(a)P	.000791	1.07	23.6	172	(20)	8.04***	+ 14.5
B(a)P	.000250	3.56	64.5	145	(20)	6.84***	+ 15.8
3NPA	3.36	.000	1.24	21	(20)	.699	+ 1.20
3NPA	1.68	.000	15.7	52	(20)	2.41***	+ 6.95
3NPA	.840	8.19	52.9	60	(20)	2.43***	+ 6.01
3NPA	.420	30.6	78.5	19	(20)	.737	+ 1.43
NC-1	Control	100.	100.	27	(40)	.427	Control
							Mean t = 3.90
Trial 2 [85]							
B(a)P	.000791	18.8	55.6	133	(20)	3.43***	+ 5.10
B(a)P	.000250	28.7	91.8	66	(19)	2.10***	+ 4.56
3NPA	2.52	2.00	21.7	74	(20)	2.82***	+ 6.63
3NPA	1.68	4.00	47.6	77	(20)	3.16***	+ 9.87
3NPA	.840	14.0	86.6	50	(20)	1.65***	+ 4.15
3NPA	.420	43.5	105.	13	(20)	.503	+ 1.24
NC-1	Control	100.	100.	38	(80)	.313	Control
							Mean t = 5.47
***p*-Phenylenediamine-2HCl** [PD, M.W. = 181.07]							
Trial 1 [37]							
B(a)P	.000791	1.60	47.6	101	(20)	4.59***	+ 9.94
B(a)P	.000250	6.80	79.7	113	(20)	5.38***	+ 13.5
PD	.110	.000	4.51	18	(20)	.702	+ .33
PD	.0552	.000	56.9	72	(20)	3.31***	+ 8.06
PD	.0276	35.6	86.0	44	(20)	1.96***	+ 4.84
PD	.0138	98.4	100.	35	(19)	1.21*	+ 2.01
NC-1	Control	100.	100.	32	(39)	.631	Control
							Mean t = 3.81
Trial 2 [89]							
B(a)P	.000791	8.13	69.0	119	(20)	4.77***	+ 10.3
B(a)P	.000250	31.9	89.1	63	(20)	2.33***	+ 5.84

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Appendix C. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c] Focus Data Type Vessels		Transformation Response[d] Foci/Vessel Focus Type	Significance[e]
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	*t*-statistic
PD	.0828	.000	65.6	45	(20)	1.80***	+ 4.88
PD	.0552	2.61	75.4	49	(18)	1.71**	+ 3.29
PD	.0276	64.2	84.4	16	(20)	.668	+ .91
PD	.0138	99.6	96.6	10	(20)	.347	.00(- .82)
NC-1	Control	100.	100.	57	(79)	.492	Control
							Mean t = 2.27
N-Phenyl-2-Naphthylamide [130668-S, M.W. = 219.30]							
Trial 1 [61]							
B(a)P	.000791	.377	32.6	94	(20)	4.28***	+ 14.3
B(a)P	.000250	9.81	69.9	43	(20)	1.92***	+ 8.76
130688-S	.227	.000	49.4	28	(20)	1.31***	+ 7.14
130688-S	.170	.000	47.9	29	(20)	1.14***	+ 4.27
130688-S	.114	6.42	65.0	26	(20)	1.08***	+ 4.95
130688-S	.0568	50.9	87.7	16	(20)	.634**	+ 2.81
NC-1	Control	100.	100.	12	(40)	.222	Control
							Mean t = 4.79
Trial 2 [87]							
B(a)P	.000791	25.1	77.2	59	(20)	2.26***	+ 5.84
B(a)P	.000250	42.2	80.2	34	(20)	1.45***	+ 5.24
130688-S	.227	1.64	75.6	36	(20)	1.50***	+ 5.28
130688-S	.170	8.18	86.3	24	(20)	.903**	+ 2.89
130688-S	.114	54.5	97.6	29	(20)	1.21***	+ 4.24
130688-S	.0568	74.9	100.	20	(20)	.787*	+ 2.43
NC-1	Control	100.	100.	43	(80)	.346	Control
							Mean t = 3.71
2,3,5,6,-Tetrachloro-4-Nitroanisole [TC4NA, M.W. = 290.91]							
Trial 1 [29]							
B(a)P	.000791	1.15	57.0	122	(20)	5.79***	+ 13.1
B(a)P	.000250	2.29	78.5	142	(20)	6.19***	+ 10.3
TCNA	.155	.000	.000	0	(3,20)	.000	.00(-5.58)
TCNA	.103	.000	.000	1	(15,20)	.047	.00(-4.42)
TCNA	.052	.000	34.3	33	(20)	1.50**	+ 3.32
TCNA	.025	12.6	76.2	26	(20)	1.03	+ 1.61
NC-1	Control	100.	100.	36	(40)	.606	Control
							Mean t = 2.47
Trial 2 [93]							
B(a)P	.000791	2.65	46.7	138	(20)	6.48***	+ 16.8
B(a)P	.000250	7.96	79.9	115	(20)	4.80***	+ 12.0
TCNA	.0859	.000	.000	0	(0,19)	.000	NA
TCNA	.0645	.000	.000	0	(3,20)	.000	.00(-7.56)
TCNA	.0430	.845	7.12	38	(18)	1.48**	+ 3.40
TCNA	.0215	8.85	82.3	10	(20)	.394	.00(-.16)
NC-1	Control	100.	100.	43	(79)	.416	Control
							Mean t = 1.70
Tetraethylthiuram Disulfide [TETD, M.W. = 296.54]							
Trial 1 [80]							
B(a)P	.000791	16.9	65.2	261	(20)	12.9***	+ 13.0
B(a)P	.000250	35.6	87.2	185	(20)	8.53***	+ 7.65
TETD	.000202	.000	1.62	141	(20)	6.79***	+ 6.86
TETD	.000101	.000	6.00	159	(20)	7.77***	+ 9.12
TETD	.0000506	11.6	77.5	107	(20)	4.82*	+ 2.43
TETD	.0000253	87.5	104.	67	(20)	2.62	.00(- .67)
NC-1	Control	100.	100.	317	(80)	3.02	Control
							Mean t = 4.60

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Appendix C. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c] Focus Data Type Vessels		Transformation Response[d] Foci/Vessel Focus Type	Significance[e]
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	*t*-statistic
Trial 2 [93]							
B(a)P	.000791	2.65	46.7	138	(20)	6.48***	+ 16.8
B(a)P	.000250	7.96	79.9	115	(20)	4.80***	+ 12.0
TETD	.000169	.000	.000	0	(0,20)	.000	ND
TETD	.0000843	1.77	.792	26	(20)	.999**	+ 3.13
TETD	.0000422	31.0	79.2	34	(20)	1.44***	+ 5.12
TETD	.0000211	46.0	108.	13	(20)	.494	+ .51
NC-1	Control	100.	100.	43	(79)	.416	Control
							Mean t = 2.92
2,6-Toluenediamine-2HCl [26TD, M.W. = 195.11]							
Trial 1 [29]							
B(a)P	.000791	1.15	57.0	122	(20)	5.79***	+ 13.1
B(a)P	.000250	2.29	78.5	142	(20)	6.19***	+ 10.3
T26D	8.20	.000	3.29	24	(19)	.825	+ .82
T26D	6.15	.000	14.2	31	(20)	1.31*	+ 2.63
T26D	4.10	1.53	43.5	40	(20)	1.54**	+ 3.06
T26D	2.05	31.7	92.7	94	(20)	4.35***	+ 9.05
NC-1	Control	100.	100.	36	(40)	.606	Control
							Mean t = 3.89
Trial 2 [44]							
B(a)P	.000791	5.07	25.9	335	(20)	15.8***	+ 16.3
B(a)P	.000250	14.2	67.2	137	(20)	6.15***	+ 7.33
T26D	4.00	5.41	30.4	91	(20)	4.23***	+ 5.29
T26D	2.00	45.6	55.6	172	(20)	7.76***	+ 8.77
T26D	1.00	79.1	64.0	180	(20)	8.42***	+ 11.3
T26D	.500	91.2	88.4	218	(19)	11.1***	+ 14.7
NC-1	Control	100.	100.	77	(40)	1.52	Control
							Mean t = 10.0

Abbreviations: BaP, benzo(a)pyrene; CC.A., co-culture clonal survival assay; Conc., concentration; mM, millimole; M.W., molecular weight; N, number of culture vessels, NC, negative control; ND; not determined; %RCE, percent relative cloning efficiency; S.A., standard clonal survival assay.

[a]Treatment condition: The experimental design for the transformation assay is described in detail in the Materials and Methods. The concentration of the positive control and test chemical treatment are presented in mM, but they can be converted to μg/ml using the molecular weight that is provided with each chemical. The solvent vehicles used for the individual test chemicals were listed in Appendix Tables A1 and A3, and the concentrations of the solvent vehicles are presented in the Materials and Methods.

[b]Cytotoxic activity: The experimental design for the standard survival assay (SA) and the co-culture clonal survival assay (CCA) were described in the Materials and Methods. The test chemical cytotoxic response was expressed as % RCE and was calculated as described in the Materials and Methods.

[c]The criteria used to evaluate the transformed foci of BALB/c-3T3 cells is described in the Materials and Methods. The number of type III foci > 2-mm in diameter per culture vessel scored are recorded in this table.

[d]Transformation response: The transformation responses are expressed as type III foci/vessel and were calculated using a $\log_{10}$ mathematical transformation procedure (refer to Materials and Methods). The arithmetic value or foci/vessel represents the antilog of the $\log_{10}$ mean transformation response minus one.

[e]Significance: The significance of test chemical transformation responses was calculated by a computer using the SAS statistical software (*22*), and the method is described in detail in Materials and Methods. The correct *t*-statistic according to the F-test is presented in this table. The *t*-statistics of each treatment dose of the test chemical in a single experiment were averaged to determine the mean *t*-statistic of the test chemical for the experiment (refer to Appendix Tables A2 and A5). The mean *t*-statistics for two or experiments for each chemical was weighted to the number of treatment doses evaluated and averaged to determine the rank *t*-statistic which was used to rank-order the test chemical transformation responses in Appendix Tables A3 and A6. Arbitrarily, transformation responses with negative (−) *t*-statistics were given a value of zero (0).

*Significant BaP or test chemical transformation response, $0.01 < p \leq 0.05$.

**Significant BaP or test chemical transformation response, $0.001 < p \leq 0.01$.

***Significant or BaP or test chemical transformation response, $p \leq 0.001$.

Appendix D.

Summary of the transformation responses of 20 cytotoxic, nonmutagenic carcinogens.

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
				Focus Data Type	Vessels	Foci/Vessel Focus Type	
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	*t*-statistic
Allyl Isothiocyanate [ALITC, M.W. = 99.16, Density = 1.0165 g/ml]							
Trial 1 [41]							
B(a)P	.000791	1.29	33.6	189	(18)	10.2***	+ 23.1
B(a)P	.000250	6.45	78.2	123	(18)	6.37***	+ 17.1
ALITC	.0133	.000	47.5	9	(18)	.339	+ .44
ALITC	.00666	12.9	69.1	11	(18)	.503	+ 1.57
ALITC	.00333	22.6	76.4	29	(18)	1.30***	+ 4.21
ALITC	.00166	63.9	91.0	7	(18)	.309	+ .27
NC-1	Control	100.	100.	13	(36)	.274	Control
							Mean t = 1.62
Trial 2 [98]							
B(a)P	.000791	3.60	51.9	91	(18)	4.63***	+ 3.24
B(a)P	.000250	4.50	77.2	37	(18)	1.73***	+ 15.0
ALITC	.0133	.901	.177	8	(16)	.414	+ 1.38
ALITC	.00666	2.70	3.89	22	(17)	1.09***	+ 4.71
ALITC	.00333	18.5	43.5	46	(18)	2.25***	+ 8.86
ALITC	.00166	73.9	94.0	10	(18)	.424	+ 1.40
NC-1	Control	100.	100.	11	(36)	.226	Control
							Mean t = 4.09
Chlorendic Acid [954870-S, M.W. = 388.83]							
Trial 1 [63]							
B(a)P	.000791	4.00	80.5	141	(20)	6.13***	+ 6.87
B(a)P	.000250	24.8	93.1	93	(20)	3.20*	+ 2.02
954870-S	4.00	32.7	30.5	45	(18)	1.78	.00 (- .29)
954870-S	2.00	37.8	38.0	55	(20)	2.34	+ 1.08
954870-S	1.00	46.1	71.3	107	(18)	4.68***	+ 4.70
954870-S	.500	94.9	96.6	63	(20)	2.76*	+ 2.05
NC-1	Control	100.	100.	84	(39)	1.92	Control
							Mean t = 1.96
Trial 2 [83]							
B(a)P	.000791	2.86	73.0	141	(20)	6.14***	+ 13.5
B(a)P	.000250	13.8	78.9	64	(20)	2.93***	+ 9.12
954870-S	3.85	27.6	37.5	12	(20)	.473	+ .73
954870-S	1.92	52.4	56.3	54	(20)	2.27***	+ 7.30
954870-S	.962	70.5	89.2	9	(20)	.347	.00 (- .02)
954870-S	.481	98.1	94.3	6	(20)	.214	.00 (- .93)
NC-1A+1B	Control	100.	100.	48	(80)	.351	Control
							Mean t = 2.01
Allyl Isovalerate [ALIV, M.W. = 142.22, Density = 0.882 g/ml]							
Trial 1 [23]							
B(a)P	.000791	.000	61.0	157	(18)	6.71***	+ 9.25
B(a)P	.000250	4.84	100.	57	(18)	3.04***	+ 8.07
ALIV	.744	6.67	98.5	8	(18)	.361	.00(-1.52)
ALIV	.372	58.8	108.	9	(18)	.370	.00(-1.40)
ALIV	.186	89.1	105.	13	(18)	.562	.00(- .43)
ALIV	.0930	90.9	87.7	16	(18)	.737	+ .32
NC-1	Control	100.	100.	23	(27)	.661	Control
							Mean t = .080

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Appendix D. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c] Focus Data Type Vessels		Transformation Response[d] Foci/Vessel Focus Type	Significance[e]
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	*t*-statistic
Trial 2 [27]							
B(a)P	.000791	4.04	33.7	170	(18)	8.90***	+ 14.8
B(a)P	.000250	13.0	47.4	73	(18)	3.18***	+ 5.71
ALIV	1.40	17.7	61.5	7	(18)	.260	.00(-1.41)
ALIV	.930	35.1	45.6	12	(18)	.513	.00(- .18)
ALIV	.465	62.7	75.6	11	(18)	.456	.00(- .43)
ALIV	.233	88.2	52.4	14	(18)	.608	+ .22
NC-1	Control	100.	100.	31	(36)	.555	Control
						Mean t = .055	
Trial 3 [31]							
B(a)P	.000791	1.87	69.1	136	(15)	8.63***	+ 11.1
B(a)P	.000250	5.14	99.9	126	(18)	6.06***	+ 8.51
ALIV	5.58	.000	.000	0	(0,18)	.000	ND
ALIV	4.34	.000	53.3	1	(4,18)	.189	.00(-2.50)
ALIV	3.10	.000	94.3	28	(17)	1.30	+ 1.18
ALIV	1.86	.000	109.	19	(18)	.834	.00(- .35)
NC-1	Control	100.	100.	43	(36)	.930	Control
						Mean t = .591	
Trial 4 [102]							
B(a)P	.000791	9.64	69.9	99	(18)	4.65***	+ 9.48
B(a)P	.000250	19.3	93.1	45	(18)	2.22***	+ 5.22
ALIV	5.89	.000	13.6	3	(4,18)	.565	.00(- .33)
ALIV	4.42	.000	83.2	41	(18)	2.05***	+ 4.83
ALIV	2.95	.419	79.2	26	(18)	1.10	+ 1.64
ALIV	1.47	7.55	91.5	19	(18)	.828	+ .59
NC-1	Control	100.	100.	64	(72)	.697	Control
						Mean t = 2.35	
Chlorinated Paraffins C23 43% Chlorine [Chlorowax 40, 499546-L, M.W.avg. = 560, Density = ND]							
Trial 1 [76]							
B(a)P	.000791	5.91	63.1	87	(18)	4.41***	+ 5.23
B(a)P	.000250	23.3	97.0	54	(18)	2.72*	+ 2.26
499546-L	ND	74.9	78.9	43	(18)	2.06	+ .72
495546-L	ND	90.0	75.9	35	(18)	1.67	.00(- .33)
495546-L	ND	82.4	83.6	67	(18)	3.02**	+ 2.68
499546-L	ND	87.7	84.8	18	(12)	1.26	.00(-1.36)
NC-1	Control	100.	100.	152	(71)	1.79	Control
						Mean t = .850	
Trial 2 [104]							
B(a)P	.000791	25.0	64.5	62	(18)	2.79***	+ 4.87
B(a)P	.000250	50.6	89.6	63	(18)	2.47***	+ 4.10
499546-L	ND	111.	98.2	17	(18)	.650	.00(- .90)
499546-L	ND	113.	99.7	10	(17)	.386	.00(-2.12)
499546-L	ND	111.	100.	21	(18)	.961	+ .31
499546-L	ND	110.	101.	14	(18)	.562	.00(-1.31)
NC-1	Control	100.	100.	83	(71)	.878	Control
						Mean t = .078	
Chlorinated Paraffins C12 60% Chlorine [Chlorowax 500c, 164848-L, M.W.avg.= 415, Density= ND]							
Trial 1 [74]							
B(a)P	.000791	3.00	69.2	43	(18)	2.02***	+ 4.51
B(a)P	.000250	9.58	78.4	27	(18)	1.29*	+ 2.49
164848-L	2.89	.000	.000	0	(0,18)	.000	ND
164848-L	1.93	.000	.000	0	(0,18)	.000	ND
164848-L	.964	.000	68.3	16	(18)	.737	+ .37
164848-L	.482	24.6	98.6	11	(18)	.456	.00(-1.00)
NC-1	Control	100.	100.	65	(71)	.657	Control
						Mean t = .185	

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Appendix D. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c] Focus Data Type	Vessels	Transformation Response[d] Foci/Vessel Focus Type	Significance[e]
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	t-statistic
Trial 2 [90]							
B(a)P	.000791	28.5	76.0	157	(18)	7.60***	+ 5.89
B(a)P	.000250	51.8	95.8	111	(18)	4.91***	+ 3.71
164848-L	1.93	6.61	58.4	15	(18)	.618	.00(-3.32)
164848-L	1.45	41.4	64.5	20	(18)	.628	.00(-3.17)
164848-L	.964	29.2	78.7	27	(18)	1.07	.00(-1.89)
164848-L	.482	94.6	107.	15	(18)	.661	.00(-4.16)
NC-1	Control	100.	100.	219	(71)	1.95	Control
							Mean t = .000
3-Chloro-2-Methylpropene [3C2MP, M.W. = 99.55, Density = 0.928 g/ml]							
Trial 1 [29]							
B(a)P	.000791	8.09	60.6	116	(18)	6.11***	+ 4.93
B(a)P	.000250	14.9	84.2	184	(18)	9.84***	+ 9.74
3C2MP	.883	.000	60.3	162	(18)	8.23***	+ 5.22
3C2MP	.442	12.8	97.0	102	(18)	4.21	+ 1.25
3C2MP	.221	56.2	97.6	60	(18)	2.53	.00 (- 1.23)
3C2MP	.110	57.4	95.1	54	(18)	2.72	.00 (- .95)
NC-1	Control	100.	100.	296	(72)	3.28	Control
							Mean t = 1.62
Trial 2 [31]							
B(a)P	.000791	15.8	73.9	54	(15)	3.36***	+ 9.58
B(a)P	.000250	35.5	82.6	24	(15)	1.50***	+ 4.90
3C2MP	1.104	.000	42.3	24	(17,18)	.928*	+ 2.23
3C2MP	.828	.000	57.5	36	(18)	1.75***	+ 5.87
3C2MP	.552	1.18	81.2	23	(18)	.805	+ 1.58
3C2MP	.276	55.8	102.	16	(18)	.582	+ .95
NC-1	Control	100.	100.	37	(67)	.406	Control
							Mean t = 2.66
Cinnamyl Anthranilate [CIN, M.W. = 253.32]							
Trial 1 [2]							
B(a)P	.000791	1.02	36.4	187	(20)	8.89***	+ 15.5
B(a)P	.000250	3.41	68.7	110	(20)	4.52***	+ 8.43
CIN	.115	.000	17.2	5	(20)	.172	.00(-2.97)
CIN	.105	3.07	17.9	37	(20)	.656	.00(- .01)
CIN	.0829	58.0	83.8	16	(20)	.668	+ .04
CIN	.0651	61.1	99.0	5	(20)	.189	.00(-2.90)
NC-1	Control	100.	100.	34	(20)	.660	Control
							Mean = .010
Trial 2 [9]							
B(a)P	.000791	3.14	38.3	108	(20)	4.93***	+ 16.2
B(a)P	.000250	8.52	87.2	47	(20)	1.92***	+ 7.04
CIN	.118	2.69	22.5	2	(16,20)	.091	.00(- .65)
CIN	.0987	20.6	51.3	2	(20)	.076	.00(- .90)
CIN	.0790	48.0	107.	2	(20)	.056	.00(-1.13)
CIN	.0592	53.4	93.3	1	(20)	.035	.00(-1.85)
NC-1	Control	100.	100.	8	(40)	.149	Control
							Mean t = .000
Trial 3 [DRI3]							
B(a)P	.000791						
B(a)P	.000250						
CIN	.142	ND	.000	0	(17)	.000	ND
CIN	.095	ND	37.3	8	(18)	.318	.00(- .45)
CIN	.0475	ND	109.	8	(20)	.275	.00(- .66)
NC-1	Control	ND	100.	5	(9)	.424	Control
							Mean t = .000

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Appendix D. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
				Focus Data Type Vessels		Foci/Vessel Focus Type	
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	*t*-statistic
Diethylstilbestrol [DES, M.W. = 268.]							
Trial 1 [42]							
B(a)P	.000791	2.41	35.7	280	(20)	13.7***	+ 20.2
B(a)P	.000250	5.72	65.4	161	(19)	7.44***	+ 9.55
DES	.0894	1.81	13.5	31	(19,20)	1.19	+ .97
DES	.0671	62.0	65.1	15	(20)	.578	.00 (- 1.10)
DES	.0447	54.5	64.4	11	(20)	.443	.00 (- 1.76)
DES	.0112	75.6	77.5	154	(19)	6.79***	+ 8.84
NC-1	Control	100.	100.	52	(40)	.861	Control
							Mean t = 2.22
Trial 2 [96]							
B(a)P	.000791	11.0	43.5	86	(20)	4.03***	+ 9.40
B(a)P	.000250	38.9	75.4	62	(20)	2.88***	+ 7.12
DES	.112	31.4	74.6	37	(20)	1.62***	+ 3.69
DES	.0838	51.5	86.5	44	(20)	1.89***	+ 4.44
DES	.0559	54.2	83.4	37	(20)	1.52**	+ 3.30
DES	.0279	59.3	79.0	32	(20)	1.19*	+ 2.07
NC-1	Control	100.	100.	62	(70)	.660	Control
							Mean t = 3.38
Dimethylvinyl Chloride [309712-L, M.W. = 90.55, Density = ND g/ml]							
Trial 1 [76]							
B(a)P	.000791	5.91	63.1	87	(18)	4.41***	+ 5.23
B(a)P	.000250	23.3	97.0	54	(18)	2.72*	+ 2.26
309712-L	4.00	.000	41.2	116	(15,18)	5.56**	+ 3.88
309712-L	2.00	.000	84.4	59	(18)	2.63	+ 1.91
309712-L	1.00	15.4	87.1	38	(18)	1.69	.00 (- .24)
309712-L	.500	58.8	94.3	41	(18)	1.62	.00 (- .43)
NC-1	Control	100.	100.	152	(71)	1.79	Control
							Mean t = 1.45
Trial 2 [102]							
B(a)P	.000791		69.9	99	(18)	4.65***	+ 9.48
B(a)P	.000250		93.1	45	(18)	2.22***	+ 5.22
309712-L	7.78	.000	27.5	19	(7,18)	2.26***	+ 3.47
309712-L	5.83	.000	77.1	84	(18)	4.04***	+ 8.63
309712-L	3.89	.000	96.5	31	(18)	1.27*	+ 2.21
309712-L	1.94	13.4	93.3	17	(18)	.834	+ .65
NC-1	Control	100.	100.	64	(72)	.697	Control
							Mean t = 3.74
Ethyl Acrylate [ETAC, M.W. = 100.12, Density = ND g/ml]							
Trial 1 [23]							
B(a)P	.000791	.000	61.0	157	(18)	6.71***	+ 9.25
B(a)P	.000250	4.84	100.	57	(18)	3.04***	+ 8.07
ETAC	.1199	.000	.000	1	(1,18)	1.00	.00 (- 4.75)
ETAC	.0799	.000	21.8	59	(18)	3.03***	+ 6.71
ETAC	.0400	4.85	84.9	20	(18)	.864	+ .77
ETAC	.0200	37.0	97.9	7	(18)	.289	.00 (- 1.90)
NC-1	Control	100.	100.	23	(27)	.661	Control
							Mean t = 2.49
Trial 2 [36]							
B(a)P	.000791	2.98	40.1	73	(9)	7.27***	+ 21.8
B(a)P	.000250	7.66	78.1	88	(18)	4.37***	+ 10.8

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Appendix D. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
Drug	Conc., mM	S.A	CC.A.	Focus Data Type Vessels III	(N)	Foci/Vessel Focus Type III	t-statistic
ETAC	.1199	1.28	55.8	45	(18)	1.87***	+ 4.96
ETAC	.0899	8.09	79.8	53	(18)	2.68***	+ 8.05
ETAC	.0599	27.7	94.8	50	(18)	2.20**	+ 5.02
ETAC	.0300	63.4	99.3	43	(18)	2.08***	+ 6.04
NC-1	Control	100.	100.	20	(36)	.424	Control
							Mean t = 6.02
Isophorone [ISPH, M.W. = 138.21, Density = 0.9229 g/ml]							
Trial 1 [25]							
B(a)P	.000791	ND	21.4	192	(18)	9.74***	+ 19.9
B(a)P	.000250	ND	73.4	59	(18)	2.55***	+ 7.52
ISPH	1.34	ND	92.2	7	(18)	.268	.00 (- 1.35)
ISPH	1.00	ND	89.6	3	(18)	.122	+ .27
ISPH	.668	ND	102.	3	(18)	.122	+ .27
ISPH	.334	ND	102.	3	(18)	.122	+ .27
NC-1	Control	ND	100.	5	(36)	.101	Control
							Mean t = .203
Trial 2 [36]							
B(a)P	.000791	2.98	40.1	73	(9)	7.27***	+ 21.8
B(a)P	.000250	7.66	78.1	88	(18)	4.37***	+ 10.8
ISPH	4.01	.000	31.5	33	(18)	1.65**	+ 5.29
ISPH	2.00	.851	92.9	17	(18)	.754	+ 1.66
ISPH	1.00	21.3	98.6	20	(18)	.950*	+ 2.60
ISPH	.501	76.6	108.	28	(18)	1.38**	+ 4.29
NC-1	Control	100.	100.	20	(36)	.424	Control
							Mean t = 3.46
Trial 3 [104]							
B(a)P	.000791	25.0	64.5	62	(18)	2.79***	+ 4.87
B(a)P	.000250	50.6	89.6	63	(18)	2.47***	+ 4.10
ISPH	5.34	6.75	80.2	34	(18)	1.68*	+ 2.61
ISPH	4.01	16.8	103.	30	(18)	1.53**	+ 2.85
ISPH	2.67	31.6	104.	36	(18)	1.66*	+ 2.45
ISPH	1.34	45.9	97.6	21	(18)	.963	+ .32
NC-1	Control	100.	100.	83	(71)	.878	Control
							Mean t = 2.06
D-Limonene [036267-L, M.W. = 136.24, Density = 0.8411 g/ml]							
Trial 1 [72]							
B(a)P	.000791	2.75	56.8	85	(18)	4.11***	+ 12.7
B(a)P	.000250	6.61	82.1	84	(18)	3.04***	+ 6.20
036267-L	.224	.000	90.7	7	(18)	.248	.00(- .30)
036267-L	.179	.000	101.	9	(18)	.392	+ .75
036267-L	.134	.000	94.4	17	(18)	.619	+ 1.54
036267-L	.089	.000	96.2	15	(18)	.572	+ 1.80
NC-1	Control	100.	100.	29	(72)	.289	Control
							Mean t = 1.02
Trial 2 [76]							
B(a)P	.000791	5.91	63.1	87	(18)	4.41***	+ 5.23
B(a)P	.000250	23.3	97.0	54	(18)	2.72*	+ 2.26
036267-L	1.57	.000	.893	1	(17,18)	.042	.00(-13.6)
036267-L	1.18	18.4	33.8	15	(18)	.557	.00(- 4.30)
036267-L	.786	49.6	104.	60	(17)	1.95	+ .27
036267-L	.393	82.4	100.	30	(18)	1.39	+ 1.15
NC-1	Control	100.	100.	152	(71)	1.79	Control
							Mean t = .473

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Appendix D. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c] Focus Data Type Vessels		Transformation Response[d] Foci/Vessel Focus Type	Significance[e]
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	*t*-statistic
Trial 3 [88]							
B(a)P	.000791	15.8	73.9	54	(15)	3.36***	+ 9.58
B(a)P	.000250	35.5	82.6	24	(15)	1.50***	+ 4.90
036267-L	1.43	.000	.167	0	(6,16)	.000	.00(-6.49)
036267-L	1.07	9.07	10.7	4	(14)	.194	.00(-1.32)
036267-L	.714	88.0	85.7	10	(18)	.392	.00(- .09)
036267-L	.357	103.	90.1	9	(18)	.392	.00(- .09)
NC-1	Control	100.	100.	37	(67)	.406	Control
							Mean t = .000
Malonaldehyde, Sodium Salt [605428-S, M.W. = 94.05]							
Trial 1 [75]							
B(a)P	.000791	7.10	66.5	149	(20)	6.35***	+ 10.9
B(a)P	.000250	28.4	85.4	67	(20)	3.10***	+ 6.56
605428-S	5.00	.000	14.2	34	(20)	1.18	+ 1.08
605428-S	3.75	2.92	66.5	19	(20)	.777	.00 (- .68)
605428-S	2.50	15.9	80.9	61	(20)	2.65***	+ 5.06
605428-S	1.25	59.3	93.1	38	(19)	1.79*	+ 2.61
NC-1	Control	100.	100.	89	(78)	.882	Control
							Mean t = 1.92
Trial 2 [97]							
B(a)P	.000791	4.74	78.0	118	(20)	5.02***	+ 12.5
B(a)P	.000250	17.4	104.	52	(20)	2.26***	+ 7.26
605428-S	5.00	.000	14.2	18	(19)	.751	+ 1.81
605428-S	3.75	.000	44.1	9	(20)	.320	.00 (- .62)
605428-S	2.50	9.49	89.2	26	(20)	1.04**	+ 3.08
605428-S	1.25	44.3	89.0	18	(20)	.652	+ 1.32
NC-1	Control	100.	100.	47	(80)	.414	Control
							Mean t = 1.55
2-Mercaptobenzothiazole [481989-S, M.W. = 167.25]							
Trial 1 [62]							
B(a)P	.000791	5.24	72.5	188	(20)	8.32*	+ 2.50
B(a)P	.000250	18.6	85.1	105	(18)	5.59	.00 (- .62)
481989-S	.294	.000	1.91	15	(20)	.543	.00 (-12.8)
481989-S	.221	.476	11.0	48	(20)	1.84	.00 (- 5.85)
481989-S	.147	16.1	70.1	56	(17)	2.90	.00 (- 5.06)
481989-S	.074	49.0	84.5	60	(20)	2.42	.00 (- 5.59)
NC-1	Control	100.	100.	261	(40)	6.02	Control
							Mean t = .00
Trial 2 [77]							
B(a)P	.000791	5.66	69.5	179	(20)	8.33***	+ 13.6
B(a)P	.000250	16.5	78.7	114	(19)	6.03***	+ 10.0
481989-S	.265	.000	.275	2	(6)	.260	.00 (- 2.24)
481989-S	.199	ND	ND	7	(10)	.490	.00 (- 1.74)
481989-S	.132	7.55	19.3	11	(2)	5.33**	+ 3.41
481989-S	.066	11.8	82.0	12	(6)	1.70	+ 1.52
NC-1A+1B	Control	100.	100.	94	(78)	.97	Control
							Mean t = 1.23
Trial 3 [89]							
B(a)P	.000791	8.13	69.0	119	(20)	4.77***	+ 10.3
B(a)P	.000250	31.9	89.1	63	(20)	2.33***	+ 5.84

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Appendix D. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
				Focus Data Type Vessels		Foci/Vessel Focus Type	
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	t-statistic
481989-S	.212	.581	.771	22	(20)	.762	+ 1.26
481989-S	.159	23.8	19.9	41	(20)	1.45***	+ 3.63
481989-S	.106	33.1	57.6	7	(14)	.385	.00 (- .52)
481989-S	.0530	47.3	84.3	6	(12)	.414	.00 (- .35)
NC-1A+1B	Control	100.	100.	57	(79)	.492	Control
							Mean t = 1.22
Methapyrilene-HCl [MEPY, M.W. = 297.88]							
Trial 1 [40]							
B(a)P	.000791	1.06	39.7	182	(19)	8.71***	+ 14.9
B(a)P	.000250	8.48	79.2	101	(18)	4.86***	+ 9.93
MEPY	1.43	.000	4.87	4	(17,20)	.177	.00(-2.22)
MEPY	.955	.707	53.1	14	(20)	.468	.00(- .34)
MEPY	.477	21.9	88.7	7	(20)	.256	.00(-1.72)
MEPY	.239	68.9	95.2	6	(19)	.208	.00(-2.00)
NC-1	Control	100.	100.	28	(40)	.533	Control
							Mean t = .000
Trial 2 [54]							
B(a)P	.000791	1.47	17.6	105	(20)	4.75***	+ 13.8
B(a)P	.000250	.490	61.1	100	(20)	4.52***	+ 13.5
MEPY	1.15	.000	.000	3	(17,20)	.130	.00(-1.10)
MEPY	.859	3.92	18.6	7	(20)	.231	.26(- .26)
MEPY	.573	20.6	81.1	11	(20)	.443	+ 1.25
MEPY	.286	33.8	81.4	3	(20)	.110	.00(-1.39)
NC-1	Control	100.	100.	15	(40)	.265	Control
							Mean t = .417
Trial 3 [DRI4]							
B(a)P	.000791						
B(a)P	.000250	ND		ND		ND	ND ND
MEPY	1.28	ND	13.2	0	(20)	.04	.00(-3.94)
MEPY	.853	ND	83.2	8	(20)	.32	.00(-1.68)
MEPY	.426	ND	100.	5	(20)	.19	.00(-2.51)
NC-1	Control	ND	100.	18	(20)	.67	Control
							Mean t = .000
Nitrilotriacetic Acid, Trisodium Salt [NTTA, M.W. = 257.1]							
Trial 1 [73]							
B(a)P	.000791	2.21	71.9	61	(19)	2.60***	+ 8.09
B(a)P	.000250	7.18	88.8	48	(20)	1.58***	+ 4.37
NTTA	7.78	.000	.836	3	(15,20)	.127	.00 (- .95)
NTTA	5.83	.000	12.7	24	(20)	.807*	+ 2.76
NTTA	3.89	14.4	87.1	28	(20)	1.03***	+ 3.72
NTTA	1.94	44.8	92.5	10	(20)	.414	+ .91
NC-1	Control	100.	100.	45	(79)	.274	Control
							Mean t = 2.46
Trial 2 [99]							
B(a)P	.000791	18.9	68.4	160	(20)	6.67***	+ 12.4
B(a)P	.000250	32.1	85.0	94	(20)	3.59***	+ 7.95
NTTA	7.00	7.92	65.0	125	(20)	5.58***	+ 11.5
NTTA	5.25	23.8	89.3	148	(20)	6.87***	+ 13.3
NTTA	3.50	61.9	108.	64	(20)	2.37***	+ 4.55
NTTA	1.75	76.6	103.	49	(20)	1.78***	+ 4.30
NC-1	Control	100.	100.	65	(80)	.586	Control
							Mean t = 8.41

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Appendix D. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c] Focus Data Type Vessels		Transformation Response[d] Foci/Vessel Focus Type	Significance[e]
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	*t*-statistic
Polybrominated Biphenyl Mixture [PBB, M.W. = 628.]							
Trial 1 [20]							
B(a)P	.000791	.000	39.4	268	(20)	13.0***	+ 26.3
B(a)P	.000250	2.34	77.3	99	(20)	4.00***	+ 9.22
PBB	.398	13.3	75.3	29	(19)	1.25***	+ 3.85
PBB	.199	33.6	92.4	23	(18)	1.07**	+ 3.30
PBB	.100	35.9	92.4	18	(20)	.668	+ 1.55
PBB	.050	53.2	94.9	11	(20)	.394	+ .16
NC-1	Control	100.	100.	21	(40)	.368	Control
							Mean t = 2.22
Trial 2 [28]							
B(a)P	.000791	2.84	28.6	189	(20)	9.02***	+ 16.9
B(a)P	.000250	6.74	68.0	62	(20)	2.78***	+ 5.73
PBB	.398	14.2	47.4	41	(20)	1.77**	+ 3.24
PBB	.199	67.8	44.3	33	(20)	1.37*	+ 2.00
PBB	.100	84.0	58.2	19	(20)	.737	.00 (- .35)
PBB	.0500	86.9	67.0	27	(20)	1.15	+ 1.31
NC-1	Control	100.	100.	41	(40)	.818	Control
							Mean t = 1.64
Reserpine [RES, M.W. = 608.70]							
Trial 1 [1]							
B(a)P	.000791	9.75	56.3	171	(20)	8.16***	+ 12.1
B(a)P	.000250	15.5	91.0	114	(20)	5.18***	+ 6.68
RES	.0230	.000	.000	0	(4,20)	.000	.00(-10.2)
RES	.0197	2.53	.000	0	(16,20)	.000	.00(-10.2)
RES	.0156	22.4	57.6	9	(20)	.301	.00(- 4.41)
RES	.0099	94.2	105.	51	(20)	2.36**	+ 2.37
NC-1	Control	100.	100.	73	(40)	1.44	Control
							Mean t = 1.38
Trial 2 [8]							
B(a)P	.000791	1.45	5.83	244	(19)	10.7***	+ 8.19
B(a)P	.000250	4.35	52.4	254	(20)	11.8***	+ 11.2
RES	.0164	28.0	.000	17	(20)	.677	.00(-4.29)
RES	.0123	68.1	91.3	37	(20)	1.60	.00(-1.36)
RES	.0082	94.2	202.	39	(20)	1.54	.00(-1.46)
RES	.0041	90.3	221.	52	(20)	2.03	.00(- .32)
NC-1	Control	100.	100.	110	(40)	2.19	Control
							Mean t = .000
Trial 3 [DRI3]							
B(a)P	.000791	ND	ND				
B(a)P	.000250	ND	ND				
RES	.0148	ND	54.5	5	(15)	.277	.00(- .61)
RES	.00986	ND	101.	32	(16)	1.31*	+ 1.80
RES	.00493	ND	106.	11	(13)	.682	+ .86
NC-1	Control	ND	100.	5	(9)	.424	Control
							Mean t = .887
Tris(2-ethylhexyl)phosphate [T2EHP, M.W. = 434.65, Density = 0.925 g/ml]							
Trial 1 [88]							
B(a)P	.000791	15.8	73.9	54	(15)	3.36***	+ 9.58
B(a)P	.000250	35.5	82.6	24	(15)	1.50***	+ 4.90

(Continued on next page)

Appendix D. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
Drug	Conc., mM	S.A	CC.A.	Focus Data Type Vessels III	(N)	Foci/Vessel Focus Type III	*t*-statistic
T2EHP	.920	.000	.000	0	(2,18)	.000	.00(-6.49)
T2EHP	.460	.000	27.4	7	(18)	.260	.00(- .97)
T2EHP	.230	.000	74.9	2	(18)	.080	.00(-2.50)
T2EHP	.115	.000	84.7	6	(18)	.260	.00(-1.00)
NC-1	Control	100.	100.	37	(67)	.406	Control Mean t = .000
Trial 2 [98]							
B(a)P	.000791	8.38	79.6	21	(18)	6.82***	+ 11.8
B(a)P	.000250	29.3	91.3	17	(18)	3.38***	+ 6.81
T2EHP	.575	.000	36.5	10	(17,18)	.453	.00(- .76)
T2EHP	.431	7.33	69.0	7	(18)	.268	.00(-1.79)
T2EHP	.287	9.42	73.8	11	(18)	.479	.00(- .65)
T2EHP	.144	6.28	67.3	13	(18)	.572	.00(- .21)
NC-1	Control	100.	100.	39	(45)	.618	Control Mean t = .000
4-Vinylcyclohexene [195579-L, M.W. = 108.20, Density = NA]							
Trial 1 [74]							
B(a)P	.000791	3.00	69.2	43	(18)	2.02***	+ 4.51
B(a)P	.000250	9.58	78.4	27	(18)	1.29*	+ 2.49
195579-L	5.23	.000	2.53	1	(4,18)	.189	.00(-1.31)
195579-L	4.18	22.8	93.1	3	(12,18)	.208	.00(-2.02)
195579-L	3.14	28.7	98.6	9	(18)	.348	.00(-1.70)
195579-L	2.09	60.5	107.	11	(18)	.456	.00(-1.00)
NC-1	Control	100.	100.	65	(71)	.657	Control Mean t = .000
Trial 2 [110]							
B(a)P	.000791	11.6	61.1	116	(18)	5.78***	+ 10.7
B(a)P	.000250	26.7	88.0	75	(18)	3.89***	+ 8.52
195579-L	5.82	.000	.000	0	(1,18)	.000	.00(-7.87)
195579-L	4.36	8.04	.712	0	(3,18)	.000	.00(-7.87)
195579-L	2.91	.322	4.24	4	(15,17)	.157	.00(-2.19)
195579-L	1.45	83.6	87.7	14	(18)	.598	.00(- .05)
NC-1	Control	100.	100.	65	(75)	.609	Control Mean t = .000

Abbreviations: B(a)P, benzo(a)pyrene; CC.A., co-culture clonal survival assay; Conc., concentration; mM, millimole; M.W., molecular weight; N, number of culture vessels, NC, negative control; ND, not determined; %RCE, percent relative cloning efficiency; S.A., standard clonal survival assay.

[a]Treatment condition: The experimental design for the transformation assay is described in detail in the Materials and Methods. The concentration of the positive control and test chemical treatment are presented in mM, but they can be converted to μg/ml using the molecular weight that is provided with each chemical. The solvent vehicles used for the individual test chemicals were listed in Appendix Tables A1 and A3, and the concentrations of the solvent vehicles are presented in the Materials and Methods.

[b]Cytotoxic activity: The experimental design for the standard survival assay (SA) and the co-culture clonal survival assay (CCA) were described in the Materials and Methods. The test chemical cytotoxic response was expressed as & RCE and was calculated as described in the Materials and Methods.

[c]The criteria used to evaluate the transformed foci of BALB/c-3T3 cells is described in the Materials and Methods. The number of type III foci > 2-mm in diameter per culture vessel scored are recorded in this table.

[d]Transformation response: The transformation responses are expressed as type III foci/vessel and were calculated using a $\log_{10}$ mathematical transformation procedure (refer to Materials and Methods). The arithmetic value or foci/vessel represents the antilog of the $\log_{10}$ mean transformation response minus one.

[e]Significance: The significance of test chemical transformation responses was calculated by a computer using the SAS statistical software (*22*), and the method is described in detail in Materials and Methods. The correct *t*-statistic according to the F-test is presented in this table. The *t*-statistics of each treatment dose of the test chemical in a single experiment were averaged to determine the mean *t*-statistic of the test chemical for the experiment (refer to Appendix Tables A2 and A5). The mean *t*-statistics for two or experiments for each chemical was weighted to the number of treatment doses evaluated and averaged to determine the rank *t*-statistic which was used to rank-order the test chemical transformation responses in Appendix Tables A3 and A6. Arbitrarily, transformation responses with negative (−) *t*-statistics were given a value of zero (0).

*Significant BaP or test chemical transformation response, $0.01 < p \leq 0.05$.

**Significant BaP or test chemical transformation response, $0.001 < p \leq 0.01$.

***Significant or BaP or test chemical transformation response, $p \leq 0.001$.

Appendix E.

Summary of the transformation responses of 30 cytotoxic, nonmutagenic noncarcinogens.

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
				Focus Data Type Vessels		Foci/Vessel Focus Type	
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	*t*-statistic
Anilazine [ANIL, M.W. = 275.53]							
Trial 1 [29]							
B(a)P	.000791	1.15	57.0	122	(20)	5.79***	+ 13.1
B(a)P	.000250	2.29	78.5	142	(20)	6.19***	+ 10.3
ANIL	.0581	5.34	7.59	9	(20)	.347	.00(-1.31)
ANIL	.0436	34.0	71.1	43	(20)	1.81***	+ 3.86
ANIL	.0290	73.7	87.3	19	(20)	.721	+ .48
ANIL	.0145	93.9	105.	13	(20)	.503	.00(- .48)
NC-1	Control	100.	100.	36	(40)	.606	Control
						Mean t = 1.09	
Trial 2 [85]							
B(a)P	.000791	18.8	55.6	133	(20)	3.43***	+ 5.10
B(a)P	.000250	28.7	91.5	66	(19)	2.10***	+ 4.56
ANIL	.0581	3.19	20.4	4	(11,20)	.110	.00(- .59)
ANIL	.0436	51.1	64.7	46	(20)	2.00***	+ 7.57
ANIL	.0290	46.7	96.7	13	(20)	.365	+ .34
ANIL	.0145	50.3	107.	14	(20)	.394	+ .52
NC-1	Control	100.	100.	38	(80)	.313	Control
						Mean t = 2.81	
L-Ascorbic Acid [ASC, M.W. = 176.14]							
Trial 1 [4]							
B(a)P	.000791	15.7	74.7	184	(20)	8.81***	+ 9.38
B(a)P	.000250	24.0	82.9	116	(20)	4.38***	+ 3.62
ASC	.341	62.0	53.5	34	(20)	1.34	.00(- .33)
ASC	.298	75.2	58.1	68	(20)	2.24	+ 1.20
ASC	.256	89.7	56.2	43	(20)	1.08	.00(- .82)
ASC	.199	100.	84.8	30	(20)	1.02	.00(-1.03)
NC-1	Control	100.	100.	118	(40)	1.51	Control
						Mean t = .300	
Trial 2 [11]							
B(a)P	.000791	1.37	34.4	128	(20)	4.94***	+ 10.5
B(a)P	.000250	7.22	36.0	37	(20)	1.62***	+ 5.38
ASC	.568	9.97	73.1	18	(20)	.525	+ 1.11
ASC	.426	23.4	86.0	6	(20)	.231	.00(- .45)
ASC	.284	63.2	90.3	5	(20)	.189	.00(- .86)
ASC	.142	78.0	64.5	9	(20)	.347	+ .28
NC-1	Control	100.	100.	21	(40)	.301	Control
						Mean t = .348	
Bisphenol A [BIS, M.W. = 228.29]							
Trial 1 [2]							
B(a)P	.000791	1.02	36.4	187	(20)	8.89***	+ 15.5
B(a)P	.000250	3.41	68.7	110	(20)	4.52***	+ 8.43
BIS	.215	2.39	56.4	8	(20)	.282	.00(-2.11)
BIS	.193	11.3	73.5	12	(19)	.449	.00(-1.04)
BIS	.167	46.4	95.5	18	(20)	.712	+ .24
BIS	.127	61.1	118.	16	(20)	.573	.00(- .41)
NC-1	Control	100.	100.	34	(40)	.660	Control
						Mean t = .060	
Trial 2 [8]							
B(a)P	.000791	1.45	5.83	244	(19)	10.7***	+ 8.19
B(a)P	.000250	4.35	52.4	254	(20)	11.8***	+ 11.2

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Appendix E. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c] Focus Data Type	Vessels	Transformation Response[d] Foci/Vessel Focus Type	Significance[e]
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	*t*-statistic
BIS	.263	.000	.000	1	(15)	.047	.00(-10.8)
BIS	.219	7.25	.000	13	(18)	.572	.00(- 4.58)
BIS	.175	22.2	1.94	55	(20)	2.15	.00(- .08
BIS	.131	43.9	27.2	89	(19)	4.31**	+ 3.45
NC-1	Control	100.	100.	110	(40)	2.19	Control
							Mean t = 1.15
Trial 3 [IP17]							
B(a)P	.000791	.000	NA	100	(15)	5.28***	+ 8.30
B(a)P	.000250	3.1	NA	84	(15)	5.28***	+ 11.4
BIS	.197	8.3	NA	4	(15)	.18	.00(-1.15)
BIS	.131	34.8	NA	15	(15)	.72	+ 1.14
BIS	.0657	76.0	NA	8	(15)	.42	+ .04
NC-1	Control	100.	NA	20	(29)	.41	Control
							Mean t = .393
Trial 4 [IP18]							
B(a)P	.000791	1.3	3.7	82	(11)	6.31***	+ 20.5
B(a)P	.000250	2.6	22.1	110	(12)	8.85***	+ 10.7
BIS	.210	.000	2.7	1	(12)	.059	.00(-1.18)
BIS	.158	3.0	19.5	10	(12)	.55	+ 1.48
BIS	.105	20.6	52.3	11	(12)	.658	+ 1.92
BIS	.0526	69.5	81.3	17	(12)	1.01*	+ 2.75
*NC-1	Control	100.	100.	6	(24)	.189	Control
							Mean t = 1.54

*Trial No. 6 was conducted in 100 mm dishes using 20000 BALB/c-3T3 Cells (refer to Exp.IP18).

Carbromal [CARB, M.W. = 237.10]

Trial 1 [35]							
B(a)P	.000791	4.51	66.1	103	(20)	4.91***	+ 6.41
B(a)P	.000250	12.3	87.5	133	(20)	6.01***	+ 6.34
CARB	5.06	2.05	12.5	12	(11,20)	.782	.00(-2.76)
CARB	3.80	7.79	72.1	67	(20)	2.96*	+ 2.02
CARB	2.53	30.7	115.	36	(20)	1.41	.00(-1.41)
CARB	1.27	59.0	111.	37	(20)	1.68	.00(- .79)
NC-1	Control	100.	100.	94	(40)	1.97	Control
							Mean t = .673
Trial 2 [44]							
B(a)P	.000791	5.07	25.9	335	(20)	15.8***	+ 16.3
B(a)P	.000250	14.2	67.2	137	(20)	6.15***	+ 7.33
CARB	4.00	9.80	22.0	156	(20)	7.00***	+ 8.03
CARB	2.00	35.1	85.2	83	(19)	3.80**	+ 4.39
CARB	1.00	89.2	84.4	68	(20)	2.60*	+ 2.26
CARB	.500	98.3	91.3	47	(20)	1.97	+ 1.09
NC-1	Control	100.	100.	77	(40)	1.52	Control
							Mean t = 3.00

Chlorpheniramine-Maleate [005004-S, M.W. = 390.87]

Trial 1 [70]							
B(a)P	.000791	1.24	48.9	11	(3)	3.38***	+ 4.23
B(a)P	.000250	11.8	81.0	19	(5)	3.62***	+ 5.73

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Appendix E. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
Drug	Conc., mM	S.A	CC.A.	Focus Data Type Vessels III	(N)	Foci/Vessel Focus Type III	*t*-statistic
005004-S	.513	.000	.000	0	(0,8)	.000	NA
005004-S	.385	.000	4.50	1	(4,11)	.119	.00(-1.15)
005004-S	.256	.000	74.3	2	(9)	.167	.00(-1.83)
005004-S	.128	8.07	105.	10	(17)	.503	.00(- .13)
NC-1	Control	100.	100.	36	(54)	.526	Control Mean t = .000
Trial 2 [85]							
B(a)P	.000791	18.8	55.6	133	(20)	3.43***	+ 5.10
B(a)P	.000250	28.7	91.8	66	(19)	2.10***	+ 4.56
005004-S	.410	.000	.000	3	(10,20)	.231	.00(- .55)
005004-S	.308	.000	34.1	20	(20)	.807**	+ 2.91
005004-S	.205	1.20	91.8	18	(20)	.737*	+ 2.56
005004-S	.103	63.1	103.	10	(20)	.374	.43
NC-1	Control	100.	100.	38	(80)	.313	Control Mean t = 1.97
C. I. Acid Red 14 [CIAR14, M.W. = 502.44]							
Trial 1 [30]							
B(a)P	.000791	1.32	60.3	158	(20)	7.23***	+ 12.3
B(a)P	.000250	2.63	101.	98	(19)	4.25***	+ 8.08
CIAR14	3.98	3.95	32.6	44	(20)	1.54*	+ 2.33
CIAR14	2.98	21.5	48.4	35	(20)	1.61**	+ 3.17
CIAR14	1.99	32.9	51.7	38	(20)	1.59**	+ 2.75
CIAR14	1.00	72.4	78.5	22	(20)	.966	+ .78
NC-1	Control	100.	100.	40	(40)	.787	Control Mean t = 2.26
Trial 2 [45]							
B(a)P	.000791	8.74	42.5	186	(20)	8.98***	+ 20.9
B(a)P	.000250	28.2	86.4	77	(20)	3.42***	+ 8.88
CIAR14	4.00	13.9	87.9	26	(20)	.848*	+ 2.15
CIAR14	2.00	89.3	103.	48	(19)	2.30***	+ 8.11
CIAR14	1.00	102.	99.2	35	(20)	1.48***	+ 4.80
CIAR14	.500	100.	103.	15	(20)	.547	+ 1.32
NC-1	Control	100.	100.	54	(59)	.732	Control Mean t = 4.09
C. I. Acid Yellow 73 [CIAY73, M.W. = 376.]							
Trial 1 [77]							
B(a)P	.000791	5.66	69.5	179	(20)	8.33***	+ 13.6
B(a)P	.000250	16.5	78.7	114	(19)	5.53***	+ 10.0
CIAY73	7.98	2.83	11.9	13	(16)	.664	.00(-1.31)
CIAY73	5.98	.000	18.7	14	(16)	.645	.00(-1.36)
CIAY73	3.99	9.91	66.0	7	(20)	.221	.00(-4.09)
CIAY73	1.99	54.2	86.4	17	(20)	.702	.00(-1.25)
NC-1	Control	100.	100.	94	(78)	.972	Control Mean t = .000
Trial 2 [83]							
B(a)P	.000791	2.86	73.0	141	(20)	6.14***	+ 13.5
B(a)P	.000250	13.8	78.9	64	(20)	2.93***	+ 9.12
CIAY73	7.98	.000	13.1	3	(13)	.173	.00(-1.01)
CIAY73	5.98	2.38	23.9	10	(13)	.406	+ .26
CIAY73	3.99	4.29	35.7	4	(12)	.230	.00(- .64)
CIAY73	1.99	34.8	78.4	6	(20)	.231	.00(- .81)
NC-1	Control	100.	100.	48	(80)	.351	Control Mean t = .065

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Appendix E. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
Drug	Conc., mM	S.A	CC.A.	Focus Data Type Vessels III	(N)	Foci/Vessel Focus Type III	*t*-statistic
Ephedrine Sulfate [213387-S, M.W. = 428.54]							
Trial 1 [71]							
B(a)P	.000791	4.48	50.7	251	(20)	11.0***	+ 12.1
B(a)P	.000250	18.1	68.7	77	(20)	3.50***	+ 7.12
213387-S	.279	73.9	80.9	18	(20)	.712	.00(-1.26)
213387-S	.209	75.7	79.4	16	(20)	.611	.00(-1.67)
213387-S	.140	80.6	87.8	37	(15)	1.96*	+ 2.14
213387-S	.0700	93.1	83.2	24	(13)	1.51	+ 1.10
NC-1	Control	100.	100.	110	(75)	1.06	Control
							Mean t = .810
Trial 2 [77]							
B(a)P	.000791	5.66	69.5	179	(20)	8.33***	+ 13.6
B(a)P	.000250	16.5	78.7	114	(19)	5.53***	+ 10.0
213387-S	2.79	.000	.367	0	(7,20)	.000	.00(-12.5)
213387-S	2.09	.000	10.6	4	(19)	.157	.00(- 6.21)
213387-S	1.40	.943	83.6	28	(20)	.880	.00(- .37)
213387-S	.698	30.2	97.6	19	(19)	.760	.00(- .92)
NC-1	Control	100.	100.	94	(78)	.972	Control
							Mean t = .000
Trial 3 [89]							
B(a)P	.000791	8.13	69.0	119	(20)	4.77***	+ 10.3
B(a)P	.000250	31.9	89.1	63	(20)	2.33***	+ 5.84
213387-S	2.09	. ?	.2 ?	0	(5,20)	.000	.00(-7.01)
213387-S	1.74	42.5 ?	2.3 ?	8	(15)	.270	.00(-1.12)
213387-S	1.40	102.	13.3	23	(19)	.704	+ .96
213387-S	.698	107.	91.9	12	(19)	.480	.00(- .06)
NC-1	Control	100.	100.	57	(79)	.492	Control
							Mean t = .320
Erythromycin Stearate [302486-S, M.W. = 1018.59]							
Trial 1 [71]							
B(a)P	.000791	4.48	50.7	251	(20)	11.0***	+ 12.1
B(a)P	.000250	18.1	68.7	77	(20)	3.50***	+ 7.12
302486-S	.118	.000	13.4	6	(16)	.242	.00(-3.18)
302486-S	.0882	.000	39.1	7	(20)	.275	.00(-4.65)
302486-S	.0588	1.79	75.1	9	(20)	.366	.00(-3.89)
302486-S	.0294	25.1	98.9	10	(16)	.461	.00(-2.13)
NC-1	Control	100.	100.	110	(75)	1.06	Control
							Mean t = .000
Trial 2 [89]							
B(a)P	.000791	8.13	69.0	119	(20)	4.77***	+ 10.3
B(a)P	.000250	31.9	89.1	63	(20)	2.33***	+ 5.84
302486-S	.118	.000	12.0	5	(20)	.172	.00(-2.55)
302486-S	.0882	.000	27.0	7	(20)	.275	.00(-1.31)
302486-S	.0588	2.61	75.4	11	(20)	.402	.00(-50)
302486-S	.0294	49.3	83.7	17	(20)	.620	+ .65
NC-1	Control	100.	100.	57	(79)	.492	Control
							Mean t = .163
Ethoxylated Dodecyl Alcohol [EDA, M.W. = ~1200., Density = 0.999 g/ml]							
Trial 1 [82]							
B(a)P	.000791	46.8	47.2	371	(18)	19.4***	+ 7.45
B(a)P	.000250	56.3	51.6	288	(18)	15.5***	+ 7.97

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Appendix E. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c] Focus Data Type Vessels		Transformation Response[d] Foci/Vessel Focus Type	Significance[e]
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	*t*-statistic
EDA	.0417	.000	.000	0	(0,18)	.000	NA
EDA	.0132	.867	67.8	125	(18)	6.42	.00(-1.74)
EDA	.00417	26.9	101.	110	(18)	5.77	.00(-2.59)
EDA	.00132	63.3	91.8	82	(18)	4.40	.00(-6.45)
NC-1	Control	100.	100.	649	(72)	8.01	Control Mean t = .000
Trial 2 [90]							
B(a)P	.000791	28.5	76.0	157	(18)	7.60***	+ 5.89
B(a)P	.000255	51.8	95.8	111	(18)	4.91***	+ 3.71
EDA	.0250	.000	.000	1	(12,18)	.059	.00(-9.90)
EDA	.0188	8.70	12.5	11	(18)	.414	.00(-4.07)
EDA	.0125	8.35	62.1	68	(18)	2.85	+ 1.44
EDA	.00625	15.7	94.3	51	(18)	2.12	+ .31
NC-1	Control	100.	100.	219	(71)	1.95	Control Mean t = .583
Ethylenediamine Tetraacetic Acid, Trisodium Salt [EDTA, M.W. = 358.22]							
Trial 1 [73]							
B(a)P	.000791	2.21	71.9	61	(19)	2.60***	+ 8.09
B(a)P	.000250	7.18	88.8	48	(20)	1.58***	+ 4.37
EDTA	2.72	.000	3.13	5	(20)	.172	.00(- .45)
EDTA	1.81	5.00	60.0	34	(20)	1.26**	+ 4.49
EDTA	1.36	45.3	79.4	14	(20)	.525	+ 1.50
EDTA	.907	80.1	98.8	19	(20)	.542	+ 1.48
NC-1	Control	100.	100.	45	(79)	.274	Control Mean t = 2.49
Trial 2 [85]							
B(a)P[h]	.000791	12.8	55.6	133	(20)	3.43***	+ 5.10
B(a)P	.000250	22.4	91.8	66	(19)	2.10***	+ 4.56
B(a)P	.000791	18.8	55.9				
B(a)P	.000250	28.7	79.4				
EDTA	2.79	.000	.000	2	(15,20)	.072	.00(-2.26)
EDTA	2.33	1.20	2.33	11	(20)	.423	+ .75
EDTA	1.67	37.5	49.1	15	(20)	.578	+ 1.68
EDTA	.837	94.6	89.7	7	(20)	.272	.00(- .29)
NC-1	Control	100.	100.	38	(80)	.313	Control Mean t = .810
Eugenol [EUG, M.W. = 164.20, Density = 1.064 g/ml]							
Trial 1 [74]							
B(a)P	.00079	?	69.2	43	(18)	2.02***	+ 4.51
B(a)P	.000250	9.58	78.4	27	(18)	1.29*	+ 2.49
EUG	.649	.000	96.3	43	(18)	1.99***	+ 4.45
EUG	.325	11.4	110.	30	(18)	1.00	+ 1.07
EUG	.162	39.5	99.1	23	(18)	1.03	+ 1.51
EUG	.0812	66.5	107.	17	(18)	.765	+ .49
NC-1	Control	100.	100.	65	(71)	.657	Control Mean t = 1.88
Trial 2 [94]							
B(a)P	.000791	.000	75.7	122	(18)	5.92***	+ 6.26
B(a)P	.000250	17.4	114.	81	(18)	3.88***	+ 4.08

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Appendix E. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c] Focus Data Type Vessels III (N)		Transformation Response[d] Foci/Vessel Focus Type III	Significance[e] t-statistic
Drug	Conc., mM	S.A	CC.A.				
EUG	1.62	.000	.000	0	(1,18)	.000	.00(-1.44)
EUG	.812	.000	59.8	32	(15)	1.52	.00(- .01)
EUG	.406	5.13	121.	22	(18)	.586	.00(-2.72)
EUG	.203	5.13	137.	15	(18)	.624	.00(-2.73)
NC-1	Control	100.	100.	150	(71)	1.52	Control
							Mean t = .000
Geranyl Acetate [GEAC, M.W. = 196.32, Density = 0.907 - 0.918 g/ml]							
Trial 1 [84]							
B(a)P	.000791	49.8	57.9	71	(18)	3.27***	+ 6.82
B(a)P	.000250	ND	71.7	57	(18)	2.44***	+ 6.14
GEAC	.560	.733	1.68	1	(16,18)	.039	.00(-5.25)
GEAC	.280	53.5	50.9	10	(18)	.446	.00(- .36)
GEAC	.140	84.2	60.6	18	(18)	.834	+ 1.59
GEAC	.0700	114.	101.	9	(18)	.339	.00(- .98)
NC-1	Control	100.	100.	50	(72)	.511	Control
							Mean t = .530
Trial 2 [92]							
B(a)P	.00250	.394	45.3	69	(18)	3.54***	+ 8.31
B(a)P	.000791	18.2	72.2	44	(18)	2.14***	+ 5.20
B(a)P	.000250	19.4	78.2	32	(17)	1.52**	+ 3.32
GEAC	.509	1.19	.000	0	(0,18)	.000	NA
GEAC	.382	14.7	19.9	9	(9,18)	.714	+ .39
GEAC	.255	61.4	66.2	14	(18)	.634	+ .18
GEAC	.127	89.5	98.1	7	(18)	.260	.00(-1.86)
NC-1	Control	100.	100.	62	(71)	.597	Control
							Mean t = .090
4-Hexylresorcinol [012776-S, M.W. = 194.27]							
Trial 1 [63]							
B(a)P	.000791	4.00	80.5	141	(20)	6.13***	+ 6.87
B(a)P	.000250	24.8	93.1	93	(20)	3.20*	+ 2.02
012776-S	.158	4.7	2.21	2	(18)	.080	.00(-11.5)
012776-S	.118	14.5	51.0	3	(9)	.220	.00(- 5.54)
012776-S	.079	49.1	82.7	16	(10)	1.35	.00(- 1.36)
012776-S	.039	76.3	103.	26	(15)	1.57	.00(- 1.00)
NC-1	Control	100.	100.	84	(39)	1.92	Control
							Mean t = .000
Trial 2 [85]							
B(a)P	.000791	12.8	55.9	133	(20)	3.43***	+ 5.10
B(a)P	.000250	28.7	79.4	66	(19)	2.10***	+ 4.56
012776-S	.147	2.00	.000	2	(19)	.076	.00(-2.85)
012776-S	.111	25.1	44.0	26	(20)	.927*	+ 3.28
012776-S	.074	48.3	80.2	12	(20)	.374	+ .40
012776-S	.037	70.3	95.2	7	(20)	.172	.00(-1.04)
NC-1	Control	100.	100.	38	(80)	.313	Control
							Mean t = .920
D,L-Menthol [MENT, M.W. = 156.27]							
Trial 1 [18]							
B(a)P	.000791	2.24	45.8	125	(20)	5.91***	+ 13.0
B(a)P	.000250	8.52	78.5	86	(20)	3.36***	+ 7.28

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Appendix E. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
				Focus Data Type Vessels		Foci/Vessel Focus Type	
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	*t*-statistic
MENT	6.40	.000	.000	7	(10,20)	.578	.00(- .34)
MENT	3.20	.000	39.8	3	(4,19)	.414	.00(- .67)
MENT	1.60	42.2	102.	14	(20)	.525	.00(- .71
MENT	.800	69.5	110.	58	(20)	.925	+ .69
NC-1	Control	100.	100.	33	(40)	.663	Control
							Mean t = .215
Trial 2 [24]							
B(a)P	.000791	.000	18.7	83	(20)	3.65***	+ 10.7
B(a)P	.000250	5.66	70.0	86	(20)	3.73***	+ 10.7
MENT	4.80	.629	.000	2	(15,20)	.097	.00(-1.92)
MENT	3.20	50.3	85.6	4	(20)	.132	.00(-1.35)
MENT	1.60	64.8	91.7	6	(20)	.231	.00(- .56)
MENT	.800	72.3	98.7	10	(20)	.347	+ .25
NC-1	Control	100.	100.	18	(40)	.308	Control
							Mean t = .083
Methoxychlor [METH, M.W. = 345.66]							
Trial 1 [37]							
B(a)P	.000791	1.60	46.0	101	(20)	4.59***	+ 9.94
B(a)P	.000250	6.80	77.2	113	(20)	5.38***	+ 13.5
METH	.231	.000	.000	0	(0,20)	.000	ND
METH	.174	.000	.000	0	(5,20)	.000	.00(-6.46)
METH	.116	.000	2.48	7	(19)	.272	.00(-2.01)
METH	.058	72.8	78.3	24	(20)	.966	+ 1.41
NC-1	Control	100.	100.	32	(39)	.631	Control
							Mean t = .705
Trial 2 [89]							
B(a)P	.000791	8.13	69.0	119	(20)	4.77***	+ 10.3
B(a)P	.000250	31.9	89.1	63	(20)	2.33***	+ 5.84
METH	.145	.000	16.8	3	(14)	.160	.00(-1.79)
METH	.108	31.1	60.9	21	(19)	.809	+ 1.45
METH	.072	63.6	84.4	30	(20)	1.30***	+ 3.54
METH	.036	90.3	85.0	15	(18)	.502	+ .05
NC-1	Control	100.	100.	57	(79)	.492	Control
							Mean t = 1.26
Methyldopa Sesquihydrate [973697-S, M.W. = 238.24]							
Trial 1 [75]							
B(a)P	.000791	7.10	66.5	149	(20)	6.35***	+ 10.9
B(a)P	.000250	28.4	85.4	67	(20)	3.10***	+ 6.56
973697-S	.119	.000	45.8	51	(20)	2.09***	+ 3.85
973697-S	.0595	55.5	71.3	39	(20)	1.37	+ 1.71
973697-S	.0298	92.3	71.0	32	(20)	1.18	+ 1.11
973697-S	.0149	100.	72.2	17	(20)	.677	.00(- .93)
NC-1	Control	100.	100.	89	(78)	.882	Control
							Mean t = 1.67
Trial 2 [91]							
B(a)P	.000791	28.9	73.6	60	(20)	2.00***	+ 5.11
B(a)P	.000250	58.1	89.9	14	(20)	.503	+ 1.31
973697-S	.167	.000	.849	5	(10,20)	.155	+ .08
973697-S	.125	.000	16.1	17	(18,20)	.596	+ 1.80
973697-S	.083	.000	84.4	5	(20)	.172	.00(-1.34)
973697-S	.042	93.5	93.1	3	(20)	.110	.00(-2.04)
NC-1	Control	100.	100.	31	(75)	.322	Control
							Mean t = .600

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Appendix E. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
Drug	Conc., mM	S.A	CC.A.	Focus Data Type Vessels III	(N)	Foci/Vessel Focus Type III	*t*-statistic
Methylphenidate [MEPH, M.W. = 269.80]							
Trial 1 [48]							
B(a)P	.00791	2.28	47.9	148	(20)	7.06***	+ 16.5
B(a)P	.00250	10.1	90.8	63	(20)	2.68***	+ 6.53
MEPH	8.36	.000	5.80	23	(20)	.927	+ 1.72
MEPH	6.27	3.91	73.6	25	(20)	1.08*	+ 2.40
MEPH	4.18	57.2	103.	18	(19)	.777	+ 1.12
MEPH	2.09	82.6	102.	9	(20)	.327	.00(-1.19)
NC-1	Control	100.	100.	29	(40)	.537	Control
							Mean t = 1.31
Trial 2 [57]							
B(a)P	.000791	3.55	30.1	162	(20)	7.55***	+ 18.7
B(a)P	.000250	5.32	69.9	37	(20)	1.63***	+ 6.91
MEPH	8.00	.000	20.3	14	(20)	.525	+ 1.60
MEPH	6.00	12.4	71.4	12	(20)	.473	+ 1.35
MEPH	4.18	68.4	81.8	3	(20)	.094	.00(-1.66)
MEPH	2.09	95.0	78.6	8	(20)	.301	+ .17
NC-1	Control	100.	100.	15	(40)	.278	Control
							Mean t = .780
Oxytetracycline-HCl [925728-S, M.W. = 496.90]							
Trial 1 [73]							
B(a)P	.000791	2.21	71.9	61	(19)	2.60***	+ 8.09
B(a)P	.000250	7.18	88.8	48	(20)	1.58***	+ 4.37
925728-S	1.80	.000	.000	0	(2,19)	.000	.00(-4.49)
925728-S	.900	.000	.000	0	(19,20)	.000	.00(-4-49)
925728-S	.450	.000	.000	1	(20)	.035	.00(-3.24)
925728-S	.225	.000	20.7	1	(20)	.035	.00(-3.24)
NC-1	Control	100.	100.	45	(79)	.274	Control
							Mean t = .000
Trial 2 [103]							
B(a)P	.000791	8.60	76.9	95	(20)	4.59***	+ 13.7
B(a)P	.000250	23.6	91.5	75	(20)	2.86***	+ 5.37
925728-S	.640	.000	20.7	38	(20)	.623	.00(- .75)
925728-S	.320	31.4	86.2	19	(19)	.777	.00(- .41)
925728-S	.160	77.8	81.4	19	(20)	.634	.00(-1.05)
925728-S	.0800	81.7	95.5	11	(19)	.389	.00(-2.31)
NC-1	Control	100.	100.	89	(79)	.874	Control
							Mean t = .000
Trial 3 [107]							
B(a)P	.000791	5.81	47.3	131	(20)	6.09***	+ 5.74
B(a)P	.000250	21.2	75.8	122	(20)	5.53***	+ 4.05
925728-S	.690	1.37	19.0	10	(20)	.347	.00(-8.60)
925728-S	.518	22.2	68.5	10	(19)	.397	.00(-8.19)
925728-S	.345	44.4	86.0	16	(20)	.619	.00(-7.09)
925728-S	.173	88.2	97.0	23	(20)	.899	.00(-5.68)
NC-1	Control	100.	100.	274	(80)	2.95	Control
							Mean t = .000
Phenol [PHENOL, M.W. = 94.11]							
Trial 1 [76]							
B(a)P	.000791	5.91	63.1	87	(18)	4.41***	+ 5.23
B(a)P	.000250	23.3	97.0	54	(18)	2.72*	+ 2.26

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Appendix E. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
				Focus Data Type Vessels		Foci/Vessel Focus Type	
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	*t*-statistic
PHENOL	4.25	.000	65.0	239	(18)	13.0***	+ 20.3
PHENOL	2.13	4.60	82.9	152	(18)	8.01***	+ 12.3
PHENOL	1.06	25.9	92.4	89	(18)	4.61***	+ 5.54
PHENOL	.53	59.1	102.	97	(18)	3.84***	+ 3.90
NC-1	Control	100.	100.	152	(71)	1.79	Control
							Mean t = 10.5
Trial 2 [90]							
B(a)P	.000791	28.5	76.0	157	(18)	7.60***	+ 5.89
B(a)P	.000250	51.8	95.8	111	(18)	4.91***	+ 3.71
PHENOL	4.25	7.65	42.5	159	(18)	8.17***	+ 8.78
PHENOL	3.19	13.6	48.6	114	(18)	5.95***	+ 7.15
PHENOL	2.13	17.7	67.9	55	(18)	2.74	+ 1.80
PHENOL	1.06	61.2	90.9	49	(18)	2.33	+ .67
NC-1	Control	100.	100.	219	(71)	1.95	Control
							Mean t = 4.60
Phenylephrine-HCl [571483-S, M.W. = 203.67]							
Trial 1 [73]							
B(a)P	.000791	2.21	71.9	61	(19)	2.60***	+ 8.09
B(a)P	.000250	7.18	88.8	48	(20)	1.58***	+ 4.37
571483-S	7.00	.000	.209	0	(3,20)	.000	.00(-4.49)
571483-S	5.25	.000	2.09	0	(19,20)	.000	.00(-4.49)
571483-S	3.50	.552	38.6	8	(20)	.275	.00
571483-S	1.75	88.4	99.2	10	(20)	.394	+ .77
NC-1	Control	100.	100.	45	(79)	.274	Control
							Mean t = .385
Trial 2 [105]							
B(a)P	.000791	5.67	63.1	59	(20)	2.43***	+ 6.70
B(a)P	.000250	18.2	86.5	40	(19)	1.93***	+ 5.54
571483-S	6.00	.000	.792	0	(10,19)	.000	.00(-8.86)
571483-S	4.50	.000	10.6	6	(20)	.172	.00(-2.68)
571483-S	3.00	9.67	89.2	20	(20)	.715	+ .69
571483-S	1.50	81.7	98.7	14	(18)	.598	+ .09
NC-1	Control	100.	100.	58	(77)	.581	Control
							Mean t = .260
Propyl Gallate [PRGA, M.W. = 212.22]							
Trial 1 [3]							
B(a)P	.000791	10.7	47.1	127	(20)	5.84***	+ 14.8
B(a)P	.000250	13.4	81.6	39	(20)	1.61***	+ 5.63
PRGA	.087	.000	32.2	33	(19)	1.04*	+ 2.60
PRGA	.075	.000	37.9	27	(20)	.977**	+ 3.31
PRGA	.059	.000	42.5	17	(20)	.658*	+ 2.24
PRGA	.038	.000	72.4	14	(20)	.556	+ 1.65
NC-1	Control	100.	100.	17	(40)	.285	Control
							Mean t = 2.42
Trial 2 [9]							
B(a)P	.000791	3.14	38.3	108	(20)	4.93***	+ 16.2
B(a)P	.000250	8.52	87.2	47	(20)	1.92***	+ 7.04
PRGA	.094	.000	44.1	25	(20)	.800**	+ 2.98
PRGA	.071	.000	66.7	7	(20)	.238	+ .85
PRGA	.047	1.35	83.1	3	(20)	.110	.00(- .46)
PRGA	.024	74.4	84.1	0	(20)	.000	.00(-3.12)
NC-1	Control	100.	100.	8	(40)	.149	Control
							Mean t = .958

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Appendix E. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
Drug	Conc., mM	S.A	CC.A.	Focus Data Type Vessels III	(N)	Foci/Vessel Focus Type III	t-statistic
Rotenone [959444-S, M.W. = 394.43]							
Trial 1 [75]							
B(a)P	.000791	7.10	66.5	149	(20)	6.35***	+ 10.9
B(a)P	.000250	28.4	85.4	67	(20)	3.10***	+ 6.56
959444-S	.00513	.000	13.7	1	(19,20)	.037	.00(- 8.85)
959444-S	.00162	.000	49.2	0	(10,20)	.000	.00(-11.2)
959444-S	.000513	2.92	45.4	2	(19)	.060	.00(- 7.10)
959444-S	.000162	5.43	85.2	9	(20)	.289	.00(- 3.09)
NC-1	Control	100.	100.	89	(78)	.882	Control Mean t = .000
Trial 2 [96]							
B(a)P	.000791	11.0	43.5	86	(20)	4.03***	+ 9.40
B(a)P	.000250	38.9	75.5	62	(20)	2.88***	+ 7.12
959444-S	.00256	.786	23.5	0	(1,20)	.000	.00(-1.02)
959444-S	.000810	2.36	51.9	0	(11,20)	.000	.00(-8.55)
959444-S	.000256	21.6	54.6	18	(20)	.813	+ .94
959444-S	.0000810	46.8	86.5	19	(20)	.677	+ .08
NC-1	Control	100.	100.	62	(70)	.660	Control Mean t = .510
Sodium Diethyldithiocarbamate [SDEDTC, M.W. = 171.27]							
Trial 1 [38]							
B(a)P	.000791	2.10	28.4	174	(20)	7.75***	+ 15.6
B(a)P	.000250	9.44	74.7	162	(20)	8.17***	+ 18.0
SDEDTC	.000467	.000	4.39	31	(20)	1.18**	+ 2.74
SDEDTC	.000234	17.1	12.9	42	(19)	1.91***	+ 5.18
SDEDTC	.000117	87.4	71.4	39	(19)	1.63**	+ 4.06
SDECTC	.0000584	96.5	84.3	19	(19)	.815	+ 1.50
NC-1	Control	100.	100.	27	(40)	.496	Control Mean t = 3.37
Trial 2 [96]							
B(a)P	.000791	11.0	43.5	86	(20)	4.03***	+ 9.40
B(a)P	.000250	38.9	75.4	62	(20)	2.88***	+ 7.12
SDEDTC	.000350	.786	4.88	41	(20)	1.75***	+ 3.99
SDECTC	.000263	7.07	11.1	46	(20)	1.98***	+ 4.64
SDEDTC	.000175	23.6	48.8	27	(20)	1.00	+ 1.45
SDEDTC	.0000876	97.4	100.	13	(20)	.49	.00(- .86)
NC-1	Control	100.	100.	62	(70)	.660	Control Mean t = 2.52
Stannous Chloride [STCL, M.W. = 189.60]							
Trial 1 [19]							
B(a)P	.000791	.000	62.5	99	(20)	4.61***	+ 13.4
B(a)P	.000250	1.67	89.0	100	(20)	4.02***	+ 10.6
STCL	.0633	.000	9.19	11	(20)	.402	+ .28
STCL	.0422	7.08	29.6	9	(20)	.327	.00(- .20)
STCL	.0211	47.9	72.4	10	(20)	.266	.00(- .58)
STCL	.0105	75.0	93.0	11	(20)	.423	+ .42
NC-1	Control	100.	100.	18	(38)	.357	Control Mean t = .175
Trial 2 [26]							
B(a)P	.000791	.382	13.5	204	(20)	9.88***	+ 17.8
B(a)P	.000250	1.15	56.2	144	(20)	6.58***	+ 10.5

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Appendix E. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c] Focus Data Type Vessels		Transformation Response[d] Foci/Vessel Focus Type	Significance[e]
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	*t*-statistic
STCL	.0527	3.82	33.6	28	(20)	1.18	+ .99
STCL	.0264	26.3	69.7	35	(20)	1.45	+ 1.77
STCL	.0132	58.8	56.9	39	(20)	1.61*	+ 2.27
STCL	.00659	78.2	68.2	25	(20)	1.04	+ .48
NC-1	Control	100.	100.	46	(40)	.907	Control
							Mean t = 1.38
Tetracycline-HCl [186206-S, M.W. = 480.94]							
Trial 1 [71]							
B(a)P	.000791	4.48	50.7	251	(20)	11.0***	+ 12.1
B(a)P	.000250	18.1	68.7	77	(20)	3.50***	+ 7.12
186206-S	.542	.000	.000	3	(20)	.110	.00(-6.87)
186206-S	.406	3.58	1.27	3	(20)	.110	.00(-6.87)
186206-S	.271	27.3	18.5	25	(20)	.988	.00(- .23)
186206-S	.135	43.5	61.1	20	(16)	1.03	.00(- .09)
NC-1	Control	100.	100.	110	(75)	1.06	Control
							Mean t = .000
Trial 2 [89]							
B(a)P	.000791	8.13	69.0	119	(20)	4.77***	+ 10.3
B(a)P	.000250	31.9	89.1	63	(20)	2.33***	+ 5.84
186206-S	.458	9.29	52.4	9	(18)	.348	.00(- .78)
186206-S	.394	56.3	58.2	9	(19)	.389	.00(- .58)
186206-S	.229	87.4	77.1	3	(19)	.116	.00(-3.52)
186206-S	.115	89.1	94.3	19	(19)	.526	+ .17
NC-1	Control	100.	100.	57	(79)	.492	Control
							Mean t = .043
Tetrakis(hydroxymethyl)phosphonium Chloride [120152-L, M.W. = 190.58, Density = 1.322 g/ml]							
Trial 1 [72]							
B(a)P	.000791	2.75	56.8	85	(18)	4.11***	+ 12.7
B(a)P	.000250	6.61	82.1	84	(18)	3.04***	+ 6.20
120152-L	.0263	.000	3.76	11	(18)	.456	+ 1.15
120152-L	.0132	.000	14.5	4	(18)	.148	.00(-1.16)
120152-L	.00658	17.1	53.1	3	(18)	.122	.00(-1.42)
120152-L	.00329	40.8	91.2	5	(18)	.212	.00(- .61)
NC-1	Control	100.	100.	29	(72)	.289	Control
							Mean t = .288
Trial 2 [90]							
B(a)P	.000791	28.5	76.0	157	(18)	7.60***	+ 5.89
B(a)P	.000250	51.8	95.8	111	(18)	4.91***	+ 3.71
120152-L	.0132	.348	4.89	101	(18)	4.93***	+ 3.88
120152-L	.00987	2.43	18.6	122	(18)	5.70***	+ 4.52
120152-L	.00658	12.2	38.1	106	(18)	5.22***	+ 5.42
120152-L	.00329	50.4	78.7	66	(18)	2.12	+ .29
NC-1	Control	100.	100.	219	(71)	1.95	Control
							Mean t = 3.53
Trial 3 [98]							
B(a)P	.000791	8.38	79.6	132	(18)	6.82***	+ 11.8
B(a)P	.000250	29.3	91.3	75	(18)	3.38***	+ 6.81
120152-L	.0132	.000	17.8	12	(18)	.489	.00(- .59)
120152-L	.00987	2.10	26.3	10	(18)	.414	.00(- .98)
120152-L	.00658	5.24	44.2	6	(18)	.240	.00(-1.99)
120152-L	.00329	17.8	71.2	5	(18)	.212	.00(-2.68)
NC-1	Control	100.	100.	39	(45)	.618	Control
							Mean t = .000

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Appendix E. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
				Focus Data Type Vessels		Foci/Vessel Focus Type	
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	*t*-statistic
Tetrakis(hydroxymethyl)phosphonium Sulfate [003374-L, M.W. = 404.32, Density = NA g/ml]							
Trial 1 [72]							
B(a)P	.000791	2.75	56.8	85	(18)	4.11***	+ 12.7
B(a)P	.000250	6.61	82.1	84	(18)	3.04***	+ 6.20
003374-L	.01098	.000	1.25	10	(18)	.361	+ .50
003374-L	.00732	.000	1.75	6	(18)	.220	.00(- .53)
003374-L	.00366	12.1	35.3	4	(18)	.148	.00(-1.16)
003374-L	.00183	51.8	68.9	1	(18)	.039	.00(-3.59)
NC-1	Control	100.	100.	29	(72)	.289	Control
							Mean t = .125
Trial 2 [84]							
B(a)P	.000791	49.8	57.9	71	(18)	3.27***	+ 6.82
B(a)P	.000250	NA	71.7	57	(18)	2.44***	+ 6.14
003374-L	.01098	.000	.635	0	(2,18)	.000	.00(-7.45)
003374-L	.00732	.000	.212	1	(16,18)	.044	.00(-5.25)
003374-L	.00366	1.47	2.96	13	(18)	.413	.00(- .52)
003374-L	.00183	11.7	51.0	6	(18)	.240	.00(-1.66)
NC-1	Control	100.	100.	50	(72)	.511	Control
							Mean t = .000
Triphenyltin Hydroxide [TPH, M.W. = 367.03]							
Trial 1 [39]							
B(a)P	.000791	1.07	23.6	172	(20)	8.04***	+ 14.5
B(a)P	.000250	3.56	64.5	145	(20)	6.84***	+ 15.8
TPTH	.000272	.000	35.1	5	(18)	.193	+ .50
TPTH	.000136	26.0	78.5	24	(20)	.955	+ .51
TPTH	.0000681	67.6	88.4	16	(20)	.533*	+ 2.30
TPTH	.0000341	86.8	98.3	13	(19)	.526	.00(-1.36)
NC-1	Control	100.	100.	27	(40)	.427	Control
							Mean t = .828
Trial 2 [93]							
B(a)P	.000791	2.65	46.7	138	(20)	6.48***	+ 16.8
B(a)P	.000250	7.96	79.9	115	(20)	4.80***	+ 12.0
TPTH	.000327	.000	17.4	1	(3,18)	.260	.00(- .49)
TPTH	.000163	.000	9.50	1	(10,20)	.072	.00(-3.35)
TPTH	.0000817	.000	73.6	45	(18)	1.58**	+ 3.33
TPTH	.0000409	34.5	86.3	25	(20)	.999**	+ 3.21
NC-1	Control	100.	100.	43	(79)	.416	Control
							Mean t = 3.27
Xylenes, (Mixed) [109591-L, M.W. = 106.17, Density = NA g/ml]							
Trial 1 [72]							
B(a)P	.000791	2.75	56.8	85	(18)	4.11***	+ 12.7
B(a)P	.000250	6.61	82.1	84	(18)	3.04***	+ 6.20
109591-L	4.77	.000	.000	0	(0,18)	.000	NA
109591-L	3.18	.000	53.8	0	(1,18)	.000	.00(-.65)
109591-L	2.39	20.9	44.3	3	(10,18)	.231	.00(- .35)
109591-L	1.59	53.4	99.7	5	(18)	.193	.00(- .76)
NC-1	Control	100.	100.	29	(72)	.289	Control
							Mean t = .000
Trial 2 [100]							
B(a)P	.000791	89.7	77.9	65	(18)	3.30***	+ 11.7
B(a)P	.000250	81.0	93.8	62	(18)	2.85***	+ 7.75

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Appendix E. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
				Focus Data Type Vessels		Foci/Vessel Focus Type	
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	*t*-statistic
109591-L	9.09	.000	.000	0	(2,18)	.000	.00(-5.11)
109591-L	6.82	29.9	54.1	1	(8,18)	.091	.00(-1.06)
109591-L	4.55	30.3	71.8	9	(18)	.348	+ .57
109591-L	2.27	57.0	92.1	12	(18)	.464	+ 1.32
NC-1	Control	100.	100.	28	(72)	.268	Control
							Mean t = .945

Abbreviations: B(a)P, benzo(a)pyrene; CC.A., co-culture clonal survival assay; Conc., concentration; mM, millimole; M.W., molecular weight; N, number of culture vessels, NC, negative control; ND, not determined; %RCE, percent relative cloning efficiency; S.A., standard clonal survival assay.

[a]Treatment Condition: The experimental design for the transformation assay is described in detail in the Materials and Methods. The concentration of the positive control and test chemical treatment are presented in mM, but they can be converted to μg/ml using the molecular weight that is provided with each chemical. The solvent vehicles used for the individual test chemicals were listed in Appendix Tables A1 and A3, and the concentrations of the solvent vehicles are presented in the Materials and Methods.

[b]Cytotoxic activity: The experimental design for the standard survival assay (SA) and the co-culture clonal survival assay (CCA) were described in the Materials and Methods. The test chemical cytotoxic response was expressed as & RCE and was calculated as described in the Materials and Methods.

[c]The criteria used to evaluate the transformed foci of BALB/c-3T3 cells is described in the Materials and Methods. The number of type III foci > 2-mm in diameter per culture vessel scored are recorded in this table.

[d]Transformation response: The transformation responses are expressed as type III foci/vessel and were calculated using a $\log_{10}$ mathematical transformation procedure (refer to Materials and Methods). The arithmetic value or foci/vessel represents the antilog of the $\log_{10}$ mean transformation response minus one.

[e]Significance: The significance of test chemical transformation responses was calculated by a computer using the SAS statistical software (*22*), and the method is described in detail in Materials and Methods. The correct *t*-statistic according to the F-test is presented in this table. The *t*-statistics of each treatment dose of the test chemical in a single experiment were averaged to determine the mean *t*-statistic of the test chemical for the experiment (refer to Appendix Tables A2 and A5). The mean *t*-statistics for two or experiments for each chemical was weighted to the number of treatment doses evaluated and averaged to determine the rank *t*-statistic which was used to rank-order the test chemical transformation responses in Appendix Tables A3 and A6. Arbitrarily, transformation responses with negative (−) *t*-statistics were given a value of zero (0).

*Significant BaP or test chemical transformation response, $0.01 < p \leq 0.05$.

**Significant BaP or test chemical transformation response, $0.001 < p \leq 0.01$.

***Significant or BaP or test chemical transformation response, $p \leq 0.001$.

Appendix F.

Summary of the transformation responses of 12 noncytotoxic carcinogens.

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
				Focus Data Type Vessels		Foci/Vessel Focus Type	
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	t-statistic
3-Amino-1,2,4-Triazole [AMT, M.W. = 84.08]							
Trial 1 [69]							
B(a)P	.000791	1.72	50.1	63	(20)	2.67***	+ 9.34
B(a)P	.000250	11.3	68.1	33	(20)	1.17**	+ 3.53
AMT	200.	.000	.000	1	(9,20)	.080	.00(-1.42)
AMT	100.	3.44	57.7	21	(20)	.798*	+ 2.53
AMT	50.0	53.3	94.2	33	(20)	1.30***	+ 4.24
AMT	25.0	88.7	75.3	27	(19)	1.14***	+ 3.86
NC-1	Control	100.	100.	15	(40)	.288	Control
							Mean t = 3.54
Trial 2 [99]							
B(a)P	.000791	18.9	68.4	160	(20)	6.67***	+ 12.4
B(a)P	.000250	32.1	85.0	94	(20)	3.59***	+ 7.95
AMT	107.	4.91	31.0	50	(20)	2.20***	+ 5.77
AMT	80.3	39.6	56.9	123	(20)	5.57***	+ 11.6
AMT	53.5	87.5	87.3	104	(20)	4.39***	+ 9.35
AMT	26.8	96.6	106.	53	(20)	2.20***	+ 5.60
NC-1	Control	100.	100.	65	(80)	.586	Control
							Mean t = 8.08
Cyclamate, Sodium Salt [CYC, M.W. = 201.22]							
Trial 1 [71]							
B(a)P	.000791	4.48	50.7	251	(20)	11.0***	+ 12.1
B(a)P	.000250	18.1	68.9	77	(20)	3.50***	+ 7.12
CYC	29.8	42.3	84.4	74	(18)	3.10***	+ 4.21
CYC	22.4	57.4	78.9	54	(18)	2.39**	+ 3.12
CYC	14.9	67.6	76.3	55	(19)	2.40**	+ 3.32
CYC	7.45	78.4	77.3	28	(20)	1.18	+ .38
NC-1	Control	100.	100.	110	(75)	1.06	Control
							Mean t = 2.76
Trial 2 [107]							
B(a)P	.000791	5.81	47.3	131	(20)	6.09***	+ 5.74
B(a)P	.000250	21.2	75.8	122	(20)	5.53***	+ 4.05
CYC	149.	8.55	31.4	61	(20)	2.57	.00(- .78)
CYC	112.	25.0	77.1	159	(19)	7.99***	+ 9.43
CYC	74.6	67.4	87.8	151	(20)	7.21***	+ 8.24
CYC	37.3	92.3	85.6	142	(20)	5.69***	+ 4.00
NA-1	Control	100.	100.	274	(80)	2.95	Control
							Mean t = 5.42
11-Aminoundecanoic Acid [11AMI, M.W. = 201.35]							
Trial 1 [17]							
B(a)P	.000791	.000	52.9	94	(20)	4.43***	+ 13.5
B(a)P	.000250	3.54	79.1	86	(20)	3.91***	+ 11.6
11AMI	.497	.000	100.	15	(20)	.578	+ 1.49
11AMI	.248	.000	102.	11	(20)	.374	+ .30
11AMI	.124	9.29	104.	17	(20)	.606	+ 1.54
11AMI	.0621	57.1	106.	12	(20)	.494	+ 1.08
NC-1	Control	100.	100.	18	(40)	.327	Control
							Mean t = 1.10
Trial 2 [24]							
B(a)P	.000791	.000	18.7	83	(20)	3.65***	+ 10.7
B(a)P	.000250	5.66	70.0	86	(20)	3.73***	+ 10.7

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Appendix F. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
Drug	Conc., mM	S.A	CC.A.	Focus Data Type Vessels III	(N)	Foci/Vessel Focus Type III	*t*-statistic
11AMI	.993	.000	105.	4	(20)	.132	.00(-1.35)
11AMI	.745	.000	104.	3	(20)	.110	.00(-1.88)
11AMI	.497	.000	106.	7	(20)	.275	.00(- .24)
11AMI	.248	.000	104.	3	(20)	.072	.00(-1.87)
NC-1	Control	100.	100.	18	(40)	.308	Control
							Mean t = .000
Trial 3 [32]							
B(a)P	.000791	10.1	51.7	197	(19)	10.1***	+ 12.6
B(a)P	.000250	6.37	77.4	115	(20)	5.46***	+ 6.94
11AMI	19.87	.000	26.2	100	(20)	4.52***	+ 4.31
11AMI	9.93	.000	75.3	87	(20)	3.77***	+ 3.11
11AMI	4.97	.000	78.1	42	(20)	1.56	.00(- .97)
11AMI	2.48	.000	74.2	53	(20)	1.79	.00(- .41)
NC-1	Control	100.	100.	91	(38)	1.99	Control
							Mean t = 1.86
Trial 4 [67]							
B(a)P	.000791	5.87	34.4	48	(20)	2.07***	+ 8.71
B(a)P	.000250	20.8	63.9	39	(20)	.969**	+ 3.32
11AMI	20.0	.000	72.0	17	(20)	.644***	+ 3.52
11AMI	10.0	.000	90.7	10	(20)	.354*	+ 2.08
11AMI	5.00	.000	86.8	10	(18)	.392*	+ 2.16
11AMI	2.50	.000	87.6	9	(20)	.301	+ 1.71
NC-1	Control	100.	100.	5	(39)	.085	Control
							Mean t = 2.37
Decabromodiphenyloxide [917884-S, M.W. = 959.22]							
Trial 1 [75]							
B(a)P	.000791	7.10	66.5	149	(20)	6.35***	+ 10.9
B(a)P	.000250	28.4	85.4	67	(20)	3.10***	+ 6.56
917884-S	4.00	83.9	80.9	22	(20)	.818	.00(- .27)
917884-S	2.00	94.8	91.0	22	(20)	.882	.00
917884-S	1.00	101.	82.8	24	(20)	.882	.00
917884-S	.500	92.7	99.4	30	(20)	1.04	+ .60
NC-1	Control	100.	100.	89	(78)	.882	Control
							Mean t = .150
Trial 2 [101]							
B(a)P	.000791	ND	64.8	108	(20)	4.63***	+ 12.7
B(a)P	.000250	ND	83.6	48	(20)	2.11***	+ 9.73
917884-S	4.17	ND	106.	6	(19)	.245	.00(- .14)
917884-S	2.08	ND	108.	7	(20)	.238	.00(- .19)
917884-S	1.04	ND	108.	12	(20)	.473	+ 1.72
917884-S	.521	ND	110.	5	(20)	.189	.00(- .68)
NC-1	Control	100.	100.	27	(78)	.260	Control
							Mean t = .430
DC Red No. 9 [DCR9, M.W. = 444.49]							
Trial 1 [43]							
B(a)P	.000791	1.02	53.0	382	(20)	18.9***	+ 26.3
B(a)P	.000250	4.75	77.5	270	(20)	13.0***	+ 19.0
DCR9	4.50	30.0	80.5	145	(20)	6.75***	+ 10.9
DCR9	2.50	71.1	85.8	108	(20)	5.12***	+ 10.5
DCR9	1.12	93.9	96.7	95	(20)	4.22***	+ 7.16
DCR9	.562	98.3	107.	75	(20)	3.16***	+ 5.26
NC-1	Control	100.	100.	44	(35)	1.05	Control
							Mean t = 8.46

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Appendix F. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c] Focus Data Type Vessels		Transformation Response[d] Foci/Vessel Focus Type	Significance[e]
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	*t*-statistic
Trial 2 [54]							
B(a)P	.000791	1.47	17.6	105	(20)	4.75***	+ 13.8
B(a)P	.000250	.490	61.1	100	(20)	4.52***	+ 13.5
DCR9	2.02	67.6	65.9	19	(18)	.817**	+ 2.96
DCR9	1.01	111.	81.8	12	(19)	.480	+ 1.39
DCR9	.526	112.	98.3	9	(20)	.270	+ .04
DCR9	.253	102.	99.3	6	(20)	.214	.00(- .41)
NC-1	Control	100.	100.	15	(40)	.265	Control
							Mean t = 1.10
Trial 3 [67]							
B(a)P	.000791	5.87	34.4	48	(20)	2.07***	+ 8.71
B(a)P	.000250	20.8	63.9	39	(20)	.969**	+ 3.32
DCR9	4.05	80.8	47.8	47	(20)	1.65***	+ 5.47
DCR9	2.02	91.4	59.3	27	(20)	.835**	+ 3.37
DCR9	1.01	93.3	65.4	13	(20)	.503**	+ 3.07
DCR9	.506	106.	74.1	9	(20)	.308	+ 1.78
NC-1	Control	100.	100.	5	(39)	.085	Control
							Mean t = 3.42
Di(2-Ethylhexyl)adipate [DEHA, M.W. = 370.57, Density = 0.928 g/ml]							
Trial 1 [88]							
B(a)P	.000791	15.8	73.9	54	(15)	3.36***	+ 9.58
B(a)P	.000250	35.5	82.6	24	(15)	1.50***	+ 4.90
DEHA	85.3	2.76	57.0	6	(18)	.181	.00(-1.53)
DEHA	27.0	3.53	71.2	3	(18)	.122	.00(-2.11)
DEHA	8.53	11.4	94.9	8	(18)	.339	.00(- .43)
DEHA	2.70	17.0	102.	6	(18)	.240	.00(-1.13)
NC-1	Control	100.	100.	37	(67)	.406	Control
							Mean t = .000
Trial 2 [108]							
B(a)P	.000791	15.5	31.3	139	(18)	7.38***	+ 13.9
B(a)P	.000250	30.0	65.7	72	(18)	4.77***	+ 5.96
DEHA	85.3	15.8	57.3	5	(18)	.212	.00(-5.88)
DEHA	27.0	22.6	83.6	3	(18)	.122	.00(-7.33)
DEHA	8.53	42.3	78.0	12	(18)	.587	.00(-3.07)
DEHA	2.70	60.0	92.0	7	(18)	.289	.00(-3.84)
NC-1	Control	100.	100.	108	(70)	1.17	Control
							Mean t = .000
Di(2-Ethylhexyl)phthalate [DEHP, M.W. = 390.54, Density = 0.981 g/ml]							
Trial 1 [36]							
B(a)P	.000791	2.98	40.1	73	(9)	7.27***	+ 21.8
B(a)P	.000250	7.66	78.1	88	(18)	4.37***	+ 10.8
DEHP	30.1	2.13	31.5	0	(10,18)	.000	.00(-5.07)
DEHP	15.1	6.81	70.3	1	(9,18)	.080	.00(-1.90)
DEHP	7.54	20.9	92.3	2	(16,18)	.071	.00(-2.49)
DEHP	3.77	28.9	94.4	7	(18)	.289	.00(- .85)
NC-1	Control	100.	100.	20	(36)	.424	Control
							Mean t = .000
Trial 2 [100]							
B(a)P	.000791	89.7	77.9	65	(18)	3.30***	+ 11.7
B(a)P	.000250	81.0	93.8	62	(18)	2.85***	+ 7.75

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Appendix F. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
Drug	Conc., mM	S.A	CC.A.	Focus Data Type Vessels III	(N)	Foci/Vessel Focus Type III	*t*-statistic
DEHP	37.7	.000	64.3	3	(17)	.130	.00(-1.14)
DEHP	18.8	3.22	74.7	0	(18)	.000	.00(-5.11)
DEHP	9.42	22.1	69.0	2	(18)	.080	.00(-1.66)
DEHP	4.71	47.4	87.0	4	(18)	.148	.00(- .99)
NC-1	Control	100.	100.	20	(72)	.268	Control Mean = .000
Dimethyl Hydrogen Phosphite [DMHP, M.W. = 110.05, Density = ND g/ml]]							
Trial 1 [84]							
B(a)P	.000791	49.8	57.9	71	(18)	3.27***	+ 6.82
B(a)P	.000250	ND	71.7	57	(18)	2.44***	+ 6.14
DMHP	200.	.000	.000	0	(3,18)	.000	.00(-7.45)
DMHP	150.	1.47	7.36	7	(18)	.248	.00(-1.56)
DMHP	100.	5.13	66.9	141	(18)	7.00***	+ 13.6
DMHP	50.0	22.7	90.4	33	(18)	1.60***	+ 4.47
NC-1	Control	100.	100.	50	(72)	.511	Control Mean t = 6.02
Trial 2 [104]							
B(a)P	.000791	25.0	64.5	62	(18)	2.79***	+ 4.87
B(a)P	.000250	50.6	89.6	63	(18)	2.47***	+ 4.10
DMHP	164.	.000	16.6	30	(18)	1.18	+ 1.01
DMHP	123.	6.96	65.7	111	(18)	5.76***	+ 9.55
DMHP	81.8	16.8	71.0	89	(18)	4.67***	+ 11.1
DMHP	40.9	63.3	94.1	32	(18)	1.50*	+ 2.06
NC-1	Control	100.	100.	83	(71)	.878	Control Mean t = 5.93
Diethylnitrosamine [DEN, M.W. = 102.14, Density = ND g/ml]							
Trial 1 [79]							
B(a)P	.000791	22.7	79.6	279	(13)	20.8***	+ 13.9
B(a)P	.000250	39.3	94.2	241	(18)	12.9***	+ 9.34
DEN	138.	.000	.000	0	(0,18)	.000	ND
DEN	104.	.000	.000	0	(0,18)	.000	ND
DEN	69.2	.826	4.05	153	(18)	8.12***	+ 4.11
DEN	34.6	2.48	78.8	207	(18)	11.1***	+ 7.71
NC-1	Control	100.	100.	430	(72)	5.12	Control Mean t = 5.91
Trial 2 [102]							
B(a)P	.000791	9.64	69.9	99	(18)	4.65***	+ 9.48
B(a)P	.000250	19.3	93.1	45	(18)	2.22***	+ 5.22
DEN	73.8	.000	.000	2	(1,18)	.063	+ 1.20
DEN	55.3	.000	1.87	61	(18)	2.46**	+ 3.78
DEN	36.9	.000	45.9	40	(18)	1.77***	+ 3.77
DEN	18.4	1.26	57.3	44	(18)	1.91***	+ 4.10
NC-1	Control	100.	100.	64	(72)	.697	Control Mean t = 3.88
Dimethylnitrosamine [DMN, M.W. = 74.08, Density = 1.01 g/ml]							
Trial 1 [31]							
B(a)P	.000791	1.87	69.1	136	(15)	8.63***	+ 11.1
B(a)P	.000250	5.14	99.9	126	(18)	6.06***	+ 8.51

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Appendix F. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
				Focus Data Type Vessels		Foci/Vessel Focus Type	
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	*t*-statistic
DMN	489.	.000	.000	1	(18)	.059	.00(-5.78)
DMN	367.	.000	34.2	31	(18)	1.49	+ 1.78
DMN	244.	.000	88.0	42	(17)	1.98**	+ 2.72
DMN	122.	10.7	113.	123	(18)	4.76**	+ 6.42
NC-1	Control	100.	100.	43	(36)	.930	Control
							Mean t = 3.64
Trial 2 [100]							
B(a)P	.000791	89.7	77.9	65	(18)	3.30***	+ 11.7
B(a)P	.000250	81.0	93.8	62	(18)	2.85***	+ 7.75
DMN	367.	.000	.000	0	(0,18)	.000	NA
DMN	244.	.000	.000	3	(16,18)	.139	.00(-1.03
DMN	122.	.000	83.9	35	(18)	1.54***	+ 4.82
DMN	61.1	18.9	88.7	30	(18)	1.49***	+ 6.50
NC-1	Control	100.	100.	29	(72)	.268	Control
							Mean t = 5.66
Dimethyl Methyl Phosphonate [267599-L, M.W. = 124.08, Density = 1.145 g/ml]							
Trial 1 [84]							
B(a)P	.000791	49.8	57.9	71	(18)	3.27***	+ 6.82
B(a)P	.000250	ND	71.7	57	(18)	2.44***	+ 6.14
267599-L	175.	.000	17.6	14	(17)	.604	+ .47
267599-L	131.	2.93	77.7	32	(18)	1.32**	+ 3.26
267599-L	87.5	11.7	88.3	20	(18)	.876	+ 1.72
267599-L	43.8	60.1	101.	27	(18)	1.25**	+ 3.21
NC-1	Control	100.	100.	50	(72)	.511	Control
							Mean t = 2.17
Trial 2 [102]							
B(a)P	.000791	9.64	69.9	99	(18)	4.65***	+ 9.48
B(a)P	.000250	19.3	93.1	45	(18)	2.22***	+ 5.22
267599-L	183.	.419	27.5	57	(18)	2.80***	+ 6.57
267599-L	137.	1.33	70.9	52	(18)	2.36***	+ 5.23
267599-L	91.7	11.3	82.1	20	(17)	.947	+ 1.07
267599-L	45.8	55.8	82.9	27	(18)	1.11	+ 1.65
NC-1	Control	100.	100.	64	(72)	.697	Control
							Mean t = 3.63
Dimethylmorpholinophosphoramidate [945355-L, M.W. = 195.18, Density = NA g/ml]							
Trial 1 [86]							
B(a)P	.000791	18.7	63.3	64	(18)	3.32***	+ 9.07
B(a)P	.000250	47.9	87.6	42	(18)	2.02***	+ 5.74
945355-L	ND	.000	38.5	75	(18)	3.48***	+ 8.69
945355-L	ND	.000	82.4	43	(18)	2.12***	+ 6.15
945355-L	ND	5.45	77.7	24	(18)	1.11**	+ 2.90
945355-L	ND	27.2	77.7	12	(18)	.441	.00(- .12)
NC-1	Control	100.	100.	47	(72)	.464	Control
							Mean t = 4.44
Trial 2 [108]							
B(a)P	.000791	15.5	31.3	139	(18)	7.38***	+ 13.9
B(a)P	.000250	30.0	65.7	72	(13)	4.77***	+ 5.96
945355-L	ND	.000	61.2	31	(16)	1.66	+ 1.36
945355-L	ND	.000	73.5	33	(11)	2.63**	+ 2.96
945355-L	ND	.000	86.7	58	(12)	4.62***	+ 8.96
945355-L	ND	1.24	97.3	29	(9)	2.70**	+ 2.78
NC-1	Control	100.	100.	108	(70)	1.17	Control
							Mean t = 4.02

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Appendix F. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
				Focus Data Type Vessels		Foci/Vessel Focus Type	
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	*t*-statistic
Dimethylmorpholinophosphoramidate [DMMP, M.W. = 195.16, Density = ND g/ml]							
Trial 1 [86]							
B(a)P	.000791	18.7	63.3	64	(18)	3.32***	+ 9.07
B(a)P	.000250	47.9	87.6	42	(18)	2.02***	+ 5.74
DMMP	16.4	.000	50.7	64	(18)	2.99***	+ 7.70
DMMP	8.21	4.09	74.0	29	(18)	1.31**	+ 3.56
DMMP	4.10	12.1	83.0	28	(18)	1.06*	+ 2.54
DMMP	2.05	49.8	82.6	14	(18)	.650	+ .98
NC-1	Control	100.	100.	47	(72)	.464	Control
							Mean t = 3.70
Trial 2 [106]							
B(a)P	.00079	27.0	56.9	134	(18)	6.88***	+ 8.00
B(a)P	.00025	44.3	77.9	91	(18)	4.53***	+ 5.56
DMMP	23.1	.000	47.3	47	(18)	2.30*	+ 2.28
DMMP	17.3	.000	61.3	66	(18)	3.13***	+ 3.56
DMMP	11.5	.000	89.1	57	(18)	2.86**	+ 3.35
DMMP	5.77	7.65	91.2	41	(18)	1.95	+ 1.54
NC-1	Control	100.	100.	74	(43)	1.30	Control
							Mean t = 2.68
Diethanolnitrosamine [DETN, M.W. = 134.14, Density = ND g/ml]							
Trial 1 [86]							
B(a)P	.000791	18.7	63.3	64	(18)	3.32***	+ 9.07
B(a)P	.000250	47.9	87.6	42	(18)	2.02***	+ 5.74
DETN	59.6	.000	66.6	38	(18)	1.83***	+ 5.39
DETN	44.7	1.17	75.2	24	(18)	1.24***	+ 3.61
DETN	29.8	26.1	87.0	21	(18)	1.04**	+ 2.75
DETN	14.9	49.8	90.5	25	(18)	1.18**	+ 3.22
NC-1	Control	100.	100.	47	(72)	.464	Control
							Mean t = 4.01
Ethylene Thiourea [ETU, M.W. = 102.16]							
Trial 1 [59]							
B(a)P	.00079	1.3	36.5	165	(20)	7.31***	+ 17.9
B(a)P	.00025	7.4	74.5	33	(19)	1.34***	+ 4.28
ETU	157.	7.4	80.8	14	(20)	.533	+ 1.62
ETU	117.	47.8	96.1	12	(20)	.473	+ 1.27
ETU	78.3	68.4	83.9	59	(20)	1.21*	+ 2.41
ETU	39.1	80.8	104.	13	(20)	.459	+ .99
NC-1	Control	100.	100.	15	(40)	.297	Control
							Mean t = 1.57
Trial 2 [65]							
B(a)P	.000791	6.95	60.7	85	(18)	4.38***	+ 13.9
B(a)P	.000250	19.2	90.2	43	(20)	1.80***	+ 6.94
ETU	157.	2.65	.000	12	(11,20)	.829**	+ 2.72
ETU	78.3	15.2	57.3	19	(20)	.772**	+ 3.18
ETU	39.1	45.7	62.5	10	(20)	.394	+ 1.10
ETU	3.91	106.	65.1	17	(20)	.611*	+ 2.22
NC-1	Control	100.	100.	14	(40)	.244	Control
							Mean t = 2.17

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Appendix F. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
				Focus Data Type Vessels		Foci/Vessel Focus Type	
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	*t*-statistic
Hexamethylphosphoramide [HMPA, M.W. = 179.2, Density = NA g/ml]							
Trial 1 [78]							
B(a)P	.000791	8.09	60.6	116	(18)	6.11***	+ 4.93
B(a)P	.000250	14.9	84.2	184	(18)	9.84***	+ 9.74
HMPA	78.1	.426	34.0	92	(18)	4.02	+ 1.01
HMPA	58.6	.426	76.5	167	(18)	8.50***	+ 5.42
HMPA	39.1	8.09	77.6	103	(18)	5.44***	+ 4.05
HMPA	19.5	48.9	94.8	64	(18)	3.13	.00(- .23)
NC-1	Control	100.	100.	296	(72)	3.28	Control
							Mean t = 2.62
Trial 2 [98]							
B(a)P	.000791	8.38	79.6	132	(18)	6.82***	+ 11.8
B(a)P	.000250	29.3	91.3	75	(18)	3.38***	+ 6.81
HMPA	83.7	1.05	26.0	10	(18)	.401	.00(-1.03)
HMPA	62.8	5.24	62.2	34	(18)	1.43**	+ 2.67
HMPA	41.9	6.28	83.5	19	(18)	.805	+ .75
HMPA	20.9	36.6	93.0	31	(18)	1.40**	+ 2.71
NC-1	Control	100.	100.	39	(45)	.618	Control
							Mean t = 1.53
Melamine [MELM, M.W. = 126.12]							
Trial 1 [43]							
B(a)P	.000791	1.02	53.0	382	(20)	18.9***	+ 26.3
B(a)P	.000250	4.75	77.5	270	(20)	13.0***	+ 19.0
MELM	31.7	8.50	45.5	55	(19)	2.32**	+ 3.23
MELM	15.9	35.0	73.1	64	(20)	2.56***	+ 4.03
MELM	7.93	63.6	75.3	51	(20)	1.61	+ 1.56
MELM	3.96	95.6	80.1	17	(20)	.69	.00(-1.48)
NC-1	Control	100.	100.	44	(35)	1.05	Control
							Mean t = 1.82
Trial 2 [58]							
B(a)P	.000791	3.92	34.3	128	(20)	6.17***	+ 22.5
B(a)P	.000250	14.7	60.6	71	(20)	1.83***	+ 4.78
MELM	32.0	14.0	68.9	26	(20)	1.12***	+ 5.97
MELM	16.0	25.2	70.7	10	(20)	.414	+ 1.97
MELM	8.00	87.9	80.7	4	(19)	.157	.00(- .33)
MELM	4.00	93.1	80.4	4	(20)	.149	.00(- .43)
NC-1	Control	100.	100.	10	(40)	.189	Control
							Mean t = 1.99
Methyl Carbamate [MEC, M.W. = 75.07]							
Trial 1 [42]							
B(a)P	.000791	2.41	35.7	280	(20)	13.7***	+ 20.2
B(a)P	.000250	5.72	65.4	161	(19)	7.44***	+ 9.55
MEC	293.	1.81	6.91	13	(20)	.400	.00(-1.67)
MEC	220.	13.6	57.8	114	(20)	4.69***	+ 6.73
MEC	147.	62.3	72.3	156	(20)	6.70***	+ 9.44
MEC	73.3	84.9	85.8	137	(20)	5.23***	+ 7.23
NC-1	Control	100.	100.	52	(40)	.861	Control
							Mean t = 5.85
Trial 2 [66]							
B(a)P	.000791	2.33	48.6	90	(20)	3.92***	+ 14.1
B(a)P	.000250	6.08	98.4	24	(20)	.795***	+ 3.69

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Appendix F. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c] Focus Data Type Vessels		Transformation Response[d] Foci/Vessel Focus Type	Significance[e]
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	*t*-statistic
MEC	200.	5.41	80.6	10	(16)	.330	+ 1.59
MEC	63.3	86.1	101.	13	(20)	.525***	+ 3.80
MEC	20.0	91.9	109.	4	(19)	.123	+ .72
MEC	6.32	105.	111.	1	(20)	.035	.00(- .41)
NC-1	Control	100.	100.	3	(38)	.056	Control
							Mean t = 1.53
Trial 3 [80]							
B(a)P	.000791	16.9	65.2	261	(20)	12.9***	+ 13.0
B(a)P	.000250	35.6	87.2	185	(20)	8.53***	+ 7.65
MEC	320.	.000	3.24	12	(20)	.473	.00(-8.62)
MEC	240.	3.86	60.6	86	(20)	3.54	+ .77
MEC	160.	52.2	76.3	179	(20)	8.36***	+ 7.72
MEC	80.0	87.8	106.	237	(20)	10.2***	+ 6.75
NC-1	Control	100.	100.	317	(80)	3.02	Control
							Mean t = 3.81
Methyl Carbamate [315183-S, M.W. = 75.07]							
Trial 1 [83]							
B(a)P	.000791	2.86	73.0	141	(20)	6.14***	+ 13.5
B(a)P	.000250	13.9	78.9	64	(20)	2.93***	+ 9.12
315183-S	325.	.000	.000	1	(18,20)	.039	.00(-3.94)
315183-S	244.	2.86	34.2	8	(20)	.301	.00(- .33)
315183-S	163.	19.5	63.5	23	(20)	.771*	+ 2.09
315183-S	81.3	71.9	87.9	48	(19)	1.65***	+ 5.02
NC-1	Control	100.	100.	48	(80)	.351	Control
							Mean t = 2.38
Trial 2 [99]							
B(a)P	.000791	18.9	68.4	160	(20)	6.67***	+ 12.4
B(a)P	.000250	32.1	85.0	94	(20)	3.59***	+ 7.95
315183-S	231.	7.92	39.8	50	(20)	1.35	+ 2.01
315183-S	173.	41.9	70.9	48	(20)	1.91***	+ 4.66
315183-S	116.	84.9	82.7	92	(20)	3.99***	+ 9.12
315183-S	57.8	103.	104.	63	(20)	2.00**	+ 3.43
NC-1	Control	100.	100.	65	(80)	.586	Control
							Mean t = 4.81
Monuron [MONU, M.W. = 198.65]							
Trial 1 [20]							
B(a)P	.000791	.000	39.4	268	(20)	13.0***	+ 26.3
B(a)P	.000250	2.34	77.3	99	(20)	4.00***	+ 9.22
MONU	7.95	.000	44.5	54	(20)	2.17***	+ 6.19
MONU	2.52	1.56	79.5	14	(20)	.385	+ .09
MONU	.795	7.03	89.7	6	(20)	.214	.00(-1.06)
MONU	.252	28.1	92.4	3	(20)	.110	.00(-2.33)
NC-1	Control	100.	100.	21	(40)	.368	Control
							Mean t = 1.57
Trial 2 [28]							
B(a)P	.000791	2.84	28.6	189	(20)	9.02***	+ 16.9
B(a)P	.000250	6.74	68.0	62	(20)	2.78***	+ 5.73
MONU	12.1	.000	19.1	7	(18,19)	.289	.00(-2.71
MONU	8.05	.000	45.9	4	(18,20)	.167	.00(-4.33)
MONU	4.03	.000	90.2	21	(20)	.807	.00(- .05)
MONU	2.01	15.6	109.	32	(20)	1.32	+ 1.81
NC-1	Control	100.	100.	41	(40)	.818	Control
							Mean t = .453

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Appendix F. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
Drug	Conc., mM	S.A	CC.A.	Focus Data Type Vessels III	(N)	Foci/Vessel Focus Type III	*t*-statistic
Phenobarbital, Sodium Salt [PHENB, M.W. = 254.22]							
Trial 1 [59]							
B(a)P	.000791	1.35	36.5	165	(20)	7.31***	+ 17.9
B(a)P	.000250	7.41	74.3	33	(19)	1.34***	+ 4.28
PHENB	7.87	21.2	23.1	12	(20)	.494	+ 1.46
PHENB	5.90	33.0	81.7	11	(20)	.414	+ .86
PHENB	3.94	59.6	89.9	14	(20)	.578	+ 1.88
PHENB	1.97	100.	100.	22	(20)	.927***	+ 3.85
NC-1	Control	100.	100.	15	(40)	.297	Control
							Mean t = 2.01
Trial 2 [109]							
B(a)P	.000791	51.9	94.3	99	(20)	4.69***	+ 5.31
B(a)P	.000250	66.2	108.	81	(20)	3.91***	+ 4.16
PHENB	3.94	69.1	104.	72	(20)	3.37	+ 1.78
PHENB	2.95	70.1	110.	128	(20)	5.99***	+ 5.76
PHENB	1.97	85.0	112.	95	(17)	5.25***	+ 4.50
PHENB	.984	86.9	112.	81	(20)	3.56*	+ 2.04
NC-1	Control	100.	100.	237	(80)	2.55	Control
							Mean t = 3.52
Saccharin, Sodium Salt [SAC, M.W. = 205.2]							
Trial 1 [75]							
B(a)P	.000791	7.10	66.5	149	(20)	6.35***	+ 10.9
B(a)P	.000250	28.4	85.4	67	(20)	3.10***	+ 6.56
SAC	136.	.835	.000	10	(20)	.394	.00(-2.50
SAC	102.	7.93	19.2	63	(20)	2.41***	+ 4.55
SAC	68.2	33.4	59.5	64	(20)	2.88***	+ 5.93
SAC	34.1	82.3	73.2	51	(20)	2.23***	+ 4.36
NC-1	Control	100.	100.	89	(78)	.882	Control
							Mean t = 3.71
Trial 2 [101]							
B(a)P	.000791	ND	64.8	108	(20)	4.63***	+ 12.7
B(a)P	.000250	ND	83.6	48	(20)	2.11***	+ 9.73
SAC	122.	.000	12.2	33	(18)	1.30**	+ 3.74
SAC	91.4	28.7	53.2	67	(17)	3.33***	+ 9.12
SAC	60.9	91.3	67.7	149	(17)	8.32***	+ 21.8
SAC	30.5	93.4	108.	48	(19)	2.05***	+ 6.43
NC-1	Control	100.	100.	27	(78)	.260	Control
							Mean t = 10.3
2,4- 2,6-Toluene Diisothiocyanate [TDIC, M.W. = 174.16, Density = 1.255 g/ml]							
Trial 1 [76]							
B(a)P	.000791	5.91	63.1	87	(18)	4.41***	+ 5.23
B(a)P	.000250	23.3	97.0	54	(18)	2.72*	+ 2.26
TDIC	8.76	115.	59.2	46	(18)	1.39	.00(- .68)
TDIC	4.38	110.	64.7	92	(18)	3.60*	+ 2.40
TDIC	1.39	106.	72.6	83	(17)	4.53***	+ 5.32
TDIC	.438	107.	83.6	65	(18)	3.02**	+ 2.70
NC-1	Control	100.	100.	152	(71)	1.79	Control
							Mean t = 2.61
Trial 2 [106]							
B(a)P	.000791	2.48	56.9	134	(18)	6.88***	+ 8.00
B(a)P	.000250	40.7	77.9	91	(18)	4.53***	+ 5.56

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Appendix F. ***Continued.***

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
				Focus Data Type Vessels		Foci/Vessel Focus Type	
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	*t*-statistic
TDIC	4.02	.000	2.19	6	(14,16)	.281	.00(-3.38)
TDIC	3.02	12.8	10.3	12	(18)	.513	.00(-2.68)
TDIC	2.01	64.8	47.7	60	(17)	3.17***	+ 3.74
TDIC	1.01	100.	92.6	78	(17)	4.33***	+ 6.89
NC-1	Control	100.	100.	74	(43)	1.30	Control Mean t = 3.54

Abbreviations: B(a)P, benzo(a)pyrene; CC.A., co-culture clonal survival assay; Conc., concentration; mM, millimole; M.W., molecular weight; N, number of culture vessels, NC, negative control; ND, not determined; %RCE, percent relative cloning efficiency; S.A., standard clonal survival assay.

[a]Treatment Condition: The experimental design for the transformation assay is described in detail in the Materials and Methods. The concentration of the positive control and test chemical treatment are presented in mM, but they can be converted to μg/ml using the molecular weight that is provided with each chemical. The solvent vehicles used for the individual test chemicals were listed in Appendix Tables A1 and A3, and the concentrations of the solvent vehicles are presented in the Materials and Methods.

[b]Cytotoxic activity: The experimental design for the standard survival assay (SA) and the co-culture clonal survival assay (CCA) were described in the Materials and Methods. The test chemical cytotoxic response was expressed as & RCE and was calculated as described in the Materials and Methods.

[c]The criteria used to evaluate the transformed foci of BALB/c-3T3 cells is described in the Materials and Methods. The number of type III foci > 2-mm in diameter per culture vessel scored are recorded in this table.

[d]Transformation response: The transformation responses are expressed as type III foci/vessel and were calculated using a $\log_{10}$ mathematical transformation procedure (refer to Materials and Methods). The arithmetic value or foci/vessel represents the antilog of the $\log_{10}$ mean transformation response minus one.

[e]Significance: The significance of test chemical transformation responses was calculated by a computer using the SAS statistical software (*22*), and the method is described in detail in Materials and Methods. The correct *t*-statistic according to the F-test is presented in this table. The *t*-statistics of each treatment dose of the test chemical in a single experiment were averaged to determine the mean *t*-statistic of the test chemical for the experiment (refer to Appendix Tables A2 and A5). The mean *t*-statistics for two or experiments for each chemical was weighted to the number of treatment doses evaluated and averaged to determine the rank *t*-statistic which was used to rank-order the test chemical transformation responses in Appendix Tables A3 and A6. Arbitrarily, transformation responses with negative (−) *t*-statistics were given a value of zero (0).

*Significant BaP or test chemical transformation response, $0.01 < p \leq 0.05$.

**Significant BaP or test chemical transformation response, $0.001 < p \leq 0.01$.

***Significant or BaP or test chemical transformation response, $p \leq 0.001$.

Appendix G.

Summary of the transformation responses of 26 noncytotoxic, noncarcinogens.

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c] Focus Data Type Vessels		Transformation Response[d] Foci/Vessel Focus Type	Significance[e]
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	t-statistic
Aldicarb [ALDC, M.W. = 190.27]							
Trial 1 [32]							
B(a)P	.000791	10.1	51.7	197	(19)	10.1***	+ 12.6
B(a)P	.000250	6.37	77.4	115	(20)	5.46***	+ 6.94
ALDC	15.8	.000	.000	3	(15,20)	.149	.00(-8.26)
ALDC	10.5	.000	38.4	52	(19)	2.36	+ .80
ALDC	5.26	6.37	95.0	29	(19)	1.41	.00(-1.85)
ALDC	2.63	58.1	85.1	35	(19)	1.58	.00(-1.00)
NC-1	Control	100.	100.	91	(38)	1.99	Control
							Mean t = .267
Trial 2 [99]							
B(a)P	.000791	18.9	68.4	160	(20)	6.67***	+ 12.4
B(a)P	.000250	32.1	85.0	94	(20)	3.59***	+ 7.95
ALDC	14.7	15.5	24.0	41	(18,20)	1.73***	+ 3.92
ALDC	11.0	26.4	97.2	43	(19)	1.74***	+ 4.09
ALDC	7.36	47.2	86.6	30	(20)	1.23**	+ 2.69
ALDC	3.68	51.7	108.	27	(20)	1.05*	+ 1.99
NC-1	Control	100.	100.	65	(80)	.586	Control
							Mean t = 3.17
Ampicillin Trihydrate [577642-S, M.W. = 403.50]							
Trial 1 [83]							
B(a)P	.000791	2.86	73.0	141	(20)	6.14***	+ 13.5
B(a)P	.000250	13.8	78.9	64	(20)	2.93***	+ 9.12
577642-S	65.0	.000	.000	0	(0,14)	.000	NA
577642-S	48.8	1.43	.257	3	(15)	.127	.00(-1.82)
577642-S	32.5	72.9	18.3	9	(17)	.395	+ .25
577642-S	16.3	111.	88.7	25	(26)	.938**	+ 2.87
NC-1	Control	100.	100.	48	(80)	.351	Control
							Mean t = 1.04
Trial 2 [105]							
B(a)P	.000791	5.67	63.1	59	(20)	2.43***	+ 6.70
B(a)P	.000250	18.2	86.5	40	(19)	1.93***	+ 5.54
577642-S	52.5	.000	.000	14	(16)	.593	+ .06
577642-S	39.4	.000	1.32	11	(12)	.740	+ .68
577642-S	26.3	11.7	13.0	45	(16)	2.07**	+ 3.60
577642-S	13.1	114.	84.0	21	(18)	.950	+ 1.75
NC-1	Control	100.	100.	58	(77)	.581	Control
							Mean t = 1.52
***o*-Anthranilic Acid** [ANT, M.W. = 137.14]							
Trial 1 [15]							
B(a)P	.000791	.851	12.4	209	(20)	8.95***	+ 15.8
B(a)P	.000250	3.83	56.7	72	(20)	3.40***	+ 14.9
ANT	36.5	68.9	95.9	7	(17)	.206	+ .15
ANT	18.2	78.7	113.	13	(19)	.379	+ 1.06
ANT	9.11	94.0	113.	24	(19)	.605	+ 1.78
ANT	4.56	94.9	114.	14	(18)	.608*	+ 2.53
NC-1	Control	100.	100.	10	(39)	.186	Control
							Mean t = 1.47
Trial 2 [22]							
B(a)P	.000791	1.86	53.4	187	(20)	8.86***	+ 16.0
B(a)P	.000250	6.52	81.4	116	(20)	5.25***	+ 9.33

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Appendix G. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
				Focus Data Type Vessels		Foci/Vessel Focus Type	
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	*t*-statistic
ANT	18.2	70.2	95.2	44	(19)	2.06***	+ 3.71
ANT	9.11	82.6	106.	52	(20)	2.11**	+ 3.42
ANT	4.56	90.4	105.	38	(20)	1.60*	+ 2.33
ANT	2.28	91.0	108.	37	(20)	1.62*	+ 2.51
NC-1	Control	100.	100.	45	(40)	.893	Control Mean t = 2.99
Benzoin [BENZ, M.W. = 212.25]							
Trial 1 [4]							
B(a)P	.000791	15.7	74.7	184	(20)	8.81***	+ 9.38
B(a)P	.000250	24.0	82.9	116	(20)	4.38***	+ 3.62
BENZ	14.1	.000	54.4	29	(20)	.849	.00(-1.42)
BENZ	9.42	.000	61.8	34	(20)	1.20	.00(- .61)
BENZ	4.71	.000	62.7	18	(20)	.711	.00(-1.91)
BENZ	2.36	2.89	75.6	30	(20)	1.12	.00(- .80)
NC-1	Control	100.	100.	118	(40)	1.51	Control Mean t = .000
Trial 2 [10]							
B(a)P	.000791	1.89	30.6	105	(20)	4.79***	+ 18.3
B(a)P	.000250	8.49	91.7	34	(20)	1.37***	+ 6.23
BENZ	9.42	.000	69.4	3	(19)	.116	+ .98
BENZ	4.71	.000	76.4	9	(20)	.308*	+ 2.13
BENZ	2.36	.000	100.	5	(20)	.189	+ 1.62
BENZ	1.18	.000	115.	10	(19)	.266	+ 1.20
NC-1	Control	100.	100.	3	(40)	.053	Control Mean t = 1.48
Benzyl Alcohol [926895-L, M.W. = 108.13, Density = 1.04013 g/ml]							
Trial 1 [81]							
B(a)P	.000791	10.2	63.9	378	(18)	20.8***	+ 15.5
B(a)P	.000250	28.0	67.8	280	(18)	15.3***	+ 10.5
926895-L	20.0	.000	10.2	59	(18)	2.41	.00(-4.95)
926895-L	15.0	3.15	72.1	167	(18)	8.66	+ 1.35
926895-L	10.0	10.8	85.0	243	(18)	13.1***	+ 7.10
926895-L	5.00	24.8	101.	136	(18)	6.00	.00(-1.51)
NC-1	Control	100.	100.	583	(72)	7.36	Control Mean t = 2.11
Trial 2 [110]							
B(a)P	.000791	11.6	61.1	116	(18)	5.78***	+ 10.7
B(a)P	.000250	26.7	88.0	75	(18)	3.89***	+ 8.52
926895-L	20.0	.000	51.1	14	(18)	.572	.00(- .17)
926895-L	15.0	8.04	84.4	29	(18)	1.25*	+ 2.39
926895-L	10.0	28.6	95.1	40	(18)	1.97***	+ 4.60
926895-L	5.00	48.2	101.	15	(18)	.645	+ .16
NC-1	Control	100.	100.	65	(75)	.609	Control Mean t = 1.79
Caprolactam [CAP, M.W. = 113.16]							
Trial 1 [5]							
B(a)P	.000791	7.76	9.41	84	(19)	3.97***	+ 15.5
B(a)P	.000250	12.5	12.5	57	(20)	2.58***	+ 12.5

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Appendix G. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
				Focus Data Type Vessels		Foci/Vessel Focus Type	
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	*t*-statistic
CAP	106.	.862	.000	1	(18,20)	.039	+ .09
CAP	88.4	.862	18.0	2	(20)	.072	+ .72
CAP	70.7	4.74	58.0	3	(20)	.110	+ 1.12
CAP	53.0	23.7	95.7	7	(20)	.256*	+ 2.26
NC-1	Control	100.	100.	2	(40)	.035	Control
							Mean t = 1.37
Trial 2 [10]							
B(a)P	.000791	1.89	30.6	105	(20)	4.79***	+ 18.3
B(a)P	.000250	8.49	91.7	34	(20)	1.37***	+ 6.23
CAP	70.7	.472	38.9	5	(20)	.172	+ 1.33
CAP	53.0	4.72	190.	3	(20)	.110	+ .90
CAP	35.3	5.66	108.	1	(20)	.035	.00(- .36)
CAP	17.7	28.8	144.	17	(19)	.419	+ 2.08
NC-1	Control	100.	100.	3	(40)	.053	Control
							Mean t = 1.80
2-Chloroethanol [2CE, M.W. = 80.52, Density = 1.200 g/ml]							
Trial 1 [78]							
B(a)P	.000791	8.09	60.6	116	(18)	6.11***	+ 4.93
B(a)P	.000250	14.9	84.2	184	(18)	9.84***	+ 9.74
2CE	74.3	.000	67.7	133	(18)	6.98***	+ 5.81
2CE	37.2	1.70	79.7	161	(18)	8.36***	+ 7.18
2CE	18.6	39.1	99.8	109	(18)	4.99*	+ 2.13
2CE	9.29	68.5	100.	56	(18)	2.72	.00(- .92)
NC-1	Control	100.	100.	296	(72)	3.28	Control
							Mean t = 3.78
Trial 2 [102]							
B(a)P	.000791	89.7	77.9	65	(18)	3.30***	+ 11.7
B(a)P	.000250	81.0	93.8	62	(18)	2.85***	+ 7.75
2CE	74.3	.000	65.8	35	(18)	1.52***	+ 4.76
2CE	37.2	.460	92.8	24	(18)	1.02***	+ 3.28
2CE	18.6	37.2	86.4	15	(18)	.671*	+ 2.58
2CE	9.29	70.8	92.6	6	(18)	.240	.00(- .22)
NC-1	Control	100.	100.	28	(18)	.268	Control
							Mean t = 2.66
(2-Chloroethyl)trimethylammonium Chloride [2CETS, M.W. = 158.07]							
Trial 1 [30]							
B(a)P	.000791	1.32	60.3	158	(20)	7.23***	+ 12.3
B(a)P	.000250	2.63	101.	98	(19)	4.25***	+ 8.08
2CETA	75.9	.000	17.8	54	(20)	1.88**	+ 3.24
2CETA	50.6	.000	62.4	51	(20)	2.18***	+ 4.36
2CETA	25.3	10.1	103.	17	(20)	.668	.00(- .54)
2CETA	12.7	65.4	101.	18	(20)	.677	.00(- .49)
NC-1	Control	100.	100.	40	(40)	.787	Control
							Mean t = 1.90
Trial 2 [45]							
B(a)P	.000791	8.74	42.5	186	(20)	8.98***	+ 19.5
B(a)P	.000250	28.2	86.4	77	(20)	3.42***	+ 7.04
2CETA	50.6	12.9	77.1	25	(20)	.988	+ 1.03
2CETA	4.00	98.1	114.	37	(20)	1.46*	+ 2.57
2CETA	2.00	100.	108.	44	(19)	1.53*	+ 2.55
2CETA	1.00	101.	116.	25	(20)	.876	+ .57
NC-1	Control	100.	100.	54	(79)	.732	Control
							Mean t = 1.68

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Appendix G. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
Drug	Conc., mM	S.A	CC.A.	Focus Data Type Vessels III	(N)	Foci/Vessel Focus Type III	*t*-statistic
C. I. Acid Orange 10 [CIA010, M.W. = 452.38]							
Trial 1 [64]							
B(a)P	.000791	6.45	26.6	98	(20)	4.47***	+ 12.7
B(a)P	.000250	16.9	69.1	32	(20)	1.37***	+ 5.13
CIA010	32.0	.000	9.35	6	(20)	.231	.00(- .45)
CIA010	16.0	20.2	78.3	4	(20)	.149	.00(-1.13)
CIA010	8.00	37.1	103.	13	(20)	.473	+ 1.10
CIA010	4.00	56.4	81.2	19	(20)	.702*	+ 2.20
NC-1	Control	100.	100.	17	(40)	.291	Control
							Mean t = .825
Trial 2 [103]							
B(a)P	.000791	8.60	76.9	95	(20)	4.59***	+ 13.7
B(a)P	.000250	23.6	91.5	75	(20)	2.86***	+ 5.37
CIA010	35.4	.000	.798	18	(20)	.751	.00(- .54)
CIA010	17.7	4.73	63.3	19	(20)	.726	.00(- .64)
CIA010	8.84	43.0	99.2	15	(20)	.588	.00(-1.32)
CIA010	4.42	82.2	104.	37	(20)	1.37	+ 1.74
NC-1	Control	100.	100.	89	(79)	.874	Control
							Mean t = .435
Dimethyl Terephthalate [DMTP, M.W. = 194.19]							
Trial 1 [103]							
B(a)P	.00791	8.60	76.9	95	(20)	4.59***	+ 13.7
B(a)P	.00250	23.6	91.5	75	(20)	2.86***	+ 5.37
DMTP	5.15	18.5	81.1	43	(20)	1.50*	+ 2.15
DMTP	2.58	24.5	83.2	45	(20)	1.83**	+ 3.12
DMTP	1.29	45.6	100.	26	(20)	1.02	+ .57
DMTP	.644	67.1	98.1	27	(19)	1.14	+ 1.00
NC-1	Control	100.	100.	89	(79)	.874	Control
							Mean t = 1.71
Trial 2 [107]							
B(a)P	.00791	5.81	47.3	131	(20)	6.09***	+ 5.74
B(a)P	.00250	21.2	75.8	122	(20)	5.53***	+ 4.05
DMTP	5.15	94.7	64.8	90	(20)	3.26	+ .55
DMTP	3.87	94.7	75.2	74	(20)	3.20	+ .48
DMTP	2.58	90.8	76.1	77	(20)	3.65	+ 1.82
DMTP	1.29	107.	87.2	101	(19)	5.02***	+ 4.59
NC-1	Control	100.	100.	274	(80)	2.95	Control
							Mean t = 1.86
Diphenylhydantoin [DPH, M.W. = 252.27]							
Trial 1 [56]							
B(a)P	.000791	2.55	32.7	122	(20)	5.54***	+ 16.5
B(a)P	.000250	2.19	61.0	33	(20)	1.47***	+ 6.97
DPH	39.6	52.6	31.4	0	(20)	.000	.00(-4.36)
DPH	12.5	55.8	45.7	1	(20)	.035	.00(-3.10)
DPH	3.96	57.3	50.4	2	(20)	.072	.00(-2.27)
DPH	1.25	66.4	55.1	5	(20)	.172	.00(- .79)
NC-1	Control	100.	100.	13	(39)	.260	Control
							Mean t = .000
Trial 2 [65]							
B(a)P	.000791	6.95	60.7	85	(18)	4.38***	+ 13.9
B(a)P	.000250	19.2	90.2	43	(20)	1.80***	+ 6.94

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Appendix G. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
Drug	Conc., mM	S.A	CC.A.	Focus Data Type Vessels III	(N)	Foci/Vessel Focus Type III	*t*-statistic
DPH	39.6	19.2	27.3	1	(20)	.035	.00(-2.66)
DPH	12.5	31.1	42.4	1	(20)	.035	.00(-2.66)
DPH	3.96	33.8	52.0	2	(19)	.076	.00(-1.86)
DPH	1.25	46.0	54.1	4	(19)	.140	.00(- .92)
NC-1	Control	100.	100.	14	(40)	.244	Control Mean t = .000
FD & C Yellow No. 6 [FDCY6, M.W. = 452.37]							
Trial 1 [34]							
B(a)P	.000791	6.91	58.7	167	(20)	8.23***	+ 14.5
B(a)P	.000250	19.3	84.5	138	(20)	6.39***	+ 7.66
FDCY6	88.4	6.18	69.5	135	(18)	7.02***	+ 8.24
FDCY6	44.2	44.0	86.1	144	(20)	6.71***	+ 7.96
FDCY6	22.1	53.8	92.4	196	(20)	8.71***	+ 9.47
FDCY6	11.1	78.5	90.4	119	(20)	5.67***	+ 7.06
NC-1	Control	100.	100.	108	(40)	2.51	Control Mean t = 8.18
Trial 2 [65]							
B(a)P	.000791	6.95	60.7	85	(18)	4.38***	+ 13.9
B(a)P	.000250	19.2	90.2	43	(20)	1.80***	+ 6.94
FDCY6	63.2	.000	47.7	40	(20)	1.54***	+ 4.81
FDCY6	20.0	70.5	47.4	61	(200	2.25***	+ 6.05
FDCY6	6.32	73.5	56.9	33	(20)	1.34***	+ 5.27
FDCY6	2.00	99.0	56.7	10	(20)	.762**	+ 3.27
NC-1	Control	100.	100.	14	(40)	.244	Control Mean t = 4.85
Trial 3 [103]							
B(a)P	.000791	8.60	76.9	95	(20)	4.59***	+ 13.7
B(a)P	.000250	23.6	91.5	75	(20)	2.86***	+ 5.37
FDCY6	88.4	.652	38.0	40	(17)	2.05***	+ 3.63
FDCY6	44.2	46.5	59.8	162	(20)	7.69***	+ 16.8
FDCY6	22.1	55.9	91.0	92	(18)	4.61***	+ 8.38
FDCY6	11.1	75.3	91.5	85	(20)	4.02***	+ 10.8
NC-1	Control	100.	100.	89	(79)	.874	Control Mean t = 9.90
D-Mannitol [MANN, M.W. = 60.07]							
Trial 1 [18]							
B(a)P	.000791	2.24	45.8	125	(20)	5.91***	+ 17.4
B(a)P	.000250	8.52	78.5	86	(20)	3.36***	+ 6.80
MANN	109.8	93.3	86.5	37	(20)	1.27*	+ 3.06
MANN	54.9	108.	103.	14	(20)	.600	.00(- .29)
MANN	27.4	102.	101.	13	(20)	.547	.00(- .58)
MANN	13.7	105.	106.	21	(20)	.792	+ .38
NC-1	Control	100.	100.	33	(40)	.663	Control Mean t = 1.72
Trial 2 [45]							
B(a)P	.000791	8.74	42.5	186	(20)	8.98***	+ 19.5
B(a)P	.000250	28.2	86.4	77	(20)	3.42***	+ 7.04
MANN	110.	90.3	98.3	46	(20)	1.93***	+ 3.91
MANN	4.00	94.8	104.	16	(20)	.556	.00(- .79)
MANN	2.00	96.8	98.6	22	(20)	.987**	+ 1.01
MANN	1.00	100.	99.0	16	(19)	.677	.00(- .24)
NC-1	Control	100.	100.	86	(98)	.732	Control Mean t = 1.23

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Appendix G. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
Drug	Conc., mM	S.A	CC.A.	Focus Data Type Vessels III	(N)	Foci/Vessel Focus Type III	*t*-statistic
Trial 3 [110]							
B(a)P	.000791	11.6	61.1	116	(18)	5.78***	+ 17.5
B(a)P	.000250	26.7	88.0	75	(18)	3.89***	+ 8.98
MANN	220.0	114.	101.	68	(20)	3.00***	+ 8.55
MANN	110.0	100.	100.	50	(20)	2.17***	+ 5.60
MANN	54.9	111.	92.	28	(20)	1.23**	+ 3.06
MANN	27.4	168.	100.	28	(20)	1.01	+ 1.53
NC-1	Control	100.	100.	65	(75)	.609	Control
							Mean t = 4.69
Methyl Methacrylate [794248-L, M.W. = 110.12, Density = 0.9433 g/ml]							
Trial 1 [79]							
B(a)P	.000791	22.7	79.6	279	(18)	20.8***	+ 13.9
B(a)P	.000250	39.3	94.2	241	(18)	12.9***	+ 9.34
794248-L	17.0	.000	.324	19	(13,17)	.615	.00(-6.94)
794248-L	12.8	.000	60.7	174	(18)	8.60**	+ 3.29
794248-L	8.50	4.13	90.8	244	(18)	13.1***	+ 9.73
794248-L	4.25	45.0	100.	83	(18)	4.42	.00(-1.38)
NC-1	Control	100.	100.	430	(62)	5.12	Control
							Mean t = 4.34
Trial 2 [106]							
B(a)P	.000791	24.8	56.9	134	(18)	6.88***	+ 8.00
B(a)P	.000250	40.7	77.9	91	(18)	4.53***	+ 5.56
794248-L	16.0	.000	1.31	3	(7)	.292	.00(-2.44)
794248-L	12.0	.000	24.7	52	(18)	2.28*	+ 2.09
794248-L	8.00	3.98	68.5	47	(18)	2.20	+ 1.99
794248-L	4.00	51.1	95.4	33	(18)	1.57	+ .69
NC-1	Control	100.	100.	74	(43)	1.30	Control
							Mean t = 1.19
Molybdenum Trioxide [MOTO, M.W. = 144.0]							
Trial 1 [47]							
B(a)P	.00791	.351	28.1	173	(20)	8.10***	+ 14.6
B(a)P	.00250	7.72	75.6	88	(20)	3.89***	+ 8.73
MOTO	9.06	.000	.000	7	(9,20)	.661	+ .29
MOTO	6.80	2.81	83.2	29	(19,20)	1.29**	+ 2.80
MOTO	4.53	36.1	96.5	7	(20)	.238	.00(-1.97)
MOTO	2.27	67.7	96.0	3	(20)	.110	.00(-3.72)
NC-1	Control	100.	100.	31	(39)	.579	Control
							Mean t = .830
Trial 2 [56]							
B(a)P	.000791	2.55	32.7	122	(20)	5.54***	+ 16.5
B(a)P	.000250	2.19	61.0	33	(20)	1.47***	+ 6.97
MOTO	11.0	1.46	.000	9	(18,20)	.339	+ .57
MOTO	8.28	70.8	75.3	12	(18,20)	.446	+ 1.04
MOTO	5.52	67.5	80.6	3	(20)	.099	.00(-1.63)
MOTO	2.76	93.8	94.6	5	(20)	.182	.00(- .79)
NC-1	Control	100.	100.	13	(39)	.260	Control
							Mean t = .000

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Appendix G. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
Drug	Conc., mM	S.A	CC.A.	Focus Data Type Vessels III	(N)	Foci/Vessel Focus Type III	*t*-statistic
4-Nitroanthranilic Acid [4NANA, M.W. = 182.15]							
Trial 1 [34]							
B(a)P	.000791	6.91	58.7	167	(20)	8.23***	+ 14.5
B(a)P	.000250	19.3	84.5	138	(20)	6.39***	+ 7.66
4NANA	9.88	8.36	59.3	41	(19)	1.99	.00(-1.62)
4NANA	6.59	16.7	81.0	55	(20)	2.53	+ .06
4NANA	4.92	38.2	89.8	41	(20)	1.80	.00(-2.12)
4NANA	3.29	86.9	83.2	45	(20)	2.00	.00(-1.57)
NC-1	Control	100.	100.	108	(40)	2.51	Control
							Mean t = .015
Trial 2 [103]							
B(a)P	.000791	8.60	76.9	95	(20)	4.59***	+ 13.7
B(a)P	.000250	23.6	91.5	75	(20)	2.86***	+ 5.37
4NANA	9.88	2.58	.000	24	(20)	.955	+ .33
4NANA	6.59	4.30	82.7	75	(20)	2.59***	+ 3.79
4NANA	4.94	5.59	85.4	55	(19)	2.42***	+ 4.52
4NANA	3.29	49.5	98.9	12	(20)	.423	.00(-2.18)
NC-1	Control	100.	100.	89	(79)	.874	Control
							Mean t = 2.16
Penicillin VK+ [519829-S, M.W. = 388.51]							
Trial 1 [80]							
B(a)P	.000791	16.9	65.2	261	(20)	12.9***	+ 13.0
B(a)P	.000250	35.6	87.2	185	(20)	8.53***	+ 7.65
519829-S	25.6	.000	1.13	29	(17,20)	1.35	.00(-3.24)
519829-S	19.2	15.1	18.6	156	(19)	7.62***	+ 6.69
519829-S	12.8	79.2	89.3	282	(20)	12.7***	+ 8.16
519829-S	6.41	97.3	98.2	144	(20)	6.22***	+ 3.80
NC-1	Control	100.	100.	317	(80)	3.02	Control
							Mean t = 2.94
Trial 2 [101]							
B(a)P	.000791	ND	64.8	108	(20)	4.63***	+ 12.7
B(a)P	.000250	ND	83.6	48	(20)	2.11***	+ 9.73
519829-S	25.6	ND	8.80	10	(19)	.334	+ .60
519829-S	19.2	ND	54.7	39	(20)	1.53***	+ 5.03
519829-S	12.8	ND	95.8	81	(18)	4.05***	+ 14.5
519829-S	6.41	ND	108.	15	(19)	.652**	+ 2.92
NC-1	Control	100.	100.	27	(78)	.260	Control
							Mean t = 5.76
Phthalamide [PHAM, M.W. = 164.18]							
Trial 1 [35]							
B(a)P	.000791	4.51	66.1	103	(20)	4.91***	+ 6.41
B(a)P	.000250	12.3	87.5	133	(20)	6.01***	+ 6.34
PHAM	48.7	4.92	97.7	28	(20)	1.17	.00(-2.24)
PHAM	24.5	20.5	111.	29	(20)	1.23	.00(- .14)
PHAM	12.2	44.3	116.	42	(20)	1.92	.00(-2.06)
PHAM	6.09	92.3	112.	52	(20)	2.28	.00(- .70)
NC-1	Control	100.	100.	94	(40)	1.97	Control
							Mean t = .000
Trial 2 [110]							
B(a)P	.000791	11.6	61.1	116	(18)	5.78***	+ 17.5
B(a)P	.000250	26.7	88.0	75	(18)	3.89***	+ 8.98

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Appendix G. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
Drug	Conc., mM	S.A	CC.A.	Focus Data Type Vessels III	(N)	Foci/Vessel Focus Type III	*t*-statistic
PHAM	60.9	8.68	61.5	9	(20)	.29	.00 (-2.06)
PHAM	30.5	51.1	89.3	20	(20)	.75	+ .64
PHAM	15.2	87.1	97.5	30	(20)	1.12	+ 1.87
PHAM	7.60	102.	102.	42	(20)	1.87***	+ 5.55
NC-1	Control	100.	100.	65	(75)	.609	Control
							Mean t = 2.02
Phthalic Anhydride [PHAN, M.W. = 148.12]							
Trial 1 [39]							
B(a)P	.000791	1.07	23.6	172	(20)	8.04***	+ 14.5
B(a)P	.000250	3.56	64.5	145	(20)	6.84***	+ 15.8
PHAN	27.0	.000	6.20	26	(15,20)	1.22	+ 2.61
PHAN	13.5	61.9	9.92	24	(20)	.955*	+ 2.27
PHAN	6.75	97.2	79.3	17	(18)	.765	+ 1.53
PHAN	3.38	95.7	96.3	11	(20)	.402	.00(- .13)
NC-1	Control	100.	100.	27	(40)	.427	Control
							Mean t = 1.27
Trial 2 [107]							
B(a)P	.000791	5.81	47.3	131	(20)	6.09***	+ 5.74
B(a)P	.000250	21.2	75.8	122	(20)	5.53***	+ 4.05
PHAN	20.3	102.	68.5	53	(20)	1.87	.00(-1.80)
PHAN	15.2	112.	75.2	34	(20)	1.06	.00(-3.69)
PHAN	10.1	114.	75.8	27	(14,19)	1.30	.00(-3.56)
PHAN	5.06	115.	82.1	46	(20)	1.91	.00(-2.34)
NC-1	Control	100.	100.	274	(80)	2.95	Control
							Mean t = .000
Roxarsone [998307-S, M.W. = 260.??]							
Trial 1 [80]							
B(a)P	.000791	16.9	65.2	261	(20)	12.9***	+ 13.0
B(a)P	.000250	35.6	87.2	185	(20)	8.53***	+ 7.65
998307-S	40.0	4.45	68.5	117	(18)	5.96***	+ 3.49
998307-S	30.0	13.1	88.7	122	(20)	5.44**	+ 3.11
998307-S	20.0	44.2	89.6	79	(20)	3.75	+ 1.67
998307-S	10.0	89.3	112.	142	(20)	5.91***	+ 3.47
NC-1	Control	100.	100.	317	(80)	3.02	Control
							Mean t = 2.94
Trial 2 [109]							
B(a)P	.000791	51.9	94.3	99	(20)	4.69***	+ 5.31
B(a)P	.000250	66.2	108.	81	(20)	3.91***	+ 4.16
998307-S	76.9	.000	.000	0	(12,20)	.000	.00(-23.0)
998307-S	57.7	.000	2.67	3	(20)	.110	.00(-14.7)
998307-S	38.5	6.05	28.4	19	(20)	.721	.00(- 5.74)
998307-S	19.2	42.7	92.6	28	(20)	1.06	.00(-4.28)
NC-1	Control	100.	100.	237	(80)	2.55	Control
							Mean t = .000
3-Sulfolene [3SULF, M.W. = 118.15]							
Trial 1 [33]							
B(a)P	.000791	3.44	2.40	214	(20)	10.0***	+ 13.6
B(a)P	.000250	5.73	51.4	130	(20)	5.86***	+ 7.74

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Appendix G. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c] Focus Data Type Vessels		Transformation Response[d] Foci/Vessel Focus Type	Significance[e]
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	*t*-statistic
3SULF	114.3	.000	64.4	59	(20)	2.54**	+ 5.80
3SULF	76.2	.000	92.9	86	(20)	3.50**	+ 4.49
3SULF	38.1	13.4	107.	53	(20)	2.32**	+ 3.63
3SULF	19.0	48.1	101.	26	(20)	1.07	.00(- .39)
NC-1	Control	100.	100.	54	(37)	1.04	Control
							Mean t = 3.48
Trial 2 [44]							
B(a)P	.000791	5.07	25.9	335	(20)	15.8***	+ 16.3
B(a)P	.000250	14.2	67.2	137	(20)	6.15***	+ 7.33
3SULF	76.2	7.09	86.5	166	(20)	7.35***	+ 8.22
3SULF	4.00	95.6	90.5	57	(20)	2.02	+ 1.11
3SULF	2.00	94.9	94.2	46	(20)	1.80	+ .67
3SULF	1.00	95.3	92.9	60	(20)	2.43*	+ 2.01
NC-1	Control	100.	100.	77	(40)	1.52	Control
							Mean t = 3.00
Sulfisoxazole [SULF, M.W. = 267.32]							
Trial 1 [19]							
B(a)P	.000791	.000	62.5	99	(20)	4.61***	+ 13.4
B(a)P	.000250	1.67	89.0	100	(20)	4.02***	+ 10.6
SULF	12.0	14.6	71.5	18	(20)	.439	+ .37
SULF	5.99	37.5	89.7	16	(18)	.682	+ 1.73
SULF	2.99	62.1	93.0	12	(19)	.471	+ .66
SULF	1.50	75.4	103.	5	(19)	.182	.00(-1.29)
NC-1	Control	100.	100.	18	(38)	.357	Control
							Mean t = .690
Trial 2 [26]							
B(a)P	.000791	.382	13.5	204	(20)	9.88***	+ 17.8
B(a)P	.000250	1.15	56.2	144	(20)	6.58***	+ 10.5
SULF	13.1	30.9	86.1	58	(20)	2.60***	+ 4.74
SULF	6.55	66.8	89.7	24	(20)	.988	+ .31
SULF	3.27	76.4	97.1	18	(20)	.713	.00(- .99)
SULF	1.64	73.3	86.5	17	(20)	.573	.00(-1.34)
NC-1	Control	100.	100.	46	(40)	.907	Control
							Mean t = 1.26
Sodium(2-ethylhexyl) Alcohol Sulfate [S2EHAS, M.W. = 232.28, Density = 1.114 g/ml]							
Trial 1 [82]							
B(a)P	.000791	46.8	47.2	371	(18)	19.4***	+ 7.45
B(a)P	.000250	56.3	51.6	288	(18)	15.5***	+ 7.97
S2EHAS	17.2	2.82	.0	1	(1,18)	.039	.00(-3.44)
S2EHAS	12.9	35.3	1.3	187	(18)	8.13	+ .07
S2EHAS	8.61	71.7	95.8	170	(18)	8.58	+ .54
S2EHAS	4.31	81.7	90.2	142	(18)	6.69	.00(-1.32)
NC-1	Control	100.	100.	649	(72)	8.01	Control
							Mean t = .180
Trial 2 [108]							
B(a)P	.000791	15.5	31.3	139	(18)	7.38***	+ 13.9
B(a)P	.000250	30.0	65.7	72	(13)	4.77***	+ 5.96
S2EHAS	17.2	17.6	90.3	48	(18)	2.44***	+ 4.24
S2EHAS	12.9	33.1	105.	45	(18)	2.27**	+ 2.96
S2EHAS	8.61	59.4	93.4	61	(18)	3.09***	+ 4.64
S2EHAS	4.31	94.9	97.3	41	(18)	1.84	+ 1.85
NC-1	Control	100.	100.	108	(70)	1.17	Control
							Mean t = 3.42

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Appendix G. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
Drug	Conc., mM	S.A	CC.A.	Focus Data Type Vessels III	(N)	Foci/Vessel Focus Type III	*t*-statistic
Titanium Dioxide [TIDI, M.W. = 79.90]							
Trial 1 [38]							
B(a)P	.000791	2.10	28.4	174	(20)	7.75***	+ 15.6
B(a)P	.000250	9.44	74.7	162	(20)	8.17***	+ 18.0
TIDI	12.5	98.3	54.2	5	(20)	.189	.00(-2.01)
TIDI	6.26	98.6	66.8	5	(19)	.182	.00(-1.97)
TIDI	3.13	92.3	79.9	5	(20)	.189	.00(-2.00)
TIDI	1.56	101.	88.4	11	(20)	.394	.00(- .56)
NC-1	Control	100.	100.	27	(40)	.496	Control
							Mean t = .000
Trial 2 [109]							
B(a)P	.000791	51.9	94.3	99	(20)	4.69***	+ 5.31
B(a)P	.000250	66.2	108.	81	(20)	3.91***	+ 4.16
TIDI	12.5	99.0	45.7	2	(20)	.072	.00(-16.4)
TIDI	6.25	87.6	55.4	8	(19)	.272	.00(- 8.31)
TIDI	3.13	97.8	72.4	28	(20)	.818	.00(- 3.95)
TIDI	1.56	104.	90.9	46	(20)	2.01	.00(- 1.35)
NC-1	Control	100.	100.	237	(80)	2.55	Control
							Mean t = .000
Tetrahydrofuran [THF, M.W. = 72.11, Density = 0.9 g/ml]							
Trial 1 [82]							
B(a)P	.000791	46.8	47.2	371	(18)	19.4***	+ 7.45
B(a)P	.000250	56.3	51.6	288	(18)	15.5***	+ 7.97
THF	111.	.000	63.4	206	(18)	9.88	+ 1.57
THF	55.5	6.50	83.1	155	(18)	7.99	.00(- .01)
THF	27.7	25.1	80.9	133	(18)	6.09	.00(-1.95)
THF	13.9	65.7	76.5	110	(18)	5.75	.00(-2.61)
NC-1	Control	100.	100.	649	(72)	8.01	Control
							Mean t = .393
Trial 2 [106]							
B(a)P	.000791	24.8	56.9	134	(18)	6.88***	+ 8.00
B(a)P	.000250	40.7	77.9	91	(18)	4.53***	+ 5.56
THF	351.	.000	.000	0	(0,18)	.000	NA
THF	263.	.306	3.06	0	(9,13)	.000	.00(-9.15)
THF	176.	3.67	68.1	15	(14)	.805	.00(-1.35)
THF	87.8	7.34	82.1	35	(10)	3.02**	+ 2.72
NC-1	Control	100.	100.	74	(43)	1.30	Control
							Mean t = 1.36
Witch Hazel [WH, M.W. = 46.07, Density = 0.790 g/ml]							
Trial 1 [81]							
B(a)P	.000791	10.2	63.9	378	(18)	20.8***	+ 15.5
B(a)P	.000250	28.0	67.8	280	(18)	15.3***	+ 10.5
WH	150000. 1500.EST.	1.05	85.2	167	(18)	8.85	+ 1.58
WH	100000. 1000.EST.	22.4	86.1	213	(18)	11.2 ***	+ 3.55
WH	50000. 500.EST.	53.8	91.7	148	(17)	8.07	+ .75
WH	25000. 250.EST.	75.5	96.9	149	(18)	7.87	+ .57
NC-1	Control	100.	100.	583	(72)	7.36	Control
							Mean t = 1.61
Trial 2 [110]							
B(a)P	.000791	11.6	61.1	116	(18)	5.78***	+ 17.5
B(a)P	.000250	26.7	88.0	75	(18)	3.89***	+ 8.98

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Appendix G. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
				Focus Data Type Vessels		Foci/Vessel Focus Type	
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	*t*-statistic
WH	150000.	80.7	97.7	26	(18)	1.11	+ 1.99
WH	100000.	99.0	101.	32	(18)	1.34*	+ 2.36
WH	75000.	97.7	95.3	23	(18)	1.07	+ 1.98
WH	50000.	102.	97.8	34	(18)	1.37*	+ 2.36
NC-1	Control	100.	100.	65	(71)	.609	Control Mean t = 2.17

Abbreviations: B(a)P, benzo(a)pyrene; CC.A., co-culture clonal survival assay; Conc., concentration; mM, millimole; M.W., molecular weight; N, number of culture vessels, NC, negative control; ND, not determined; %RCE, percent relative cloning efficiency; S.A., standard clonal survival assay.

[a]Treatment Condition: The experimental design for the transformation assay is described in detail in the Materials and Methods. The concentration of the positive control and test chemical treatment are presented in mM, but they can be converted to μg/ml using the molecular weight that is provided with each chemical. The solvent vehicles used for the individual test chemicals were listed in Appendix Tables A1 and A3, and the concentrations of the solvent vehicles are presented in the Materials and Methods.

[b]Cytotoxic activity: The experimental design for the standard survival assay (SA) and the co-culture clonal survival assay (CCA) were described in the Materials and Methods. The test chemical cytotoxic response was expressed as & RCE and was calculated as described in the Materials and Methods.

[c]The criteria used to evaluate the transformed foci of BALB/c-3T3 cells is described in the Materials and Methods. The number of type III foci > 2-mm in diameter per culture vessel scored are recorded in this table.

[d]Transformation response: The transformation responses are expressed as type III foci/vessel and were calculated using a $\log_{10}$ mathematical transformation procedure (refer to Materials and Methods). The arithmetic value or foci/vessel represents the antilog of the $\log_{10}$ mean transformation response minus one.

[e]Significance: The significance of test chemical transformation responses was calculated by a computer using the SAS statistical software (*22*), and the method is described in detail in Materials and Methods. The correct *t*-statistic according to the F-test is presented in this table. The *t*-statistics of each treatment dose of the test chemical in a single experiment were averaged to determine the mean *t*-statistic of the test chemical for the experiment (refer to Appendix Tables A2 and A5). The mean *t*-statistics for two or experiments for each chemical was weighted to the number of treatment doses evaluated and averaged to determine the rank *t*-statistic which was used to rank-order the test chemical transformation responses in Appendix Tables A3 and A6. Arbitrarily, transformation responses with negative (−) *t*-statistics were given a value of zero (0).

*Significant BaP or test chemical transformation response, $0.01 < p \leq 0.05$.

**Significant BaP or test chemical transformation response, $0.001 < p \leq 0.01$.

***Significant or BaP or test chemical transformation response, $p \leq 0.001$.

Appendix H.

Summary of the transformation responses of 7 very noncytotoxic chemicals.

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
				Focus Data Type Vessels		Foci/Vessel Focus Type	
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	t-statistic
Acetone [ACET, M.W. = 58.08, Density = 0.786 g/ml]							
Trial 1 [82]							
B(a)P	.000791	46.8	47.2	371	(18)	19.4***	+ 7.45
B(a)P	.000250	56.3	51.6	288	(18)	15.5***	+ 7.97
ACET	1377.	.000	.000	0	(0,18)	.000	ND
ACET	1033.	.000	.000	0	(0,18)	.000	ND
ACET	689.	.000	.000	10	(6,18)	1.15	.00(-4.78)
ACET	344.	1.08	75.7	397	(18)	21.0***	+ 8.19
NC-1	Control	100.	100.	649	(72)	8.01	Control
							Mean t = 8.19
Trial 2 [102]							
B(a)P	.000791	9.64	69.9	99	(18)	4.65***	+ 9.48
B(a)P	.000250	19.3	93.1	45	(18)	2.22***	+ 5.22
ACET	517.	.000	.000	6	(13,18)	.347	.00(-1.66)
ACET	344.	.000	37.3	28	(18)	1.30*	+ 2.40
ACET	172.	1.26	76.8	55	(18)	2.67***	+ 6.15
ACET	86.1	34.8	79.5	22	(18)	.974	+ 1.20
NC-1	Control	100.	100.	64	(72)	.697	Control
							Mean t = 3.25
Dimethyl Sulfoxide [DMSO, M.W. = 78.13, Density = 1.100 g/ml]							
Trial 1 [41]							
B(a)P	.000791	1.29	33.6	189	(18)	10.2***	+ 23.1
B(a)P	.000250	6.45	78.2	123	(18)	6.37***	+ 17.1
DMSO	851.	.000	.000	0	(13,18)	.000	.00(-4.11)
DMSO	568.	22.9	27.4	69	(18)	3.15***	+ 7.36
DMSO	284.	76.8	77.1	15	(18)	.587	+ 1.59
DMSO	142.	88.4	89.9	17	(18)	.754**	+ 2.81
NC-1	Control	100.	100.	13	(36)	.274	Control
							Mean t = 3.92
Trial 2 [100]							
B(a)P	.000791	89.7	77.9	65	(18)	3.30***	+ 11.7
B(a)P	.000250	81.0	93.8	62	(18)	2.85***	+ 7.75
DMSO	563.	27.1	.000	31	(18)	1.42***	+ 5.84
DMSO	426.	57.0	11.0	18	(18)	.720*	+ 2.19
DMSO	282.	91.5	83.9	16	(18)	.634*	+ 2.25
DMSO	141.	94.3	88.7	16	(18)	.586	+ 1.61
NC-1	Control	100.	100.	28	(72)	.268	Control
							Mean t = 2.97
Ethanol [ETOH, M.W. = 46.07, Density = 0.790 g/ml]							
Trial 1 [81]							
B(a)P	.000791	10.2	63.9	378	(18)	20.8***	+ 15.5
B(a)P	.000250	28.0	67.8	280	(18)	15.3***	+ 10.5
ETOH	866.	.000	.000	0	(0,18)	.000	NA
ETOH	650.	.000	2.93	6	(11,18)	.422	.00(-13.2)
ETOH	433.	.000	49.1	159	(18)	8.07	+ .74
ETOH	217.	22.7	90.0	263	(18)	13.7***	+ 5.31
NC-1	Control	100.	100.	583	(72)	7.36	Control
							Mean t = 2.01
Trial 2 [108]							
B(a)P	.000791	15.5	31.3	139	(18)	7.38***	+ 13.9
B(a)P	.000250	30.0	65.7	72	(13)	4.77***	+ 5.96

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Appendix H. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
Drug	Conc., mM	S.A	CC.A.	Focus Data Type Vessels III	(N)	Foci/Vessel Focus Type III	*t*-statistic
ETOH	606.	.000	1.68	1	(10)	.072	.00(-7.45)
ETOH	455.	2.46	47.0	25	(14)	1.59	+ 1.15
ETOH	303.	36.5	99.5	48	(10)	3.92***	+ 4.34
ETOH	152.	114.	91.1	47	(16)	2.61***	+ 3.44
NC-1	Control	100.	100.	108	(70)	1.17	Control
							Mean t = 2.23
Glycerol [GLY, M.W. = 92.09, Density = 1.25245 g/ml]							
Trial 1 [82]							
B(a)P	.000791	46.8	47.2	371	(18)	19.4***	+ 7.45
B(a)P	.000250	56.3	51.6	288	(18)	15.5***	+ 7.97
GLY	434.	.000	1.31	91	(18)	4.04	.00(-3.65)
GLY	326.	1.30	44.2	308	(18)	16.2***	+ 5.79
GLY	217.	16.3	91.8	220	(18)	11.5**	+ 2.95
GLY	109.	70.2	77.8	191	(18)	9.64	+ 1.47
NC-1	Control	100.	100.	649	(72)	8.01	Control
							Mean t = 2.55
Trial 2 [108]							
B(a)P	.000791	15.5	31.3	139	(18)	7.38***	+ 13.9
B(a)P	.000250	30.0	65.7	72	(13)	4.77***	+ 5.96
GLY	434.	30.0	56.0	49	(18)	2.21**	+ 2.67
GLY	326.	55.6	82.0	72	(17)	3.32***	+ 4.48
GLY	217.	72.6	96.5	84	(18)	4.01***	+ 5.74
GLY	109.	102.	92.3	37	(18)	1.75	+ 1.65
NC-1	Control	100.	100.	108	(70)	1.17	Control
							Mean t = 3.64
Sodium Chloride [NaCl, M.W. = 58.44]							
Trial 1 [80]							
B(a)P	.000791	16.9	65.2	261	(20)	12.9***	+ 13.0
B(a)P	.000250	35.6	87.2	185	(20)	8.53***	+ 7.65
NaCl	154.	16.9	56.7	496	(20)	24.1***	+ 20.6
NaCl	116.	68.8	84.9	463	(20)	22.2***	+ 17.3
NaCl	77.0	78.3	84.1	216	(20)	10.0***	+ 8.86
NaCl	38.5	86.9	100.	86	(20)	4.00*	+ 2.04
NC-1	Control	100.	100.	317	(80)	3.02	Control
							Mean t = 12.2
Trial 2 [109]							
B(a)P	.000791	51.9	94.3	99	(20)	4.69***	+ 5.31
B(a)P	.000250	66.2	108.	81	(20)	3.91***	+ 4.16
NaCl	171.	22.9	19.4	9	(15,17)	.962	.00(-6.63)
NaCl	128.	49.4	86.7	65	(19)	.134	+ .44
NaCl	85.6	69.1	86.7	84	(20)	.893*	+ 2.13
NaCl	42.8	83.8	94.5	65	(20)	.641	+ .85
NC-1	Control	100.	100.	237	(80)	2.55	Control
							Mean t = .855
Trial 3 [R1]							
MCA	.00186	NA	NA	45	(20)	1.61**	+ 3.49
MNNG	.00850	NA	NA	95	(20)	2.77***	+ 4.53
NACL	128.	NA	NA	35	(20)	3.17***	+ 8.25
NACL	85.6	NA	NA	32	(20)	2.07**	+ 3.45
NACL	42.8	NA	NA	7	(20)	.771	+ 1.36
NC-1	Control	100.	100.	23	(40)	.416	Control
							Mean t = 4.35

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Appendix H. *Continued.*

Treatment Condition[a]		Cytotoxic Activity[b] RCE (%)		Transforming Activity[c]		Transformation Response[d]	Significance[e]
				Focus Data Type Vessels		Foci/Vessel Focus Type	
Drug	Conc., mM	S.A	CC.A.	III	(N)	III	*t*-statistic
Sucrose [SUC, M.W. = 342.30]							
Trial 1 [101]							
B(a)P	.000791	ND	64.8	108	(20)	4.63***	+ 12.7
B(a)P	.000250	ND	83.6	48	(20)	2.11***	+ 9.73
SUC	438.	.000	.000	0	(0,20)	.000	NA
SUC	219.	28.7	61.1	205	(19)	10.3***	+ 25.2
SUC	110.	91.3	112.	38	(18)	1.67***	+ 5.19
SUC	54.8	93.4	112.	9	(20)	.347	+ .75
NC-1	Control	100.	100.	27	(78)	.260	Control
							Mean t = 10.3
Trial 2 [107]							
B(a)P	.000791	5.81	47.3	131	(20)	6.09***	+ 5.74
B(a)P	.000250	21.2	75.8	122	(20)	5.53***	+ 4.05
SUC	292.	7.52	38.4	53	(20)	2.45	.00(-1.11)
SUC	219.	31.5	49.8	115	(20)	5.34***	+ 3.85
SUC	146.	65.3	77.7	114	(20)	5.37***	+ 5.09
SUC	73.0	96.4	93.0	60	(20)	2.60	.00(- .72)
NC-1	Control	100.	100.	274	(80)	2.95	Control
							Mean t = 2.24
Urea [UREA, M.W. = 60.07]							
Trial 1 [109]							
B(a)P	.000791	51.9	94.3	99	(20)	4.69***	+ 5.31
B(a)P	.000250	66.2	108.	81	(20)	3.91***	+ 4.16
UREA	416.	.000	.243	0	(20)	.000	.00(-23.0)
UREA	312.	43.0	17.5	43	(20)	1.85	.00(- 1.80)
UREA	208.	80.6	69.2	102	(20)	4.40**	+ 3.39
UREA	104.	90.8	74.1	80	(20)	3.57*	+ 2.05
NC-1	Control	100.	100.	237	(80)	2.55	Control
							Mean t = 1.81

Abbreviations: B(a)P, benzo(a)pyrene; CC.A., co-culture clonal survival assay; Conc., concentration; mM, millimole; M.W., molecular weight; N, number of culture vessels, NC, negative control; ND, not determined; %RCE, percent relative cloning efficiency; S.A., standard clonal survival assay.

[a]Treatment Condition: The experimental design for the transformation assay is described in detail in the Materials and Methods. The concentration of the positive control and test chemical treatment are presented in mM, but they can be converted to μg/ml using the molecular weight that is provided with each chemical. The solvent vehicles used for the individual test chemicals were listed in Appendix Tables A1 and A3, and the concentrations of the solvent vehicles are presented in the Materials and Methods.

[b]Cytotoxic activity: The experimental design for the standard survival assay (SA) and the co-culture clonal survival assay (CCA) were described in the Materials and Methods. The test chemical cytotoxic response was expressed as & RCE and was calculated as described in the Materials and Methods.

[c]The criteria used to evaluate the transformed foci of BALB/c-3T3 cells is described in the Materials and Methods. The number of type III foci > 2-mm in diameter per culture vessel scored are recorded in this table.

[d]Transformation response: The transformation responses are expressed as type III foci/vessel and were calculated using a $\log_{10}$ mathematical transformation procedure (refer to Materials and Methods). The arithmetic value or foci/vessel represents the antilog of the $\log_{10}$ mean transformation response minus one.

[e]Significance: The significance of test chemical transformation responses was calculated by a computer using the SAS statistical software (*22*), and the method is described in detail in Materials and Methods. The correct *t*-statistic according to the F-test is presented in this table. The *t*-statistics of each treatment dose of the test chemical in a single experiment were averaged to determine the mean *t*-statistic of the test chemical for the experiment (refer to Appendix Tables A2 and A5). The mean *t*-statistics for two or experiments for each chemical was weighted to the number of treatment doses evaluated and averaged to determine the rank *t*-statistic which was used to rank-order the test chemical transformation responses in Appendix Tables A3 and A6. Arbitrarily, transformation responses with negative (−) *t*-statistics were given a value of zero (0).

*Significant BaP or test chemical transformation response, $0.01 < p \leq 0.05$.

**Significant BaP or test chemical transformation response, $0.001 < p \leq 0.01$.

***Significant or BaP or test chemical transformation response, $p \leq 0.001$.