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Detection of carcinogens as mutagens in the *Salmonella*/microsome test: Assay of 300 chemicals: Discussion*

(prevention of cancer and genetic defects/somatic mutation/environmental insult to DNA)

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ABSTRACT **About 300 carcinogens and non-carcinogens of a wide variety of chemical types have been tested for mutagenicity in the simple *Salmonella*/microsome test. The test uses bacteria as sensitive indicators of DNA damage, and mammalian liver extracts for metabolic conversion of carcinogens to their active mutagenic forms. There is a high correlation between carcinogenicity and mutagenicity: 90% (157/175) of the carcinogens were mutagenic in the test, including almost all of the known human carcinogens that were tested. Despite the severe limitations inherent in defining non-carcinogenicity, few "non-carcinogens" showed any degree of mutagenicity [McCann *et al.* (1975) *Proc. Nat. Acad. Sci. USA* 72, 5135–5139]. In the present paper, carcinogens negative in the test and apparent false positives are discussed. We also discuss evidence that chemical carcinogens and radiation, likely to initiate most human cancer and genetic defects, do so by damage to DNA. The *Salmonella* test can play a central role in a program of prevention: to identify mutagenic chemicals in the environment (all indications are there are many) and to aid in the development of non-mutagenic products to prevent future human exposure.**

This paper is a discussion of Part I (1) in which we reported results obtained testing about 300 carcinogens and non-carcinogens for mutagenicity in the simple and rapid *Salmonella*/microsome microbial test (1). We undertook this study to determine the correlation between carcinogenicity and mutagenicity and the utility of the *Salmonella* test, at this stage in its development, for detecting chemicals likely to be mutagens and carcinogens for humans.

Classification of chemicals as to carcinogenicity

Carcinogens. We selected as standard carcinogens and non-carcinogens almost 300 chemicals which had been tested for carcinogenicity in animals or were known human carcinogens. Since virtually every chemical known to cause cancer in humans also causes cancer in animals (2–5), the simplest assumption is that any chemical which is a carcinogen in an animal test is likely to be a human carcinogen, though there are many uncertainties in determining the risk to humans from animal data (2, 6, 7). In general, chemicals carcinogenic in one species are carcinogenic in other species (3, 5), although the carcinogenic potency of a particular chemical can vary considerably depending upon the animal species in which it is tested, and the manner in which the chemical is administered (3, 4, 8–10). In evaluating carcinogenicity data we have considered a positive result to take precedence over a negative result. In a few cases, chemicals have been designated carcinogens in limited studies (?+), when the test procedure used indicates the result should be confirmed by other methods. We have also designated a few chemicals weak carcinogens (*w*+).

Non-Carcinogens. Classification as to non-carcinogenicity is usually difficult because of the varying completeness and modes of treatment in many studies and the statistical limitations inherent in animal tests (4, 8–10). Recent criteria for adequate carcinogenicity tests are much more stringent (4, 8–10). The test should be of adequate duration (lifetime preferred in rodents) in at least two animal species, at several dose levels, and positive controls should be of the same general chemical type as the chemical under test. The application of such criteria in evaluating non-carcinogenicity would mean that only relatively few chemicals classified as "non-carcinogens" in this study could be considered non-carcinogens with a high degree of certainty. Some method is clearly needed which would permit a more quantitative evaluation of negative cancer data. Some kind of completeness index might be useful which would permit expression of negative data as a "less-than" figure which would take into account limitations of the particular experimental system, such as duration of the experiment, numbers of animals used, and dose. We have used a "less than" figure (e.g., <0.01 revertant/nmol) to express negative results in the *Salmonella* mutagenicity test (1).

Mutagenicity of carcinogens

Carcinogens (classified +, *w*+, or ?+ in Part I) of a broad range of chemical structures were tested and 90% (157/175)† were mutagenic in the test.

Human Carcinogens. Of the few known, or suspected, human carcinogens (3, 4, 11–13), almost all which have been tested are positive. These include: β-naphthylamine (**A21**), 4-aminobiphenyl (**A26**), benzidine (**A29**), vinyl chloride (**B4**), chloroprene (**B7**)‡, *bis*-chloromethylether (**B8**), cyclophosphamide (**B12**), chlornaphazin (**B14**), melphalan (**B15**), polycyclic components of coal tar (**C**) (3), 4-nitrobiphenyl (**E2**), aflatoxins (**H**), cigarette smoke condensates (**I1**), and soot (D. Streitwieser and B. N. Ames, unpublished). *Auramine* (**A43**) and *para-rosaniline* (**A35**) (a component of magenta) are suspected human carcinogens (3) and are negative in the *Salmonella* test. However, in both cases, human cancer occurred as a consequence of exposure to highly impure dye mixtures, which, in the case of auramine, were also used in carcinogenicity tests in animals (3). The hormonal carcinogen diethylstilbestrol (**F19**) could not be adequately tested (see *Bacterial toxicity*).

Mutagenic Potency. We have calculated the number of revertants per nanomole from linear dose-response curves (1). Comparisons between the mutagenic potency of different chemicals must be undertaken with caution because: (*1*)

Abbreviations: bold-faced symbols (e.g., **A21**) refer to chemicals listed in the table in Part I.

* Part II; Part I is ref. 1.

† Slightly modified from Part I because of **C6**, which we classified as a non-carcinogen in limited studies, but which should be classified as a weak carcinogen in limited studies (14).

‡ There is a controversy about **B7** (see Part I).

The mutagenic potency of each chemical is based on the reversion of that tester strain most sensitive to it; each strain has been independently optimized for detection of a particular class of mutagens and thus potency in the test will change as additional strain improvements are made (15–17). (2) The standard assay represents a compromise between various factors, e.g., method of induction and amount of microsomal enzymes (S-9) (18, 19). (3) There can be higher effective mutagen concentration in the bacteria due to active transport, e.g., azaserine (20), or to bacterial (rather than liver) metabolic activation, e.g., nitrofurans (21, 22). Nevertheless, expressing results on one potency scale is useful, and factors affecting relative potency even by 10-fold do not greatly obscure the order of magnitude because the scale varies over a range of about 10^6: e.g., aflatoxin B_1 (**H1**) (7057 revertants/nmol) and benzyl chloride (**B20**) (0.02 revertants/nmol).

It is of interest to see to what extent mutagenic potency in the test parallels carcinogenic potency, despite the hazards of such a comparison. Chemicals of very similar structure can differ greatly in carcinogenic potency. We have examined (1) a number of these, mostly isomers, and changes which decrease carcinogenicity (references in ref. 1) also decrease mutagenicity: **A3** versus **A7, A8, A9, A10, A11; A19** versus **A20; A21** versus **A22; A24** versus **A23; A26** versus **A28; A29** versus **A30, A31; C3** versus **C8; C34** versus **C35; H1, H3,** versus **H2, H4, H5, H6, H7.** An exception is **C11** versus **C10.** Also of interest is a comparison of the mutagenic and carcinogenic potencies of chemicals of very different structure. In general, animal carcinogenicity tests have not been designed for this type of comparison and few data are available. M. Meselson and K. Russell (personal communication) have found sufficient carcinogenic potency data for about 10 chemicals (including several human carcinogens) and have plotted these data against our mutagenic potency values, and the results show an encouraging agreement. It seems imperative for animal carcinogenesis studies to deal more with potency as an aid for human risk assessment, as an encouragement to design more rigorous animal tests, and as a standard for attempts to adjust the sensitivity of mutagenesis and transformation assays to parallel as closely as possible carcinogenic risk to humans.

Carcinogens negative in the test

Ten percent of the carcinogens (18/175) were non-mutagenic in the test. These 18 chemicals (italicized for clarity) are discussed below.

(1) *Some chlorinated hydrocarbons,* such as *carbon tetrachloride* (**B2**), *1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene* [*DDE*] (**B23**), and *dieldrin* (**B24**), are not detected and presumably require metabolic activation (dehalogenation?) for mutagenic activity. These three have been shown to mutate or to interact with the DNA of mammalian cells (23–25). We are attempting to modify the *in vitro* metabolic activation system for their detection. Many important industrial chemicals, e.g., ethylene dichloride (26) and a large number of pesticides are chlorinated hydrocarbons.

(2) *Chemicals activated by bacterial flora. Cycasin* (**G21**), a β-glucoside of methylazoxymethanol (**G22**), is inactive in the test because neither *Salmonella* nor mammalian microsomes contain a β-glucosidase necessary for converting it to the active alkylating agent methylazoxymethanol. *Cycasin* is non-carcinogenic in germfree animals (3, 4). A similar case may be *1,2-dimethylhydrazine* (**K5**) which gives markedly fewer colon tumors in germfree animals (27). However, nitro carcinogens are detected in the *Salmonella* test (see nitrofurantoin discussion).

(3) *"Carcinogens" which may not be carcinogens. Auramine* and *para-rosaniline* are discussed under *Human Carcinogens.* The carcinogenicity studies in animals of *para-rosaniline* (**A35**) (3) and *4-aminoantipyrene* (**J3**) (28) are not definitive. *Acetamide* (**D28**) is carcinogenic in rats, but enormous doses were given (>1% in the diet for a year) (3), and this raises questions about possible impurities.

(4) *3-Amino-1,2,4-triazole* (**J5**) and the weak carcinogens *thioacetamide* (**D27**) and *thiourea* (**D19**) all possess goitrogenic activity and cause thyroid tumors and a non-mutagenic mechanism has been suggested (4).

(5) *Dimethylamino carcinogens.* Though the potent carcinogen dimethylnitrosamine (**G1**) as well as dimethylaminoazobenzene (**L6**) was detected, their activity was very weak (see also **L5, L10, K9, K10,** and **G2**) and it was necessary to preincubate the chemicals with the *S-9 Mix* (see ref. 1). Their enzymatic activation appears to involve a demethylation (11) and the *in vitro* system may need to be improved for this activity. The lack of mutagenic activity of the carcinogens *Natulan* (**K7**) [a mutagen in mice (29)] and *1,2-dimethylhydrazine* (**K5**) (discussed above) may also be related to this problem.

(6) *Miscellaneous. Ethionine* (**F7**), which might act through *S*-adenosylethionine by ethylating nucleic acids at natural methylation sites (30), has been shown to be a mutagen in *Coprinus* (30). *Safrole* (**F15**) and *1'-hydroxysafrole* (**F16**) are negative, but 1'-acetoxysafrole (**F17**), a carcinogenic metabolite, is quite mutagenic, and a mutagenic metabolite of *safrole* can be detected (H. Rosenkranz, personal communication) in the *Salmonella*/urine test (31, 32). *Phenobarbital* (**J1**), a carcinogen in the mouse (33), is also a promoter and a potent inducer of microsomal enzymes in liver responsible for activating carcinogens. *Urethane* (**D21**) has been reported to be mutagenic in other organisms (34), to cause chromosome damage in rats, and to be converted to metabolites which can react with DNA (3).

In conclusion, 90% of the 175 carcinogens examined were detected as mutagens. Of the 18 carcinogens that were not mutagenic in the test, many are either mutagenic in other systems or produce mutagenic metabolites. It is striking that so few carcinogens of the 175 examined remain with no evidence for ability to damage DNA. We suspect that even a higher percentage of carcinogens will be detected in the test after further improvements in the *in vitro* activation system or in the tester strains. The *Salmonella* test has been adapted for the testing of urine (31, 32, 35) and colonic contents (36) as a supplemental way of detecting mutagenic metabolites and some of the carcinogens negative in the standard test might also be detected in this way (see *safrole*).

Non-mutagenicity of non-carcinogens

Despite the difficulties in determining "non-carcinogenicity" (see *Non-Carcinogens*) 87% (94/108)[§] of the "non-carcinogens" (0, ?0, c0) are non-mutagenic in the test. The "non-carcinogens" fall into two general categories: 62 chemicals, most of which are fairly closely related and even isomeric to carcinogens, and 46 common biochemicals.

Common Biochemicals. All 46 common biochemicals showed no mutagenic activity (<0.01 revertant/nmol) using the criteria for non-mutagenicity described (1). Few common biochemicals have been examined for carcinogenicity

[§] **A10** and **C6** classification changed from Part I (see *text*).

and most of the 46 we selected have some negative cancer data, though in most cases these are quite limited. The common biochemicals tested were: adenosine, cytidine, L-ascorbic acid, diaminopimelic acid, nicotinamide, *d*-pantothenic acid, riboflavin, thiamine, thioctic acid, L-arabinose, dextran, L-fucose, D-galactose, D-glucose, D-glucosamine, potassium gluconate, glycogen, inositol, lactose, maltose, D-ribose, sucrose, L-asparagine, L-glutamic acid, glycine, L-methionine, L-phenylalanine, L-tyrosine, L-tryptophan, L-lysine, glutathione, ammonium chloride, magnesium chloride, potassium chloride, sodium bicarbonate, sodium chloride, disodium phosphate, ammonium acetate, ethyl acetate, citric acid, glycerol, propylene glycol, sodium potassium tartrate, indole, spermidine, and putrescine.

"Non-carcinogens" positive in the test

Thirteen percent (14/108)[§] showed some degree of mutagenic activity and these "false positives" (italicized for clarity) are discussed below.

(*1*) *Weak mutagens: mostly close relatives of carcinogens.* Two close relatives (**A7, A10**) of 2-acetylaminofluorene (**A3**) when tested showed weak but definite mutagenic activity (several hundred- to several thousandfold less). Thus, though the discrimination of the test for these "non-carcinogenic" derivatives was excellent (see *Mutagenic Potency*), by our criteria we initially classified these as false positives. Clearly, even a trace of 2-acetylaminofluorene impurity could account for the results, and for this reason we analyzed these by high-pressure liquid chromatography. In fact, both **A7** and **A10** contain this contaminant and the purified material shows no significant activity, <0.03 revertant/nmol (V. Donahue, J. McCann, and B. N. Ames, manuscript in preparation). The fact that these chemicals were determined to be "non-carcinogens," but actually contained some potent carcinogen, dramatically illustrates both the statistical limitations of animal carcinogenesis testing and the power of the *Salmonella* test.

The statistical limitations inherent in animal carcinogenicity tests limit their usefulness for the detection of weak carcinogens. More extensive animal carcinogenicity tests may therefore be required to determine if the "false positives" showing weak mutagenic activity are really non-carcinogens. These are *4-acetylaminofluorene* (**A11**), *α-naphthylamine* (**A22**), *styrene oxide* (**D13**), *glycidol* (**D14**), *1,2-epoxybutane* (**D16**), *5-nitro-2-furamidoxime* (**E18**), *5-nitro-2-furoic acid* (**E20**), and *N-hydroxy-4-aminoazobenzene* (**L9**). *Sodium nitrite* (**K13**), among the weakest mutagens (0.01 revertant/nmol) in the test, has been subjected to extensive carcinogenicity tests with negative results, though it does show some activity in *in vitro* transformation tests (37). It is both highly reactive and rapidly detoxified by mammalian enzymes.

(*2*) *"Non-carcinogens" which are potent mutagens.* The captan-type fungicides, including the closely related captan (**B21**), *folpet* (**B22**), and Difolatan, are widely used and it has been estimated that milligram amounts could be consumed per person per day as residues in food (38). They are potent mutagens in the *Salmonella* test and are mutagenic and teratogenic and cause chromosome abnormalities in higher organisms as discussed in a review by Bridges (38). Captan and *folpet* were negative in a carcinogenicity study in mice (39), but preliminary evidence from another study in mice on captan indicates it may be a potent carcinogen (H. Rosenkranz, personal communication). The strains of mice were different in the two studies and it is also possible that captan and *folpet*, which would react readily with protein, reacted with the gelatin used as a vehicle in the negative study. Further tests are in progress on captan at the National Cancer Institute.

ICR-191 (**B18**), the prototype, and the first reactive frameshift mutagen characterized (40), has since been shown to be a potent mutagen in mammalian cells (41) as well, and is positive in a DNA repair test in human fibroblasts (42) used to detect chemical mutagens and carcinogens. Though two closely related chemicals of similar mutagenic potency (**B16** and **B17**) were positive in the lung tumor test in strain A mice, *ICR-191* was negative (R. Peck, personal communication).

Nitrofurantoin (**E19**), a widely used drug, is strongly mutagenic in the test. It was non-carcinogenic in one fairly extensive feeding study in rats (22) but, as was the case for the Japanese food additive furylfuramide (AF-2) (**E16**) (43), more extensive tests might show carcinogenicity. The test is unusually sensitive for the detection of the nitro carcinogens (**E**), apparently because they are activated directly by nitro reductases in the *Salmonella*. Nitro reductases from both mammalian liver and gut bacteria are thought to play a role in activating nitro carcinogens. It is possible that *Salmonella* may contain nitro reductases not present in liver or in the *Escherichia coli* normally present in human gut. We think this is unlikely, as *Salmonella* and *E. coli* are very close relatives (nitrofurantoin is mutagenic in *E. coli*, ref. 22). If activation by the tester strains is found to be a problem they could be modified; several groups have isolated mutants of *E. coli* or *Salmonella* lacking particular nitro reductases (reviewed in refs. 22 and 21).

Dibenz[a,h]anthracene-5,6-oxide (**C12**) was not carcinogenic when tested under conditions where the parent compound, dibenz[*a,h*]anthracene (**C11**), was positive; however, it is as mutagenic in the *Salmonella* test as the parent hydrocarbon (which requires liver activation). Epoxides and diol-epoxides are formed as metabolites of polycyclic hydrocarbons and are thought to be the active carcinogenic forms. A number of these are mutagenic in *Salmonella* (44–46) and mammalian cells (46, 47) and are active in *in vitro* transformation (reviewed in ref. 48). Those that have been tested show relatively slight activity as carcinogens in some of the standard animal tests (48) (e.g., **C5**), possibly because they are reactive, unstable compounds.

Sodium azide (**K11**) is a very potent mutagen in the *Salmonella* test, and in barley (49). It was found negative in a thorough lifetime carcinogenicity test in rats where it was administered either in the diet or by gastric intubation, at two dose levels (50). It would be of both practical (it is used industrially) and theoretical (it may not act as an electrophile) interest to test it by other routes and in other species.

Bacterial toxicity

Ten chemicals could not be thoroughly tested for mutagenicity because of bacterial toxicity (1). The limits of non-mutagenicity for each chemical, e.g., **C28** = <16 revertants/nmol and **F14** = <0.13 revertant/nmol, are determined by the maximum level that could be tested without inhibition. We have not included these 10 chemicals in the statistics since they could not be classified as mutagens or non-mutagens, but, practically, they add three carcinogens (**D8, F19, H14**) that could not be detected by the test (and five non-carcinogens: **F14, F18, G9, G10, H13**), though this does not change the statistics appreciably. Mitomycin C (**H14**) has been shown to be a mutagen in *Salmonella* under different conditions (17).

Chemicals of unknown carcinogenicity which were mutagenic.

Hundreds of chemicals of unknown carcinogenicity have been tested in the *Salmonella* test, and in general most are negative. A few that were mutagenic, and are in Part I, are **A15, A16, A18, A28, A44, B9, C15, G17, L1, L13, L17;** and we have reported on others (e.g., refs. 26 and 51). Of unusual interest is ethidium bromide (**A44**), a very potent mutagen after microsomal activation (1, 52). It causes a variety of biological effects, presumably related to its ability to intercalate in DNA, and is widely used in physico-chemical studies with nucleic acids. Proflavin (**A18**) and 9-aminoacridine (**A16**) are used clinically and Dexon (**L13**) is a pesticide. All should be handled with caution.

Uses of the *Salmonella*/microsome test

We believe that the test can play a central role in a long-term program of cancer prevention aimed at identifying, and minimizing human exposure to, environmental carcinogens and mutagens. It is a complement to traditional animal carcinogenicity tests, as it can be used in a variety of ways not feasible with the animal tests. (*1*) Chemical and drug companies can now afford to test routinely all new compounds at an early stage of development so that mutagens can be identified and this information taken into consideration before there is a large vested interest in the compound. The *Salmonella* test is now being used by over 50 major chemical and drug companies. (*2*) If a drug is found to be mutagenic, a variety of derivatives can be synthesized to find a non-mutagenic form (53, 54). (*3*) The mutagenicity of a chemical may be due to a trace impurity and such knowledge could save a useful chemical (R. Gustafson, American Cyanamid, personal communication). (*4*) Complex mixtures or natural products with carcinogenic activity can be investigated, using the test as a bioassay for identifying the mutagenic ingredients; e.g., cigarette smoke condensate is mutagenic (55) and numerous tobacco companies are trying to identify the chemicals responsible. (*5*) Colonic contents (36) and human feces (W. R. Bruce, personal communication) and urine (31, 32, 35) can be monitored to see if ingested products or drugs are giving rise to mutagens. (*6*) The variety of substances that humans are exposed to, both pure chemicals and mixtures, is being assayed for mutagenicity by hundreds of laboratories: e.g., water supplies; soot from city air; hair dyes (51) and cosmetics; drugs; food additives; food; mold toxins; pesticides; industrial chemicals; fumigants. Many substances have been found to be mutagenic and several which have been tested have since been shown to be carcinogenic (43, 56), e.g., **A17, B3, E12, E16,** and probably **B21.** (*7*) The active metabolic forms of chemical carcinogens, and their metabolism, can be determined using the test as a bioassay (e.g., 26, 44, 46). (*8*) The test system is useful in clarifying basic mechanisms of mutagenesis by chemical carcinogens, e.g., the demonstration that many aromatic carcinogens are reactive frameshift mutagens with particular base sequence specificity (15, 16, 40, 45, 57), and the clarification of the role of different repair systems in mutagenesis by various carcinogens (15, 17). (*9*) The sensitivity of the *Salmonella* test may make it particularly useful for detecting chemicals which have weak carcinogenic activity and would be difficult to identify in animal tests because of statistical limitations. Weak carcinogens could be of great importance to the human population where millions of individuals could be exposed.

Do chemical carcinogens cause cancer through damage to DNA?

On the basis of our work and other evidence, listed below, we find compelling the theory (see ref. 34 for historical review) that radiations and chemical carcinogens cause cancer through damage to DNA (somatic mutation). (*1*) It is known that cell regulation can be altered by mutation, and that a heritable change in cell regulation is a characteristic property of a cancer cell. (*2*) The theory is simple and consistent with facts in cancer biology (58, 59). (*3*) It is supported by studies on the genetics of cancer (60). (*4*) There are human mutants lacking DNA repair systems who are extremely prone to cancer (61). (*5*) There is a correlation between capacity for repair of DNA damage and the occurrence of organ-specific cancer (62–64). (*6*) Active forms of many carcinogens are electrophiles capable of interacting with DNA (34). (*7*) Almost all carcinogens tested have been shown to be mutagens (1). (*8*). Many potent aromatic carcinogens are unusually potent frameshift mutagens and it is our hypothesis that the structural basis for this is that they are DNA affinity reagents containing both an aromatic ring system capable of a strong stacking interaction with DNA and an electrophilic moiety (19, 40, 45, 57). (*9*) In addition to the many chemical carcinogens whose active forms have an electrophilic interaction with DNA and are mutagens there is a diverse collection of carcinogens such as asbestos (65), metal carcinogens (66), and a variety of radiations that have no obvious connection other than their ability to damage DNA.

Public health and insult to DNA

It has been estimated that environmental factors (2, 58, 67) initiate almost all human cancer and it is becoming increasingly apparent that many environmental factors are mutagens, e.g., cigarette smoke, asbestos, ultraviolet light, x-rays, known human chemical carcinogens (see *Human Carcinogens*). It seems clear that many more chemicals will be added to the list of human carcinogens, as we are being exposed to an increasing flood of chemicals that have not been tested for carcinogenicity or mutagenicity, from flame retardants in our children's pajamas to pesticides accumulating in our body fat. In general, the approach to this problem has been to ignore it and even very large volume chemicals, involving extensive human exposure, have been produced for decades without adequate carcinogenicity or mutagenicity tests, e.g., vinyl chloride (2.5 billion kg/yr, U.S.A.) and ethylene dichloride (3.5 billion kg/yr, U.S.A.) (26), and a host of pesticides. A small fraction of these chemicals is now being tested in animals, but for the vast bulk of them the only experimental animals are humans, and epidemiological studies on humans are impractical in most cases.

Damage to DNA by environmental mutagens may be the main cause of death and disability in advanced societies (58). We believe that this damage, accumulating during our lifetime, initiates most human cancer and genetic defects and is quite likely a major contributor to aging (68, 69) and heart disease (70, 71) as well. The solution is prevention: identifying environmental mutagens and minimizing human exposures. Rapid, accurate, *in vitro* tests, such as the *Salmonella*/microsome test, should play a crucial role in realizing this goal.

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1. McCann, J., Choi, E., Yamasaki, E. & Ames, B. N. (1975) *Proc. Nat. Acad. Sci. USA* **72,** 5135–5139.
2. Epstein, S. S. (1974) *Cancer Res.* **34,** 2425–2435.
3. *IARC Monograph on the evaluation of carcinogenic risk of chemicals to man* (1972–1975) (IARC, Lyon), Volumes 1–9.
4. Weisburger, J. H. (1973) in *Cancer Medicine*, eds. Holland, J. F. & Frei, E., III (Lea and Febiger, Philadelphia, Pa.), pp. 45–90.
5. Tomatis, L., Partensky, C. & Montesano, R. (1973) *Int. J. Cancer* **12,** 1–20.
6. Hoel, D. G., Gaylor, D. W., Kirschstein, R. L., Saffiotti, U. & Schneiderman, M. A. (1975) *J. Toxicol. Environ Health* **1,** 133–151.
7. Mantel, N. & Schneiderman, M. A. (1975) *Cancer Res.* **35,** 1379–1386.
8. Sontag, J., Page, N. P. & Saffiotti, U. (1975) *Guidelines for carcinogen bioassay in small rodents* (Div. of Cancer Cause & Prevention, NCI).
9. Weisburger, E. K. (1975) *J. Clin. Pharmacol.* **15,** 5–15.
10. *Carcinogenesis Testing of Chemicals* (1974) ed. Golberg, L. (CRC Press, Cleveland, Ohio).
11. Heidelberger, D. (1975) *Annu. Rev. Biochem.* **44,** 79–121.
12. Sieber, S. M. & Adamson, R. H. (1975) in *Pharmacological Basis of Cancer Chemotherapy*, the 27th Ann. Symp. on Fundamental Cancer Research (Williams & Wilkins Co., Baltimore, Md.), pp. 401–468.
13. Khachatryan, E. A. (1972) *Probl. Oncol.* **18,** 85–86.
14. Cook, J. W. & Schoental, R. (1952) *Br. J. Cancer* **6,** 400–406.
15. Ames, B. N., Lee, F. D. & Durston, W. E. (1973) *Proc. Nat. Acad. Sci. USA* **70,** 782–786.
16. Isono, K. & Yourno, J. (1974) *Proc. Nat. Acad. Sci. USA* **71,** 1612–1617.
17. McCann, J., Spingarn, N. E., Kobori, J. & Ames, B. N. (1975) *Proc. Nat. Acad. Sci. USA* **72,** 979–983.
18. Ames, B. N., McCann, J. & Yamasaki, E. (1975) *Mutat. Res.* **31,** 347–364.
19. Ames, B. N., Durston, W. E., Yamasaki, E. & Lee, F. D. (1973) *Proc. Nat. Acad. Sci. USA* **70,** 2281–2285.
20. Ames, G. F. (1964) *Arch. Biochem. Biophys.* **104,** 1–18.
21. Rosenkranz, H. S. & Speck, W. T. (1975) *Biochem. Biophys. Res. Commun.* **66,** 520–525.
22. Tazima, Y., Kada, T. & Murakami, A. (1975) *Mutat. Res.* **32,** 55–80.
23. Rocchi, P., Prodi, G., Grilli, S. & Ferreri, A. M. (1973) *Int. J. Cancer* **11,** 419–425.
24. Kelly-Garvert, F. & Legator, M. S. (1973) *Mutat. Res.* **17,** 223–229.
25. Markaryan, D. S. (1966) *Genetika* **2,** 132–137.
26. McCann, J., Simmon, V., Streitwieser, D. & Ames, B. N. (1975) *Proc. Nat. Acad. Sci. USA* **72,** 3190–3193.
27. Reddy, B. S., Narisawa, T., Wright, P., Vukusich, D., Weisburger, J. H. & Wynder, E. L. (1975) *Cancer Res.* **35,** 287–290.
28. Boyland, E., Busby, E. R., Dukes, C. E., Grover, P. L. & Manson, D. (1964) *Br. J. Cancer* **18,** 575–581.
29. Ehling, U. H. (1973) *Mutat. Res.* **21,** 217–218.
30. Talmud, P. J. & Lewis, D. (1974) *Genet. Res. Camb.* **23,** 47–61.
31. Durston, W. E. & Ames, B. N. (1974) *Proc. Nat. Acad. Sci. USA* **71,** 737–741.
32. Commoner, B., Vithayathil, A. J. & Henry, J. I. (1974) *Nature* **249,** 850–852.
33. Thorpe, E. & Walker, A. I. T. (1973) *Fd. Cosmet. Toxicol.* **11,** 433–442.
34. Miller, E. C. & Miller, J. A. (1971) in *Chemical Mutagens: Principles and Methods for their Detection*, ed. Hollaender, A. (Plenum Press, New York), Vol. I, pp. 83–119.
35. McCann, J. & Ames, B. N. (1975) *Ann. N.Y. Acad. Sci.* **269,** 21–25.
36. Wheeler, L. A., Carter, J. H., Soderberg, F. B. & Goldman, P. (1975) *Proc. Nat. Acad. Sci. USA* **72,** 4607–4611.
37. Tsuda, H., Inui, N. & Takayama, S. (1973) *Biochem. Biophys. Res. Commun.* **55,** 1117–1124.
38. Bridges, B. A. (1975) *Mutat. Res.* **32,** 3–34.
39. Innes, J. R. M., Ulland, B. M., Valerio, M. G., Petrucelli, L, Fishbein, L., Hart, E. R., Pallotta, A. J., Bates, R. R., Falk, H. L., Gart, J. J., Klein, M., Mitchell, I. & Peters, J. (1969) *J. Nat. Cancer Inst.* **42,** 1101–1114.
40. Ames, B. N. & Whitfield, H. J., Jr. (1966) *Cold Spring Harbor Symp. Quant. Biol.* **31,** 221–225.
41. Preud'homme, J.-L., Birshtein, B. K. & Scharff, M. D. (1975) *Proc. Nat. Acad. Aci. USA* **72,** 1427–1430.
42. Stich, H. F. (1975) in *Molecular Mechanisms for Repair of DNA (Part B)*, eds. Hanawalt, P. C. & Setlow, R. B. (Plenum Press. New York), pp. 773–784.
43. Nomura, T. (1975) *Nature* **258,** 610–611.
44. Malaveille, C., Bartsch, H., Grover, P. L. & Sims, P. (1975) *Biochem. Biophys. Res. Commun.* **66,** 693–700.
45. Ames, B. N., Sims, P. & Grover, P. L. (1972) *Science* **176,** 47–49.
46. Wood, A. W., Goode, R. L., Chang, R. L., Levin, W., Conney, A. H., Yagi, H., Dansette, P. M. & Jerina, D. M. (1975) *Proc. Nat. Acad. Sci. USA* **72,** 3176–3180.
47. Huberman, E., Sachs, L., Yang, S. K. & Gelboin, H. V. (1976) *Proc. Nat. Acad. Sci. USA* **73,** 607–611.
48. Sims, P. & Grover, P. L. (1974) *Adv. Cancer Res.* **20,** 165–274.
49. Kleinhofs, A., Kleinschmidt, M., Sciaky, D. & Von Broembsen, S. (1975) *Mutat. Res.* **29,** 497–500.
50. Ulland, B., Weisburger, E. K. & Weisburger, J. H. (1973) *Toxicol. Appl. Pharmacol.* **25,** 446.
51. Ames, B. N., Kammen, H. O. & Yamasaki, E. (1975) *Proc. Nat. Acad. Sci. USA* **72,** 2423–2427.
52. Mattern, I. E. (1975) *Abstr. 5th Meeting European Environmental Mutagen Soc.* (Tecnico Scientifica, Pisa), p. 86.
53. Hartman, P. E. & Hulbert, P. B. (1975) *J. Toxicol. Environ. Health* **1,** 243–270.
54. Bueding, E. (1975) *J. Toxicol. Environ. Health* **1,** 329–334.
55. Kier, L. D., Yamasaki, E. & Ames, B. N. (1974) *Proc. Nat. Acad. Sci. USA* **71,** 4159–4163.
56. Ames, B. N. & McCann, J. (1975) in *Screening Tests in Chemical Carcinogenesis* eds. Montesano, R., Bartsch, H. & Tomatis, L. (IARC, Lyon), in press.
57. Ames, B. N., Gurney, E. G., Miller, J. A. & Bartsch, H. (1972) *Proc. Nat. Acad. Sci. USA* **69,** 3128–3132.
58. Cairns, J. (1975) *Sci. Am.* **233** (November), 64–78.
59. Cairns, J. (1975) *Nature* **255,** 197–200.
60. Knudson, A. G., Jr. (1973) *Adv. Cancer Res.* **17,** 317–352.
61. Cleaver, J. E. & Bootsma, D. (1975) *Annu. Rev. Genet.* **9,** 19–38.
62. Goth, R. & Rajewsky, M. F. (1974) *Proc. Nat. Acad. Sci. USA* **71,** 639–643.
63. Nicoll, J. W., Swann, P. F. & Pegg, A. E. (1975) *Nature* **254,** 261–262.
64. Kleihues, P. & Margison, G. P. (1974) *J. Nat. Cancer Inst.* **53,** 1839–1841.
65. Sincock, A. & Seabright, M. (1975) *Nature* **257,** 56–58.
66. Nishioka, H. (1975) *Mutat. Res.* **31,** 185–189.
67. Higginson, J. (1969) *Can. Cancer Conf.* **8,** 40–75.
68. Burnet, M. (1974) *Intrinsic Mutagenesis: A genetic approach to ageing* (Medical & Technical Publ. Co. Ltd., Lancaster, U.K.).
69. Linn, S., Kairis, M. & Holliday, R. (1976) *Nature*, in press.
70. Benditt, E. P. & Benditt, J. M. (1973) *Proc. Nat. Acad. Sci. USA* **70,** 1753–1756.
71. Pearson, T. A., Wang, A., Solez, K. & Heptinstall, R. H. (1975) *Am. J. Pathol.* **81,** 379–388.