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SANTA BARBARA • SANTA CRUZ

DEPARTMENT OF BIOCHEMISTRY

BERKELEY, CALIFORNIA 94720

10 June 1977

Dr. George E. Stapleton
Division of Biomedical Research
E.R.D.A.
Washington, D.C. 20545

Dear Dr. Stapleton:

I enclose my request for renewal of my ERDA contract EY-76-03-0034, P.A.156 formerly E(04-3)34 PA156. I am enclosing my 3 year progress report and a justification for an increased level of funding for the new period beginning October 1, 1977. My budget for the previous 12 month period was about $102,000 (direct costs): this included a $40,000 supplement that I had requested. I would like to increase this for the coming year to $164,237 (direct costs), $211,141 (total costs). The increased level of funding over that previously asked for is for work on the following new or expanded projects:

1. To continue improving the Salmonella/microsome test.
We are currently detecting 90% of the carcinogens tested. With further improvements in the test, we hope to be able to raise this figure to 95% or more, although we do not expect to reach 100% as several carcinogens do not act through a mutagenic mechanism. Personnel: Ms. Yamasaki and Ms. Haroun. See Part 4 of our Research Proposal.

2. To establish an index of carcinogenic potency in animal cancer tests and to calculate the potency of carcinogens.

3. To use the index of carcinogenic potency for establishing human risk from carcinogens in the environment.

4. To investigate the relation of carcinogenic potency to mutagenic potency in the Salmonella/microsome test.

5. To determine how much of organ specific cancer and sex specific cancer in chemical carcinogenesis in animals can be explained by metabolic activation. This is an offshoot of project 4.

Projects 2-5 are a new area for us. We believe carcinogenic potency is the most important question in the area of estimating human hazard from carcinogens and mutagens and in trying to set priorities among the many carcinogenic chemicals and mixtures with which we are surrounded. This is a sophisticated area and we discuss this project in detail in Part 1 of the Research Proposal. The personnel involved are Dr. Charles Sawyer, Dr. N.K. Hooper, and Mr. Alan Friedman. Dr. J. McCann and Dr. Arlene Blum will be involved in aspects of human risk assessment.

6. To determine the best methods of examining mutagenicity in complex mixtures, particularly in aqueous fluids.

DISCLAIMER

Hundreds of laboratories are using our test system for looking at complex mixtures, from shale oil and coal fractions to possible mutagens in human feces that may be the cause of colon cancer in man. We plan to adapt our method for looking at human urine to other aqueous fluids, such as water effluents (Dr. Hooper). We are continuing to serve as a clearinghouse for work in particular areas (Mrs. Maron).

7. To detect unsuspected mutagens/carcinogens in the environment that may be of major importance for human health.

This work has expanded considerably. Dr. Blum will continue our work on the flame retardants in children's polyester sleepwear. We are becoming interested in hair dyes once again, as positive cancer results are coming out on some of the ingredients we had identified as mutagens and I expect more controversy. I have hired an undergraduate student to work in this area (Miss C. Gold). We are also monitoring several other environmental chemicals. See Research Proposal Part 5.

8. To do theoretical work on DNA repair systems and other aspects of mutagenesis.

9. Advising government and industry in the general area of environmental carcinogens.

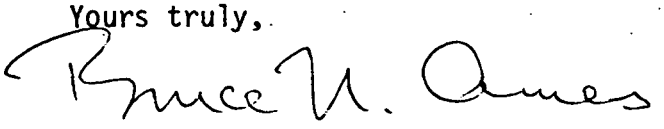
This has expanded greatly in the last year as there is a tremendous interest in environmental causes of cancer and genetic defects. I have built up a first rate group of people who are quite knowledgeable in this area. This is discussed in Part 3 of our Research Proposal. Personnel: Drs. McCann, Blum, Hooper, Sawyer, Ms. Haroun; secretarial help from Mrs. McAllan and Ms. Stark-Dean.

10. Service to universities, government agencies and industry on the use of our test system.

This service has expanded greatly in recent years due to the ever increasing interest in our test system. Most of the major chemical and drug industries in the world appear to be using the system (See Appendix A). We feel it is important to continue this. We discuss it in Part 2 of our Research Proposal. The personnel involved are Mrs. Dorothy Maron and Mr. Dennis Ing, with secretarial help from Mrs. Susan McAllan and Ms. Gaylee Stark-Dean.

11. The Biochemistry Department has had a building grant from N.I.H. that paid for the personnel that operated the central building services such as the media kitchen, the electron microscope, the carpentry and machine shops, and the animal room. This grant has been phased out and the department is now recharging me for my share of the personnel of the central services. As I use these services heavily, particularly the media kitchen where we are the heaviest user, I have requested some money to pay for my share of these services, which were previously subsidized by the department grant.

Yours truly,



Bruce N. Ames
Professor of Biochemistry

BNA/spbm

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Professor B.N. Ames
Biochemistry Dept.
University of California
Berkeley, CA 94720

COMPREHENSIVE PROGRESS REPORT

ERDA Contract E(04-3)-34 P.A. 156 AT03-76ET70156

(For the 3 years, 3 months: July 1, 1974 to Sept 30, 1977, written May, 1977)

(Work done since the last Yearly Progress Report & related materials are marked by an asterisk.)

1. Main Research Accomplishments with Special Reference to Originally Stated Objectives.

The original proposal to the AEC on The Detection and Analysis of Mutagens listed two objectives: 1) to develop a "set of strains that can be used by government agencies and other interested parties to screen pesticides, herbicides, food additives, drugs, etc. as mutagens," and 2) "to use these strains for investigating the mode of action of various mutagens and in particular for finding mutagens that make specific changes in the DNA". We believe we have been very successful in objective 1, partially successful in objective 2, and quite successful in a number of side projects that developed out of our general interest in mutations and mutagens. A major advance was in showing that a wide range of carcinogens are mutagens and can be detected with our system. We have also contributed to the somatic mutation theory of cancer and identified a number of important environmental carcinogens.

*The potential hazard for humans of mutagens and carcinogens in the environment is very high, and it is important to screen large numbers of compounds and mixtures of compounds to which humans are exposed. However, it is impractical for both technical and monetary reasons to do mutagen screening by using mammals. Carcinogenicity testing is also extremely expensive and takes years for adequate tests. Microorganisms have been used extensively in mutagenesis testing because they offer great technical advantages and because of the fact that DNA is the genetic material in both bacteria and man. Clearly many different types of test systems will have to be developed and each will have particular advantages and disadvantages. The Salmonella/liver system we have described is simple, inexpensive, and extremely sensitive, and, with the addition of the urine test recently developed, we believe it will detect a high percentage of the environmental chemicals that cause cancer and mutations in man.

*The test is particularly useful for the detection of mutagens/carcinogens in complex mixtures (such as air pollution, cigarette smoke, water, food, and urine). It is also useful in the development of drugs and industrial chemicals where large numbers of chemicals must be screened. Most of the major drug and chemical companies in the world are now using the test system and are starting to make economic decisions on the basis of it, e.g. DuPont has recently decided (at considerable economic loss) not to use two freons in spray cans (they are the available replacements for the freon that is damaging the ozone layer) because they were mutagens in our test.

We have described our test system for mutagen and carcinogen detection in the papers from the 3 years of this contract (1-24). I will give a summary of some of these results, omitting references to other work which is credited in our original papers.

Improvements of bacterial tester strains. Our earlier set of tester strains (TA1535, 1537, 1538) were detecting a wide variety of carcinogens (perhaps 75%), but a few important classes of carcinogens were not detected as mutagens. We have now (4) extended the utility of the method by introducing two new bacterial strains which can detect with great sensitivity many important carcinogens which we did not detect before or detected with less sensitivity. Among these carcinogens are aflatoxin B_1, sterigmatocystin, benzyl chloride, benzo(a)pyrene, 7,12-dimethylbenzanthracene, 1'-acetoxysafrole, and the nitrofuran food additive furylfuramide (AF-2). The new strains TA100 and TA98 contain an R factor plasmid, pKM101, in our standard tester strains TA1535 and TA1538. The R factor increases mutagenesis with certain mutagens, but not others. We presented evidence that the mutagens that become more effective work through an error-prone recombinational repair(4,24).

A method for detecting mutagens that cause deletions has been developed (3).

*A new tester strain, TA94, that detects mitomycin C (an antibiotic carcinogen) and malondialdehyde (a product of lipid peroxidation and a possible contributory cause of breast cancer) has been developed (19). This strain contains the uvrB repair system which is necessary for a few DNA crosslinking-type carcinogens to show mutagenicity.

Improvements of test system. In addition to the sensitive tester strains which can detect mutagens, the test incorporates an aspect of mammalian metabolism so that carcinogens can be converted to their active forms. A crude rat (or human) liver homogenate (a 9000 x g cell supernatant or "S-9") is used which contains active microsomes. We have investigated the nature of the cofactors for activation and the optimum procedure for inducing the animals from which the S-9 is prepared (7). We prefer Aroclor 1254 (PCB's) as a good general inducer of rat microsomal enzymes (7). We have also investigated several different tissues as a source of S-9 for the metabolic activation of various carcinogens and prefer rat liver for general screening (7). We have also compared human autopsy liver tissue with rat liver and find no qualitative differences in the compounds we have investigated (5,7,18). General technical points have been discussed in detail in a Methods paper (7).

*Analysis of mutagenicity of urine and various aqueous fluids. Another approach to the analysis of mammalian metabolites is to examine mutagenic metabolites in urine. Our earlier work (reviewed in 11) showed that human urine, or urine from animals used in toxicology tests, could be assayed in the mutagenicity test. Drawbacks were the sensitivity of adding urine directly to the petri plates (because of volume limitations) or of solvent extraction of the urine (because of technical difficulties). We have now developed (20) a simple method for concentrating mutagens/carcinogens about 200-fold from human or animal urine. The method utilizes a column of the polystyrene XAD resin which has been shown to adsorb a wide variety of non-polar mutagens/carcinogens from urine. The column can then be easily eluted with a few ml of acetone which is then taken to dryness. The method should have wide applicability for surveying human urine or assaying urine of animals in toxicology tests, and for examining a wide variety of aqueous fluids such as waste and drinking water, beverages, and industrial and fuel plant effluents. We have used the method for showing that cigarette smokers have mutagenic urine.

*Assay of the mutagenicity of complex mixtures. We have shown the mutagenicity of cigarette smoke condensate and most of the standard fractions into which it has been separated(1). This bioassay is now being used by the tobacco industry to identify the mutagens in the condensate. We have also shown that car exhaust, air pollution, soot and coal tar fumes (unpublished results) are mutagenic. (These materials are known to have polycyclic hydrocarbons and a wide variety of other chemicals.) One of the advantages of the Salmonella test is the ability to have a rapid bioassay for complex mixtures.

The utility of high pressure liquid chromatography in mutagenicity testing has been shown by demonstrating that a variety of chemicals (many of industrial importance) have mutagenic impurities (21). (We have been informed recently by the Canadian group that did the cancer tests on saccharin that they have found a mutagenic impurity in saccharin and we have confirmed their result in a sample they sent us.)

*Validation of the Salmonella/microsome test. We have published extensive data on the validation of the test with over 300 chemicals (8,14,19) under standard conditions. Ninety percent (157/175)of carcinogens tested for mutagenicity were shown to be mutagens, and these 157 carcinogens cover a wide variety of classes of chemicals known to be carcinogenic. Also, almost all of the known or suspected human carcinogens were shown to be mutagenic: i.e. 4-aminobiphenyl, chlornaphazine, β-naphthylamine, benzidine, cigarette smoke condensates, bis-chloromethyl ether, aflatoxin B_1, vinyl chloride, 4-nitrobiphenyl, and cyclophosphamide. We tested 108 "non-carcinogens" (including 46 common biochemicals, none of which were mutagens) and, despite the severe statistical limitations in defining "non-carcinogenicity" in the tests using small numbers of animals, few (13%) of them showed any degree of mutagenicity. The test is highly selective in discriminating between carcinogens and closely related "non-carcinogenic" analogs (8,14,19). Our test system has been validated independently with similar results in a blind study by ICI (the largest chemical company in Europe) and by the National Cancer Research Institute in Tokyo.

Environmental mutagens. Several environmental mutagens have been found that may represent serious public hazards. We have shown (5) that 89% (150/169) of commercial oxidative-type (hydrogen peroxide) hair dye formulations are mutagenic. Of the 18 components of these hair dyes, nine showed various degrees of mutagenicity: 2,4-diaminoanisole, 4-nitro-o-phenylenediamine, 2-nitro-p-phenylenediamine, 2,5-diaminoanisole, 2-amino-5-nitrophenol, m-phenylenediamine, o-phenylenediamine, 2-amino-4-nitrophenol, and 2,5-diaminotoluene. Three hair dye components (p-phenylenediamine, 2,5-diaminotoluene, and 2,5-diaminoanisole) become strongly mutagenic after oxidation by H_2O_2: the mutagenic product of p-phenylenediamine was identified as the known trimer, Bandrowski's base. 2,4-Diaminotoluene, a hair dye component until recently, was also shown to be mutagenic: this compound has been shown to be a carcinogen in rats, and is used in large amounts in the polyurethane foam industry. About 20,000,000 people (mostly women) dye their hair in the U.S. and the hazard could be considerable as there is evidence that hair dye and similar chemicals can be efficiently absorbed through human skin. A number of the hair dye ingredients are under test by N.C.I. and the preliminary test results that I have seen suggest that a number of them are carcinogens as we expected. The first of these, o-phenylenediamine has now been declared a carcinogen by N.C.I.

*Tris-(2,3-dibromopropyl)phosphate, the main flame retardant in children's sleepwear has been shown to be a mutagen in our test system by Prival and Rosenkranz and ourselves (18). We have discussed the implications for the 30 million children who have worn garments treated with Tris, the ease of migration of chemicals through human skin and the alternatives to add-on chemicals in preventing burns (18). The original findings on mutagenicity were 1 1/2 years ago. Within the last few months the N.C.I. has obtained the final results on a 2 year animal cancer test. Tris is a strong carcinogen in both sexes of rats and mice. We have spent considerable time on a letter (22) to the Consumer Product Safety Commission discussing the meaning of these cancer results and requesting a ban on Tris. They have now banned it. We have recently shown (M.D. Gold & B.N. Ames, unpublished) that a proposed replacement for Tris in children's sleepwear tris-(1,3-dichloroisopropyl) phosphate, and its presumptive metabolites 1,3-dichloroisopropanol and 1,3-dichloroacetone are mutagenic, the latter being extraordinarily so.

*Ethidium bromide, a known DNA-intercalating agent that is widely used in physical chemical studies with DNA has been shown to be mutagenic (19).

*A variety of anti-cancer drugs, many of them known carcinogens, have been shown to be mutagenic (23).

*Mutagenesis theory. Work from this laboratory has clarified some of the aspects of why the RTF (Resistance transfer factor) increases error prone repair in the tester strains (24). This study also explored the relation of the various DNA repair systems to mutagenesis.

Metabolism. We have examined several large-volume industrial chemicals and their possible metabolic products for mutagenic activity in the Salmonella test, and have found that chloroacetaldehyde, a proposed metabolic product in mammals of the human carcinogen vinyl chloride, is a potent mutagen for strain TA100 (6). Chloroacetaldehyde is also a likely metabolic product of chloroethanol, a common industrial chemical. We pointed out that there is evidence to suggest that 1,2-dichloroethane (ehtylene dichloride), an industrial precursor of vinyl chloride, produced in excess of 8 billion lbs/yr in the U.S. and used as a lead-scavenging agent in gasoline, is metabolized to chloroethanol and chloroacetaldehyde. The mutagenicity of chloroacetaldehyde, the structural similarity between dichloroethane and dibromoethane, a known carcinogen, and the huge amounts of dichloroethane produced annually indicate that there may be a human hazard from it. The National Cancer Institute has finished its animal cancer test on dichloroethane and we expect that its carcinogenicity will be announced shortly (we have done our own calculations on the animal data).

*The relation of somatic mutation to cancer and of environmental carcinogens/mutagens to public health. We have discussed these subjects in several review articles on our test system (2,10,12,13,14,16,17,19).

*Carcinogenic potency and human risk assessment. We (C. Sawyer, N.K. Hooper, A. Friedman, unpublished) have made good progress on 1) developing a scale for carcinogenic potency in animal feeding studies, 2) in calculating potency in a wide variety of animal carcinogenicity tests from the literature and from the chemicals in the N.C.I. bioassay program, and 3) in establishing a thoroughness index for negative animal cancer tests.

Some aspects of human risk assessment in the area of carcinogenicity have been discussed in a N.A.S. report on pesticides (9).

2. Plans for continuation of present objectives and possible new objectives.

Our main objectives at the present time are discussed in the research proposal and are:

1) To improve the Salmonella/microsome test. We are currently detecting 90% of carcinogens. We hope to be able to raise this figure to 95% or more, though there are reasons for believing that we will never reach 100%.
2) To establish an index of carcinogenic potency in animal cancer tests and to calculate the potency of carcinogens.
3) To use the index of carcinogenic potency for establishing human risk from carcinogens in the environment.
4) To investigate the relation of carcinogenic potency to mutagenic potency in the Salmonella/microsome test.
5) To determine how much of organ specific cancer and sex specific cancer in chemical carcinogenesis can be explained by metabolic activation.
6) To determine the best methods of examining mutagenicity in complex mixtures, particularly in aqueous fluids.
7) To detect unsuspected mutagens/carcinogens in the environment that may be of major importance for human health.
8) To do theoretical work on DNA repair systems and other aspects of mutagenesis.
9) To interact with industry and government agencies in the general area of public policy on the detection of environmental carcinogens.
10) To help industry and government in the use of our test system.

3. Graduate Students and Postdoctoral Trainees. (7/1/74 to 9/30/77)

Larry Kier, Graduate student, Ph.D. December 1974 (part time on project).

* Joyce McCann, Postdoctoral 1974-1975; Senior Postdoctoral, 1976-
* Arlene Blum, Senior Postdoctoral, 1976, 1977-
* N.Kim Hooper, Senior Postdoctoral, 1977-
* Charles Sawyer, Postdoctoral, 1977-
* Goran Löfröth, Visiting Professor from Sweden, 1976-1977.
* John Katznellenbogen, Visiting Professor from University of Illinois, 1977-1978.

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Niel Spingarn, undergraduate

Joan Kobori, undergraduate

David Streitwieser, undergraduate

Edmund Choi, undergraduate

George Jen, undergraduate

*Debbie Gold, undergraduate

*Dennis Enomoto, undergraduate

*Allan Friedman, undergraduate

(These undergraduates did considerable work on the project as part of an honors research project.)

4. Bibliography July 1, 1974 to September 30, 1977 (written May 1977). (Work supported by this ERDA contract.)

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5. Present State of Knowledge in the Field and Significance.

It seems likely that the major part of human cancer and genetic defects arises from damage to DNA by environmental mutagens/carcinogens, which may contribute in a significant way to aging and heart disease as well. Already 5% or so of human births are believed to show some sort of genetic abnormality. We are living in a sea of new (and old) chemicals that have not been tested at all for their mutagenic potential. There are mutagens (both natural and man-made) in our diet, in the large number of industrial chemicals to which we are exposed, in hair dyes, in cigarettes, in our drinking water, and in air pollutants.

It seems clear that many more chemicals will be added to the list of human carcinogens, as since the late 1950's we have been exposed to a flood of chemicals that were not tested before use for carcinogenicity or mutagenicity, from flame retardants in our children's pajamas to pesticides accumulating in our body fat. In the past this problem has been largely ignored 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 (5 billion lbs/yr, U.S.A.) and ethylene dichloride (8 billion lbs/yr, U.S.A.), 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 still are humans, and epidemiological studies on humans are impractical in most cases. As the 20 to 30 year lag time for chemical carcinogenesis in humans is almost over, a steep increase in human cancer may be the outcome if too many of the thousands of new chemicals to which humans have been exposed turn out to be powerful mutagens and carcinogens. It seems likely that we will soon know how dearly we will have to pay in increased cancer and birth defects for the modern world of industrial chemicals, pesticides, food additives, and plastics.

We believe that this major area of public health can best be attacked by prevention: identifying environmental mutagens/carcinogens, making a rough estimate of human risk based on potency and amount of human exposure, and minimizing human exposure to the more dangerous of these agents. We must identify these agents, but existing animal bioassays (and human epidemiology) alone are inadequate because of expense, time, and the difficulty of dealing with the complex mixtures to which we are exposed.

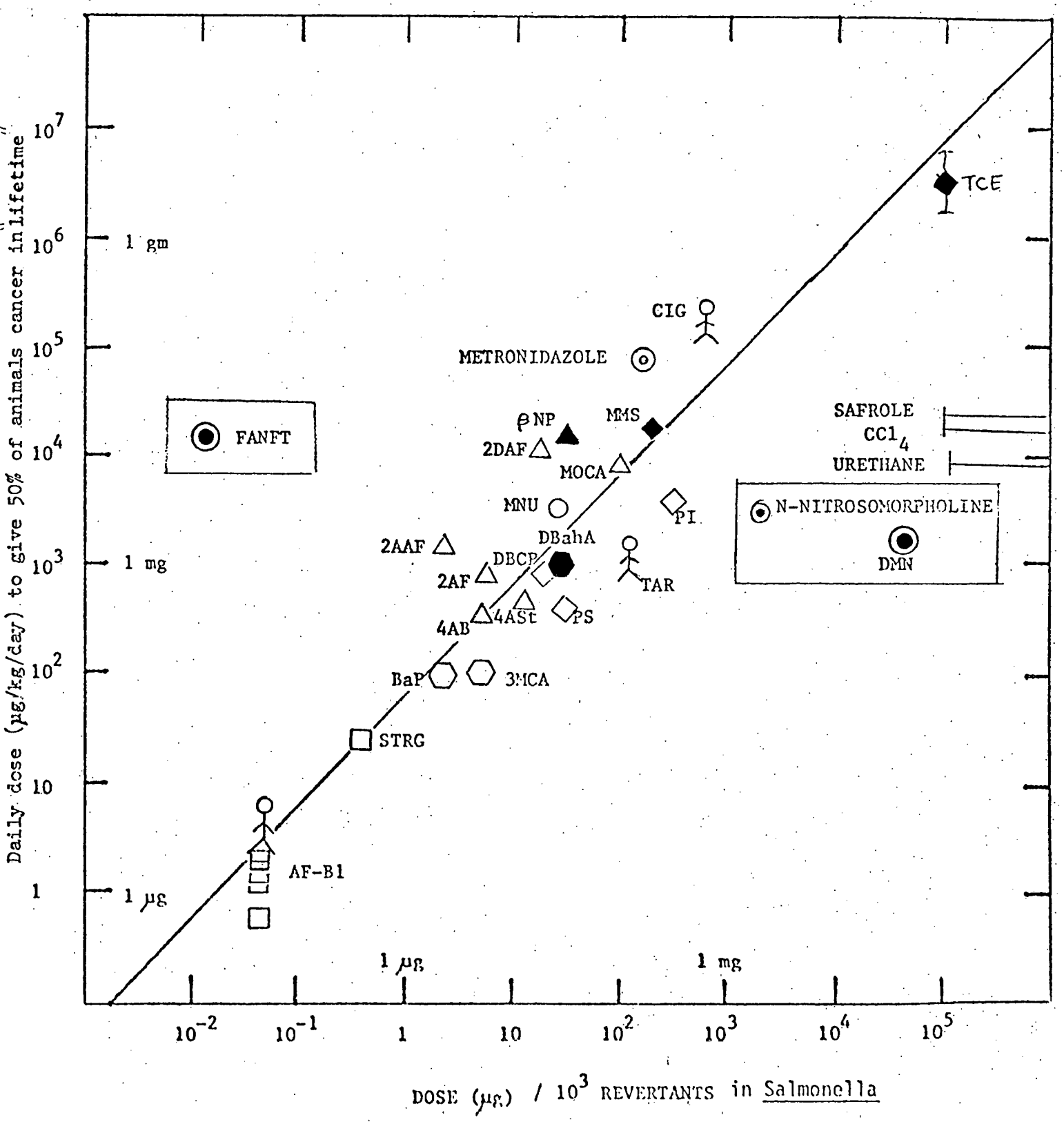
It appears likely however, that the new methods and discoveries from basic biology that have been developed over the last decade and that are being developed at present will help in making future decisions more rational. Over the last 10 years (with ERDA support) we have developed and validated a sensitive, inexpensive and rapid in vitro method for identifying chemical mutagens and we have shown that almost all chemical carcinogens are mutagens. A number of other in vitro tests such as transformation and mutagenesis with animal cells, sister chromatid exchange, and unscheduled DNA synthesis in cells, have been developed in other labs and are in the process of validation. These tests, and the belated realization that we must test chemicals before they come on the market, and that we must evaluate the biological consequences of new technologies, should contribute to a more rational risk - benefit analysis.

6. Federal Support for My Research Program.

USPHS grant, Regulation of Gene Function and Protein Synthesis, GM-19993. $79,393.00 for 1977 (direct costs).
This grant supports my other project on the regulation of bacterial genes.

Relation between Carcinogenic and Mutagenic Potency

C.B. Sawyer and B.N. Ames (preliminary results) 2/77



Research Proposal

1 October 1977 to 30 September 1978

Renewal of ERDA Contract E(04-3)-34 PA156

Our main plans for the coming year are:

1. Carcinogenic Potency, Mutagenic Potency and Their Relation.

We propose to investigate the relation between mutagenic potency in the *Salmonella* test and carcinogenic potency in animals. There are a number of reasons why one should not expect a very close relationship, nevertheless there is a million fold range in both carcinogenic potency and mutagenic potency (aflatoxin B_1 is about a million times stronger as both a mutagen and a carcinogen than trichloroethylene), and even a rough quantitative correlation would be extremely useful. Preliminary work on this problem, by Meselson and Russel (25) and by ourselves, indicates that there is a significant correlation, across a broad range of chemical classes. We believe the area of carcinogenic potency is extremely important for human risk assessment, independently of its relation to mutagenic potency.

To investigate this relationship, we need to define carcinogenic potency. At present, there is no good index of potency: for example the Iball index (see 6) does not even consider the dose. Recent work of Peto and Lee (7) and their forerunners makes this an approachable problem. We plan to work, and have already made significant progress, in several areas:

(a) definition of an acceptable index of carcinogenic potency analogous to the present index of mutagenic potency;

(b) determination of carcinogenic potencies from bioassay results supplied by the National Cancer Institute (NCI) as well as from experiments in the literature;

(c) human carcinogenicity: determination of the carcinogenic potency and mutagenicity of the relatively few substances for which good human data exist;

(d) determination of mutagenicity values for those compounds for which suitable carcinogenicity data exist but for which the mutagenicity is unknown; and

(e) correlating the indices of carcinogenic and mutagenic potencies, with appropriate statistics.

If the correlation is as significant as is indicated by preliminary results, we then aim to:

(f) develop rules for using results of mutagenicity assays for estimation of carcinogenic hazards of new compounds and complex mixtures. We are surrounded by mutagenic complex mixtures (car exhaust, industrial effluents, etc) where even a rough idea of carcinogenic potency would be useful.

These points are expanded on below:

(a) An index of carcinogenic potency has been proposed by Meselson and Russell (25), and we have only slightly modified it. This index, the TD_{50}, is the daily dose required to decrease the probability of a treated animal being cancer free at the end of a standard lifetime (two years in rats or mice) to one-half that of an untreated animal. This definition is adopted in preference to others because the dose thus required is not much different from the dose actually given in a meaningful bioassay, making the choice of dose-response functions (Mantel-Bryan or linear [26]) insignificant. The TD_{50} is calculated when possible using the regression parameters of Peto and Lee (27) which in turn are computed directly from the experimental results. Other methods of treating carcinogenic potency (see 28) seem less satisfactory for a variety of reasons. We propose to see if the TD_{50} is an acceptable index by discussion with other experts in the field such as R. Peto, M. Meselson, E. Scott and A. Whittemore, by testing with actual data on carcinogenic potency and by comparing it with alternative indices such as life-shortening dose.

(b) Determination of carcinogenic potency has been done on 14 compounds by Meselson and Russell (25) and plotted against our published results on mutagenicity. We have done 24 carcinogens and plotted the results against our values on mutagenicity (Fig. 1). Meselson and Russell follow Peto and Lee (27) in assuming that the cancer incidence rate follows a Weibull distribution. We also prefer this to a log normal (Mantel-Bryan), but in any case it would not change the results very much (26). We have extended Meselson and Russell's analysis by applying the techniques of Pike (29), Cox (30), and Peto and Lee (27) for determination of the regression parameters defined by Peto and Lee (26), and have used this to calculate TD_{50}. Meselson and Russell have set the criteria for an acceptable cancer bioassay suitable for calculation. These include route of administration (feeding studies are acceptable but not skin painting or sub-cutaneous) and specify dosing schedule (approaching continuous), length of experiment, number of tumor bearing animals, etc. We agree with their criteria.

We propose to gather animal cancer data suitable for calculating carcinogenic potency from two sources. We have made an arrangement with N.C.I. to obtain their computer tapes of the bioassays in progress as they are released and we propose to write programs for a direct potency analysis of the several hundred tests that will be released in the next year. We are also scanning the literature in a systematic way for animal bioassays that meet our criteria for calculations and we propose to write the authors of most recent published papers that appear suitable, but which have insufficient information for doing our calculations. We plan to augment our current computer programs for analyzing this data. We propose to calculate confidence limits on the points plotted in Fig. 1.

We propose to extend the TD_{50} concept to negative cancer data. It would be useful to express a negative cancer test by its "thoroughness", that is, what is the least potent carcinogen that could have been detected in the test. We propose to write computer programs to express confidence limits on TD_{50} based on the data.

We propose to explore whether the TD_{50} concept can be extended to other routes of administration and shorter exposure times.

(c) Human cancer data, where a cause and a dose are known, is quite rare. Whittemore (31) has done a thorough job on cigarette smoking and human lung cancer. She shows that the Weibull distribution fits the results as well as log normal. We have determined the mutagenicity of cigarette smoke condensate with rat liver (1). We have also done a preliminary calculation on the epidemiological results on aflatoxin and human cancer (32) and on data on coal tar fume exposure and lung cancer among roofers (33) and plotted these against our mutagenicity data. We propose to follow up our preliminary results with the mutagenicity of coal tar fumes and cigarette smoke condensate and to repeat the results with human autopsy liver which we have obtained. (Our past experience with rat liver and human liver shows no great differences for most chemicals.) We also plan to collect another sample of coal tar fumes and reassay the mutagenicity. There are some other possible candidates (9) and we plan to explore these and others.

(d) Mutagenic potency has been determined for a large variety of chemicals (most results from this laboratory[8,14]). We have determined potency for complex mixtures such as cigarette smoke condensates (1), coal tar fumes (Sawyer and Ames, unpublished), and air pollution samples.

We would determine mutagenicity by our standard methods (1) for any chemicals for which we can calculate a carcinogenic potency. We also propose to find cases in the literature where the carcinogenic potency differs between species (mostly rats vs. mice) or between sexes, and determine how much of this can be explained by differential activity of rat liver vs. mouse liver, or of male vs. female liver in a mutagenicity test; we would then test the chemical using human autopsy liver.

(e) Correlation. After plotting new points, and calculating confidence limits on points for Fig. 1, we propose to determine whether a linear relationship is sufficient to describe the correlation. If not we will explore other models. We propose to do a regression analysis on the data.

(f) Human risk estimation. The use of carcinogenic potency calculations for estimating the hazard of chemicals to humans is in its infancy and the field needs a reliable index of carcinogenic potency. Companies and government agencies are starting to make decisions based on the qualitative results of mutagenicity in *Salmonella*. No work has been done on quantitative results with *Salmonella*, though we have now predicted successfully the carcinogenic potency of *tris*-(dibromopropyl)phosphate, the main flame retardant added to 30 million children's pajamas in the U.S. (18) (N.C.I. cancer bioassay results: TD_{50} calculated in this laboratory [22]). We propose to investigate the utility of the TD_{50} concept of carcinogenic potency for human risk estimation as it relates to government decisions on carcinogenic chemicals in the environment. We also hope to see what classes of chemicals show a correlation between mutagenic potency and carcinogenic potency and to improve the *Salmonella* test for those classes not showing a good correlation, if possible. For example, nitrofurans are too mutagenic relative to their carcinogenicity and we know this is due to the nitroreductases of the bacteria which activate these chemicals. It is possible to eliminate these enzymes from the bacteria and to substitute liver activation instead. Certain nitroso carcinogens are too weak as mutagens and we are investigating why the test is deficient in this. Eventually, we hope the investigation of the relation between carcinogenic potency and mutagenic potency might make it possible to get a rough estimate of human risk from complex mixtures of carcinogens that we face in our environment and for which animal cancer tests are impractical. If the correlation between the two potencies is good enough it also should be useful for risk estimations during the two or more years that the animal test is underway. Unfortunately mutagenicity information was not taken seriously in the two cases where the results on bacterial mutagenicity preceded those on animal carcinogenicity by a year or more for chemicals with widespread human exposure: the Japanese food additive, furylfuramide (see 19) and the flame retardant, Tris (18,22).

2. Service to universities, government agencies, and industry in mailing out our tester strains and helping them to set up the test system.

There has been an ever increasing interest in the test system by both industry and research scientists. This is due, in part, to our validation of the test system (8,14,19) showing that 90% of carcinogens are mutagens, and that few "non-carcinogens" are mutagens. This validation has been confirmed by an independent blind-study using all the promising in vitro test systems by ICI (the largest chemical company in Europe [34]). It has also been confirmed in a large study by the group of Dr. Sugimura, the Director of the National Cancer Institute of Japan (35) and it is being required by the Japanese for all pesticides. Many large companies such as Merck are testing all their new chemicals with the system. Due to the volume of mail requesting strains and information on setting up the test system, I have had to hire an additional technician, Dorothy Maron, and a student, Dennis Ing, whose main function is to handle the letters and phone calls from industry and research labs, and to mail out the strains. We receive about 20 letters a week requesting strains and/or information and many phone calls each day.

In addition, Mrs. Maron has been coordinating the information on specific uses of the test. For example many people have requested the strains for the purpose of examining waste water, or shale oil effluents, or coal burning effluents and wastes. We try and keep these people aware of the other groups using the test for each of these specific purposes so that they can contact each other. I enclose a few sample letters.

We propose to continue to do this.

3. Aid to government and industry in the general area of environmental mutagens and carcinogens.

We have been advising Congressional Committees, E.P.A., N.C.I., E.R.D.A., Consumer Product Safety Commission, N.I.O.S.H., N.I.E.H.S., F.D.A., the State of California, the World Health Organization, and various foreign governmental agencies that have requested aid, about problems on the detection, human risk assessment, and regulatory decisions in the general area of mutagens and carcinogens. I am on the National Cancer Advisory Board and was on the Search Committee for a new N.C.I. director. Dr. McCann, of my laboratory, is on the F.D.A. Toxicology Advisory Board, and is currently on the Saccharin Commission set up by Congress. Both of us have testified before Congressional Committees and have been involved in several World Health Organization Committees.

We have also received numerous requests from industry for advice which we give without charge. (I do not do paid consulting for industry because of my government advising). For example, DuPont, ICI and Allied recently had to make a decision as to possible replacements (for use in spray cans) of the Freon that is harming the ozone layer and is being phased out. The two freons that were possible replacements were both very weakly mutagenic in our test system and the companies decided, as a consequence, not to go ahead with them for spray can use, at considerable economic loss; they will be without a substitute for several years. Dr. McCann and I spent several days going over the data and discussing the results with them. Dr. Blum, Miss Gold and I have also had numerous discussions with many industries in connection with the whole flame retardant (in sleepwear) controversy. All three of us are in very close contact with the Consumer Product Safety Commission on this matter.

Dr. Hooper has been involved with the EPA on pesticide risk assessment and on setting priorities for the new toxic substances law. He has also interacted with the Consumer Product Safety Commission on flame retardants and with the State of California Water Board.

I propose to continue spending some part of my time in this public service activity. Drs. McCann, Blum and Hooper, senior researchers in my laboratory, will spend part of their time in this type of interaction with government.

4. Test improvements.

About 10% of organic carcinogens are not detected as mutagens in our test. We propose to investigate the chemicals that do not work to try and understand why. Among the groups of chemicals are some hydrazines, some chlorinated chemicals, such as chloroform and carbon tetrachloride, and some methylated amino chemicals such as hexamethylphosphoramide. We are trying several approaches:

1) *Salmonella-somes.* We are in the process of fusing the membranes of the microsomes from the rat liver with the outer membrane of the bacteria, using methods developed for fusing animal cells. By coating the bacteria with the microsomal activation system we hope that short-lived active species will be more likely to reach the DNA of the bacteria.

2) We are investigating the metabolic activity of the rat liver mitochondria, a fraction that we have traditionally thrown away in making our liver 9000 x g supernatant fraction.

3) A number of new repair systems have been described in bacteria that also appear to exist in mammals. We plan to put mutations for these various systems in our tester strains. We will then see if this increases the sensitivity of the test system.

4) We are picking those chlorinated pesticides and industrial chemicals that are fairly strong carcinogens (see carcinogenic potency) and attempting to optimize the dechlorination metabolism. We plan to try anaerobic metabolism, mouse liver vs. rat liver and 1), 2), and 3) above. This is a particularly important project because of the importance of organic chlorinated chemicals in industry and in chlorinated drinking water.

5) Nitrosamines. Nitrosamines are a particularly potent class of carcinogens that are showing up as only weak mutagens in our system. We plan to investigate the reasons for this. We will try the above possible improvements on this class.

6) We are investigating the possibility of making tester strains with new repetitive sequences in the DNA at the sight of the mutation. We have a strain with a sequence of $\frac{GGGGGGG}{CCCCCCC}$ (+1) that may be useful in a new tester strain.

7) We will explore the utility of making *Salmonella* with a constitutive β-glucuronidase for experiments with urinary metabolites.

8) Metals. Dr. Lofroth has explored various ways of getting carcinogenic metals to work in the *Salmonella* system. He has shown that Cr^{III} is a mutagen. We will continue to investigate this area.

9) Fecal enzymes. Certain carcinogens are activated by enzymes in fecal bacteria (e.g. β-glucosidase). We plan to collaborate with Professor Chang of the Nutrition Department on a study using an enzyme extract he has made by sonicating human feces (feces are mostly bacteria) to see if this is useful in adding to our test system for activating this class of carcinogens.

5. Mutagens/Carcinogens in the environment.

Although our main interest is in the further improvement of the test we will continue to try and discover important unsuspected mutagens/carcinogens in the environment such as hair dyes and flame retardants in children's sleepwear.

We are continuing our work on flame retardants in children's sleepwear. We have recently shown that Fyrol FR2, a proposed replacement for the banned *tris*-(2,3-dibromopropyl)phosphate, is a mutagen. Now that we have eliminated Fyrol FR2, a new add-on, hexabromocyclododecane, is being proposed. We propose to test it thoroughly as it looks like another horror. The Consumer Product Safety Commission has not fully understood that it is unacceptable to put large amounts of brominated and chlorinated add-on chemicals in children's polyester sleepwear. We plan to keep an eye on future developments in the sleepwear controversy (18,22), and also on the 100,000,000 lbs of flame retardants of a variety of kinds (mostly brominated and chlorinated) that are used in the U.S.

We are also currently investigating brominated vegetable oils. These are added to soft drinks (e.g. Fresca) in reasonably large amounts to change the density of the fat droplets so that they float and the drink looks like a fruit juice. We are also investigating a variety of other brominated organics that are used as fumigants.

We have found one type of widely used suntan lotion that contains a mutagen and we will investigate this further.

6. Complex mixtures.

We hope to continue to work out methods so that the test can be used for complex mixtures such as air pollution samples, water contaminants, oil fractions and car exhaust samples. We will try to determine if our method for urine analysis using XAD columns is suitable for other aqueous fluids such as waste water (in collaboration with the State of California Water Board).

References:

1-24 are listed in the COMPREHENSIVE PROGRESS REPORT

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PROPOSED BUDGET (12 months, 10/01/77 - 09/30/78)

1. Salaries:

Dr. Bruce N. Ames, Principal Investigator (1 month summer salary)	$ 4,103#
Dorothy Maron Staff Research Associate I (80% time)	$ 11,519*
Lynne Haroun Staff Research Associate I (100% time)	$ 14,742*
Edith Yamasaki Associate Specialist I (100% time)	$ 17,450+
Dr. Charles Sawyer Postgraduate Research Biochemist I (100%, 3 months)	$ 3,027*
Dr. N.K. Hooper (Senior Postdoctoral) Assistant Research Biochemist I (100%)	$ 17,762+
Dr. Arlene Blum (Senior Postdoctoral) Assistant Research Biochemist I (100%, 6 months)	$ 8,881+
Dr. Joyce McCann (Senior Postdoctoral) Assistant Research Biochemist I (100%, 6 months)	$ 8,881+
C. Gold (undergraduate student) Lab Helper (100%, 3 months)	$ 1,926@
A. Friedman (undergraduate student) Lab Helper (100%, 3 months)	$ 1,926@
D. Ing (undergraduate student) Lab Assistant (62.5%)	$ 5,601@
Gaylee Stark Dean (Secretary) Principle Typist Clerk I (50%)	$ 5,010*
Susan Barnes McAllan (Secretary) Administrative Assistant II (50%)	$ 5,718*
Recharges by department for my share of personnel of media kitchen, animal room, electron microscope, shops	$ 15,000*
TOTAL SALARIES	$ 121,546

2. Fringe Benefits:

21% for academic salaries marked with +	$ 11,125
20% for staff salaries marked with *	$ 10,403
1.76% for faculty summer salary marked with #	$ 72
0.96% for undergraduate student salaries marked with @	$ 91
	$ 21,691

3. Supplies:

Petri dishes, media, chemicals, biochemical supplies	$ 12,000

4. Equipment:

Small items of equipment under $1,000 each	$ 2,500

5. Travel:

Domestic Scientific meetings	$ 1,500
6. Publication costs, xeroxing, books, journals, and telephone:	$ 5,000
TOTAL DIRECT COSTS	$ 164,237
MTDC (29%, all items except equipment)	$ 46,904
TOTAL AMOUNT REQUESTED OF E.R.D.A. AND TOTAL PROJECT COSTS	$ 211,141

AMOUNT REQUESTED ON 2nd and 3rd YEARS OF CONTRACT:

We request the same amount for the second year and the third year with a 7% increase each year to cover merit increases and cost of living increases in personnel. We expect that slightly less money will be used for postdoctoral personnel the second and third year and slightly more money for the purchase of equipment such as accessories for high pressure liquid chromatography and for a gas chromatography apparatus with an electron capture detector for the detection of chlorinated and brominated chemicals.

2nd year = $ 164,237 + 7% = $ 175,734 direct costs ($226,697 total)

3rd year = $ 175,734 + 7% = $ 188,035 direct costs ($242,565 total)

(Dr. Stapleton has indicated that no more detail is necessary for the 2nd and 3rd year budgets.)

CURRICULUM VITAE

NAME:	Bruce N. Ames	
DATE & PLACE OF BIRTH:	December 16, 1928. New York, New York.	
MARITAL STATUS:	Married (Dr. Giovanna Ferro-Luzzi Ames) Two children	
EDUCATION:	Cornell University, B.A. (Chemistry Major, Biology Minor)	1946-1950
	California Institute of Technology, Ph.D. (Biochemistry Major, with Prof. H.K. Mitchell; Chemistry and Genetics Minors)	1950-1953
PROFESSIONAL EXPERIENCE:	Professor of Biochemistry, Biochemistry Department University of California at Berkeley, 94720 Tel. (415) 642-5165	1968-
	Chief, Section of Microbial Genetics, Laboratory of Molecular Biology, N.I.A.M.D., National Institutes of Health.	1962-1967
	Sabbatical year as N.S.F. Senior Fellow in Laboratories of F.H.C. Crick in Cambridge, England and F. Jacob in Paris, France.	1961
	Biochemist at the National Institutes of Health	1954-1960
	Postdoctoral Fellow (U.S.P.H.S.) at National Institutes of Health with Dr. B.L. Horecker	1953-1954
PROFESSIONAL SOCIETIES:	American Chemical Society American Society of Biological Chemists Genetics Society of America American Society for Microbiology Environmental Mutagen Society American Association for Cancer Research	
AWARDS & HONORS:	National Academy of Sciences	1972
	American Academy of Arts and Sciences	1970
	Lewis Rosenstiel Award	1976
	Arthur Flemming Award (as outstanding young Government Employee)	1966
	Eli Lilly Award of the American Chemical Society	1964
	FASEB/3M Award for Research in Life Sciences	1976
	E.R.D.A. Distinguished Associate Award	1976
	Environmental Mutagen Society Award	1977
	Cal. Tech. Distinguished Alumni Award	1977

urriculum Vitae - Bruce N. Ames (Continued)

SERVICE ON BOARDS AND COMMITTEES:

National Cancer Advisory Board	1976-
Governing Council, Environmental Mutagen Society.	1971-1975
Subcluster on Environmental Health and Toxicology of President's Biomedical Research Panel.	1975
Consultative Panel on Hazards of Chemical Pesticides, National Research Council, National Academy of Sciences.	1974
Advisory Committee, Earl Warren Legal Institute.	1971-1975
Organizer, 1st International Conference on Environmental Mutagens, Asilomar, California.	1973
Member of National Research Council (representative of Genetics Society).	1964-1969
Nominating Committee of the American Society of Biological Chemists.	1967-1969
Program Committee of the ASBC.	1963-1967
Nominating Committee of the Genetics Soceity	1971
Editorial Board, Archives of Biochemistry and Biophysics.	1964-1969
Editorial Board, Journal of Biological Chemistry.	1965-1971
Search Committee for director of N.C.I.	1977

RESEARCH INTERESTS: Detection of environmental chemicals causing damage to DNA,
chemical mutagenesis and carcinogenesis,
biochemical genetics of bacteria,
regulation of gene expression, operons,
histidine biosynthesis.

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70a. Smith, G.R. and Tong, B. (1974) Construction of ØSO dhis Carrying Salmonella typhimurium Histidine Operon Mutations. J. Bacteriol. 120, 1223-1226.

71. Ames, B.N. and Hartman, P.E. (1974) The Histidine Operon of Salmonella typhimurium. in Handbook of Genetics, ed. R.C. King (Plenum Press, New York) Vol. 1 pp. 223-235.

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81. McCann, J., Choi, E., Yamasaki, E., & Ames, B.N. (1975) Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals. Proc. Nat. Acad. Sci. USA 72, 5135-5139.

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85. McCann, J. and Ames, B.N. (1976) A Simple Method for Detecting Environmental Carcinogens as Mutagens. Ann. NY Acad. Sci., 271, 5-13.

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87. McCann, J. and Ames, B.N. (1976) Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals: Discussion. Proc. Nat. Acad. Sci. USA 73, 950-954.

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94. Weppelman, R., Kier, L.D., and Ames, B.N. (1977) Properties of Two Phosphatases and a Cyclic Phosphodiesterase of Salmonella typhimurium. J. Bacteriol. 130, 411-419.

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95a. Walker, G. (1977) Plasmid (pKM101)-Mediated Enhancement of Repair and Mutagenesis: Dependence on Chromosomal Genes in Escherichia coli K-12. Molec. gen. Genet. 152, 93-103.

96. Hooper, N.K. and Ames, B.N. (1977) Letter to Chairman John Byington, U.S. Consumer Product Safety Commission, Washington, D.C.

97. Ames, B.N. (1977) The Detection of Environmental Mutagens/Carcinogens. Federation Proc. 36, 1630.

98. Yamasaki, E. and Ames, B.N. (1977) The Concentration of Mutagens from Urine by XAD-2 Resin Adsorption: Cigarette Smokers have Mutagenis Urine. Proc. Natl. Acad. Sci. USA, in press.

99. Donahue, E.V., McCann, J., and Ames, B.N. (1977) Impurities, High Pressure Liquid Chromatography, and Mutagenicity Testing. Cancer Research, in press.

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Physical Chemistry, 1966-67.
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Stanford University, Stanford, California, NIH Postdoctoral Fellow, Department of Biochemistry, (with Dr. R.A. Baldwin)

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Research Experience:

Study of Mutagenicity of Chemical Flame Retardants (1976) Univ. of California, Berkeley

NMR Study of Ribonuclease Folding (1973-1975), Stanford Univ.

Isolation and Spectroscopy (ORD-CD,UV, IR) of tRNAs and Oligonucleotides, (1967-1971), Univ. of California, Berkeley

Chemical Analysis of Fumarole Emanations from Mt. Hood Oregon (1966), Reed College Senior Thesis

Spectroscopic Study of Palladium and Platinum Nucleic Acid Complexes, (1966) Reed College.

Synthesis and NMR Spectroscopy of Picrate Compounds, (1964,65) Argonne National Laboratory.

Teaching Experience:

Assistant Professor of Chemistry, Wellesley College, Wellesley, Massachusetts, February, 1977 - May, 1977.

Acting Assistant Professor, Stanford University, Stanford California, 1975.

Teaching Assistant, University of California, Berkeley, California, 1967-1969.

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Publications:

J. A. Weil, A. Blum, A. H. Heiss, and J. K. Kinnaird (1967), "Conformation and Internal Rotation of Nitroaromatic Amines in Solution as Detected by Proton Magnetic Resonance II, Polynitro Acetanilides", J. Chem. Phys. 46, 3132.

A. Blum, (1971) "A Circular Dichroism Study of Nine Species of Transfer Ribonucleic Acid", Ph. D. Thesis, University of California, Berkeley.

A. D. Blum, O. C. Uhlenbeck, and I. Tinoco, Jr. (1972) "Circular Dichroism Study of Nine Species of Transfer RNA", Biochemistry 11, 3248.

A. Blum, S. H. Smallcomb, and R. L. Baldwin, (1975) " Temperature Jump NMR Study of Intermediates in Refolding of Ribonuclease", Biophyscial Journal 15, 192a.

A. Blum and B. N. Ames (1977) "Flame-Retardant Additives as Possible Cancer Hazards", Science 195, 17.

A. Blum, S.H. Smallcomb, and R.L. Baldwin (1977) "Direct Evidence for Intermediates in Ribonuclease Refolding", in preparation.

Fellowships:

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References:

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EDUCATION

B.A. with Distinction, 1966, University of Colorado, Boulder. (Chemistry major/Math-Physics minor.) Phi Beta Kappa.

Ph.D., 1972, University of Colorado, Boulder. (Biology.) Doctoral Dissertation: Colicin E2 induced DNA degradation in *Escherichia coli*.

PROFESSIONAL EXPERIENCE

Senior Fellow (American Cancer Society) 1976-1978, Biochemistry Department, University of California, Berkeley.

Postdoctoral Fellow with Dr. Bruce N. Ames, 1973-1975, Biochemistry Department, University of California, Berkeley.

PROFESSIONAL SOCIETIES

Environmental Mutagen Society
American Chemical Society

HONORS & AWARDS

NIH Traineeship, 9/66-8/67; NDEA Fellowship, 9/67-8/70; NSF Fellowship, 9/70-8/71; American Cancer Society, California Division Postdoctoral Junior Fellowship, 1/74-2/76; American Cancer Society, California Division Senior Fellowship, 3/76-2/78.

SERVICE ON BOARDS AND COMMITTEES

Cancer Testing Technology and Saccharin Panel, U.S. Congress Office of Technology Assessment, 1977.

Special Review Group, National Research Service Award (NRSA) Program, National Institute of Environmental Health Sciences, 1977.

Food and Drug Administration Toxicology Advisory Committee, 1976-80.

World Health Organization, International Agency for Research on Cancer (IARC) Working Group on the Evaluation of Carcinogenic Risk of Chemicals to Man, 1976.

Councilmember, Environmental Mutagen Society, 1977.

INVITED LECTURES (1976-1977)

Birth Defects: Detection & Prevention, Fort de France Martinique. Sponsored by l'Institute de la Vie & NCI, 26-28 Jan. '76.

School of Public Health, Univ. of California, Berkeley, 14 Apr. '76.

Third International Symposium on Detection & Prevention of Cancer, New York. Sponsored by the International Study Group for the Detection & Prevention of Cancer, 26 Apr. - 1 May '76.

Michigan State Univ., East Lansing. 3 May '76.

American Chemical Soc., Akron Division, Akron, Ohio, 4 May '76.

Food Research Inst., Univ. of Wisconsin, Madison, 11-12 May '76.

Toxicity Testing *in vitro*, Jefferson, Arkansas. Sponsored by the National Center for Tox. Res., 19-21 May '76.

Chronic Toxicity Testing in the Food Industry, Aspen, Colorado. Organized by the Eppley Inst. for Res. on Cancer, 22-27 Aug. '76.

Origins of Human Cancer, Cold Spring Harbor, NY. Organized by Harvard Sch. of Pub. Health & Cold Spring Harbor Lab., 7-14 Sept. '76.

Department of Nutrition, Univ. of California, Berkeley, 17 Nov. '76.

Short Term Tests for Carcinogenicity, Hunt Valley, Maryland. Sponsored by the Toxicology Forum, 7-9 Feb. '77.

Inter-Agency Nutrition Council of Alameda County, 28 Feb. '77.

Soc. of Cosmetic Chemists, Golden Gate Chapter, 8 Mar. '77.

Panel member. Public Discussion Session on the Toxic Substances Control Act, Los Angeles, Ca., 15 Mar. '77.

Sonoma State College, CA., 17, Mar. '77.

Society of Cosmetic Chemists. National Meeting, Montreal, Canada, 4-5 May '77.

PUBLICATIONS

McCann, J., N.E. Spingarn, J. Kobori & B.N. Ames (1975) Detection of carcinogens as mutagens: bacterial tester strains with R factor plasmids. *PNAS USA 72:*979-983.

McCann, J., V. Simmon, D. Streitwieser & B.N. Ames (1975) Mutagenicity of chloroacetaldehyde, a possible metabolic product of 1,2-dichloroethane (ethylene dichloride), chloroethanol (ethylene chlorohydrin), vinyl chloride & cyclophosphamide. *PNAS USA 72:*3190-3193.

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Ames, B.N., J. McCann & E. Yamasaki (1975) Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test. *Mutation Res. 31:*364-374.

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Ames, B.N., J. McCann & C. Sawyer (1976) Mutagens and Carcinogens. *Science 194:*132-133.

McCann, J. (1976) Carcinogenesis & Mutagenesis. *Chem. Tech. 6:*682-687.

McCann, J. & B.N. Ames (1977) The *Salmonella*/microsome test: predictive value for animal carcinogenicity. IN *Origins of Human Cancer* (Cold Spring Harbor Lab., NY) in press.

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Donahue, E.V., J. McCann, & B.N. Ames (1977) Impurities, high pressure liquid chromatography, and mutagenicity testing. Submitted to Cancer Research.

CURRICULUM VITAE

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Public Schools in Ann Arbor, Michigan.
B.S. in Chemistry, Cornell University, Ithaca, New York; June 1961.
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Positions Held:

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1972-75	Research Associate, Department of Biology, University of Oregon, Eugene, Oregon; with Dr. David L. Barker.
1968-71	National Institutes of Health Postdoctoral Fellow, Department of Biochemistry, University of California, Berkeley, California; with Dr. Bruce N. Ames.
1963-65	Resident Tutor in Chemistry and Biochemistry, Lowell House, Harvard Unviersity, Cambridge, Massachusetts.
1962-66	National Institutes of Health Predoctoral Fellow, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts.
Summer, 1961	Research Assistant, Dutch Central Institute for Brain Research, Amsterdam, The Netherlands; with Dr. Jos. Schadé.
Summer, 1960	National Science Foundation Summer Traineeship, Department of Neurophysiology, California Institute of Technology, Pasadena, California; with Dr. Anton van Harreveld.
Summer, 1959	Research Assistant, Brain Research Laboratory, University of Michigan; with Dr. James Olds.

Grants:

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Assistant Research Scientist, Water REsources Grant, State of California, "Mutagenicity of Selected California Wastewaters," January, 1977.

CURRICULUM VITAE: N.K. Hooper

Publications:

1. Hooper, N.K. and van Harreveld, A., "Brain Electrolytes and Cortical Impedance," Am. J. Physiol. 201, 139 (1961).

2. Hooper, N.K. and Law, J.H., "Biosynthesis of Cyclopropane Compounds VII. Synthesis of Cyclopropane and Cyclopropene Fatty Acids by Hibiscus Seedlings," Biochem. Biophys. Res. Commun. 18, 426 (1964).

3. Hooper, N.K. and Law, J.H., "Mass Spectrometry of Derivatives of Cyclopropene Fatty Acids," J. Lipid Res. 9, 270-275 (1966).

4. Thesis: "Biosynthesis of Cyclopropene Fatty Acids," Harvard University, June, 1968.

5. Hooper, N.K. and Barker, D.L., "Synthesis of Dopamine and Octopamine in the Crustacean Stomatogastric Nervous System," Society for Neurosciences, Meeting Abstracts, 1975 (manuscript in preparation).

6. Hackforth-Jones, J., Hooper, N.K., Janos, D., Miller, G., and Janzen, D.H., "Mammalian Influence on Seed Shadow of Tropical Legume," Ecology, 1976 (in press).

Other Research:

1. Literature research on mutagenicity and carcinogenicity of selected environmental and industrial chemicals, e.g. pesticides, water pollutants, etc. in preparation for paper with Dr. B.N. Ames.

2. Compilation of dossier on mutagenicity of chemicals for WHO/IARC project to evaluate carcinogenic risk of chemicals with significant human exposure, with Dr. Joyce McCann, 1976.

Administrative:

1. Supervisor, laboratory work in neurochemistry, Department of Biology, University of Oregon, 1972-75.

2. Faculty Sponsor, NSF study of occupational health of reforestation workers in Oregon, 1975.

3. Founder, reforestation and timber management company, 1975.

Consulting:

1. Advisory Committee on Occupational Carcinogens, Department of Health, State of California, 2151 Berkeley Way, Berkeley, California, 1976-77.

2. Consultant to Battelle Memorial Laboratories, 505 King Ave., Columbus, Ohio for EPA contract "Scientific Review on Pesticide Health Effects

Literature," 1976-77.

3. Consultant to Clement Associates, Washington, D.C., 1976-77.

Seminar:

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Sawyer, C.B. and Kirsch, J.F. (1975) Kinetic isotope effects for reactions of methyl formate-methoxyl-^{18}O. J. Amer. Chem. Soc. 95, 7375-7381.

Sawyer, C.B. and Kirsch, J.F. (1975) Kinetic isotope effects for the chymotrypsin catalyzed hydrolysis of ethoxyl-^{18}O labeled specific ester substrates. J. Amer. Chem. Soc. 97, 1963-1964.

Sawyer, C.B. and Kirsch, J.F. Kinetic Isotope Effects for the Chymotrypsin Catalyzed Hydrolysis of Ethoxy-^{18}O Labeled Specific Ester Substrates: Effect of pH. in preparation.

McCann, J., Sawyer, C., and Ames, B.N. (1976) The Salmonella/microsome test: predictive value for animal carcinogenicity. Abstracts of papers presented at the meeting on Origins of Human Cancer, September 7-14, 1976. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, p. 66.

Ames, B.N., McCann, J., and Sawyer, C. (1976) Mutagens and Carcinogens. Science 194, 132-133.

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1. Yamasaki, E.F., Swindell, R., and Reed, D.J. Some Aspects of Catalysis by the Amine Oxidase of Pea Seedlings. Biochemistry 9, 1206 (1970).

2. Brot, N., Yamasaki, E., Redfield, B., and Weissbach, H. The Binding of Aminoacyl-tRNA and Poly U to a Soluble Factor(s) Extracted from Ribosomes. Biochem. Biophys. Res. Comm. 40, 698 (1970).

3. Brot, N., Yamasaki, E., Redfield, B., and Weissbach, H. The Properties of an E. coli Ribosomal Protein which is Required for the Function of Factor G. Arch. Biochem. Biophys. 148, 148 (1972).

4. Weissbach, H., Redfield, B., Yamasaki, E., Davis, R.C., Jr., Pestka, S., and Brot, N. Studies on the Ribosomal Sites Involved in Factors Tu and G Dependent Reactions. Arch. Biochem. Biophys. 149, 110 (1972).

5. Weissbach, H., Redfield, B., Yamasaki, E., and Brot, N. Interaction of a Phe-tRNA-Tu-GTP Complex with Ribosomal Subunits. Arch. Biochem. Biophys. 149, 560 (1972).

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7. Brot, N., Marcel, R., Yamasaki, E., and Weissbach, H. Further Studies on the Role of 50S Ribosomal Proteins in Protein Synthesis. J. Biol. Chem. 248, 6952 (1973).

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11. Ames, B.N., McCann, J., and Yamasaki, E. Methods for Detecting Carcinogens and Mutagens with the Salmonella/Mammalian-Microsome Mutagenicity Test. Mutation Res. 31, 347 (1975).

12. Ames, B.N., McCann, J. and Yamasaki, E. Carcinogens are Mutagens: A Simple Test System. Mutation Res. 33, 27 (1975).

13. McCann, J., Choi, E., Yamasaki, E., and Ames, B.N. Detection of carcinogens as Mutagens in the Salmonella/microsome test: Assay of 300 Chemicals. Proc. Nat. Acad. Sci. USA 72, 5135 (1975).

14. Sugimura, T., Yahagi, T., Nagao, M., Takeuchi, T., Hara K., Yamasaki, E., Matsushima, T., Hashimoto, Y., and Okada, M. Validity of Mutagenicity Tests Using Microbes As A Rapid Screening Method for Environmental Carcinogens. (IARC Scientific Publication No. 12) 81 (1976).

15. Yamasaki, E. and Ames, B.N. The Concentration of Mutagens from Urine by XAD-2 Resin Adsorption: Cigarette Smokers have Mutagenic Urine. Proc. Natl. Acad. Sci. USA, in press.

Appendices:

A. List of Scientist and Organizations who have requested our Tester Strains between March, 1976 and May, 1977.

B. New York Times article on the test system.

C. A few of the letters we have received within the year including some concerned with energy matters.

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SCIENTIST AND ORGANIZATIONS WHO HAVE REQUESTED OUR TESTER STRAINS

March, 1977

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SCIENTIST AND ORGANIZATIONS WHO HAVE REQUESTED OUR TESTER STRAINS

March, 1977

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May, 1977

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Bacteria Use Changes Product Cancer Tests

By VICTOR K. McELHENY
Special to The New York Times

BERKELEY, Calif. — Scientists working here with colonies of tiny bacteria in little laboratory dishes are brewing a revolution in the ways that chemical and pharmaceutical companies test their new products for cancer risks.

Up to now, chemical and drug companies and the Government agencies that regulate them have depended on life-time doses of chemicals administered to laboratory rats and mice. This procedure for uncovering cancer risks takes about three years, and can cost $150,000.

Over the last 12 years, on the fourth floor of the biochemistry building at the University of California here, Dr. Bruce N. Ames and a few colleagues have developed tests using colonies of bacteria called salmonella. These produce readings in two or three days at a cost of only a few hundred dollars.

In a process that many scientists think is similar to the induction of cancer, the single-cell bacteria can undergo a mutation, that is, a change in inherited characteristics, from some chemicals and not from others.

Thus, the salmonella, variants of which can cause various diseases in man and domestic animals, are serving as an early warning system for cancer risks from the hundreds of new chemicals introduced each year to the market place.

Presently, Dr. Ames and such colleagues as Dr. Joyce McCann and Dr. Kim Hooper are refining the salmonella mutation test. They want to find which classes of cancer-causing chemicals, such as some hormones, their test misses. They also want to develop the test further as a measure of a chemical's potency as a cancer-causing agent.

But even now, because of results from tests with the bacteria, plans by such concerns as Merck & Company and E. I. du Pont de Nemours & Company to market certain chemicals have been modified or canceled.

Other industries, such as the hair-dye companies, whose products contain many substances that produce bacterial mutations in the Ames test, have been slower to act, Dr. Ames said in an interview. He noted that the National Cancer Institute has included hair-dye components among some 200 substances being tested on animals to see if they incuce cancer.

Use of the Ames test and other short-duration tests are expected to expand rapidly because of the new Federal law regulating toxic substances. The law went into effect Jan. 1.

Focusing further attention on the Ames test was confirmation from cancer induction in laboratory animals of the risks from a bromine-containing fire retardant called Tris that has been used heavily since 1972 in children's sleepwear.

The Consumer Product Safety Commission did not act to remove some 20 million Tris-coated sleep garments from store shelves until last month, a year and a half after the substance proved mutagenic in the Ames test.

One finding that Tris was mutagenic was published in Science last January, along with a special article by Dr. Ames and his colleague, Dr. Arlene Blum, pleading for an immediate ban on Tris in sleepwear.

In February, the Velsicol Chemical Corporation, which recently merged with the Michigan Chemical Corporation, the main manufacturer of Tris, announced it would no longer sell it for use in sleepwear.

Alterations in Characteristics

Mutations are alterations in the inherited characteristics of living things, such as single-cell bacteria of the sort used in the Ames test. The mutations result from damage to the genetic chemical deoxyribonucleic acid, or DNA, that encodes instructions for living processes.

Scientists like Dr. Ames are increasingly convinced that such mutations, brought about by substances at large in the man-made environment of an industrial society, cause most human cancer.

Dr. Ames expressed worry that massive human exposure to man-made chemicals, particularly pesticides and other synthetics containing chlorine and bromine, over the last 30 years could soon begin to drive up human cancer rates as cigarette smoking has done.

Adding to the worry, he said, is recent work by a British scientist, Dr. Richard Peto, an associate of the widely known cancer statistician, Sir. Richard Doll. Dr. Peto has found that halting exposure to cancer-causing agents—as when a person stops smoking—only levels off the risk of getting cancer, rather than rolling back the risk as earlier studies indicated.

Liver Extract With Enzymes

The Ames test involves putting about one billion salmonella bacteria into Petri dishes, along with a liver extract from either rodent or human autopsies. The liver extract, containing various worker proteins called enzymes, are designed to carry out some of the alterations of chemicals that regularly go on in humans or closely related mammals like rats and mice.

The salmonella bacteria are of special types, or mutants. They have outer membranes that chemicals can penetrate more easily than usual. They also have lost their ability to make their own supplies of a protein sub-unit, or amino acid, called histidine. Thus, the mutants require histidine as a food supplement to grow and multiply.

When a mutagenic chemical is put into the dish with the bacteria, it causes some, or many of them to mutate back to a form that can make its own histidine. Colonies of such revertants multiply rapidly in the dish among their crippled rivals. Spots showing the fast-growing colonies crop up in a cloud around the place where a drop of the tested chemical was put.

In a continued search for new types of mutants to increase the test's sensitivity, Dr. Ames said, the Berkeley group is using the much publicized "gene-splicing" or genetic recombination, techniques on which strict controls are being imposed.

Dr. Ames said, "It's really an exciting time. People ask what molecular biology has ever contributed. We could never done this without the techniques of molecular biology."

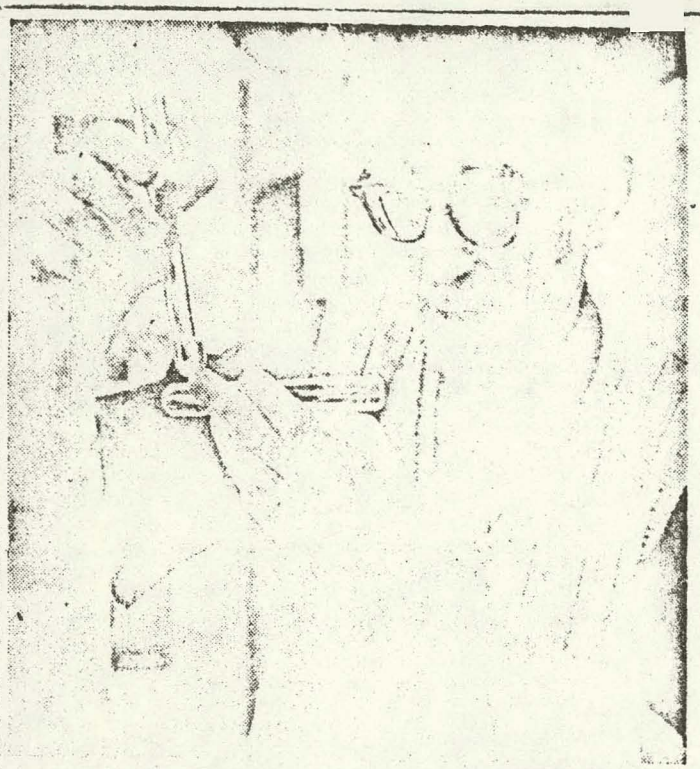
The test can be performed in 48 to 72 hours, at a cost of a few hundred dollars. Lifetime tests with hundreds of laboratory rodents take two to three years, and cost $150,000.

Last year, the test was used on two types of freon that Du Pont was considering as substitutes for freon 11 and 12 spray-can propellants. These chlorofluorocarbon propellants are to be removed from the market as a threat to the ozone in the stratosphere, which absorbs mutagenic ultraviolet rays from the sun, to which some types of skin cancer are attributed.

One of the substitute freons, called 22, is used in the cooling systems of refrigerators. The other called 142B, is not in wide commercial use, James Howell, a Du Pont spokesman said. Both were found mutagenic in 72-hour runs of the Ames test that Du Pont workers think may have done this without the techniques of

tors. The other, called 142B, is not in wide

stead of solids or liquids.



The New York Times/Sandy Solomon

Dr. Bruce N. Ames at work in laboratory at University of California at Berkeley. He is doing research on chemicals that could cause cancer.

Shifted to Other Freon Forms

Du Pont told Federal regulatory agencies, competitors and customers of its decision not to market the freons pending tests in animals. The company switched its attention to other forms of freon, but Mr. Howell said that marketing was at least two years away. "We're in a tough position," he said.

He added: "The Ames test is more helpful than damaging. The need is to develop skills both in government and in industry to find out where the yellow warning flags are. We didn't want to overreact to the Ames test. We took this decision at some risk. But if we didn't do it, it would have been really wrong."

Other companies also deciding to hold off on marketing mutagenic freons are Imperial Chemical Industries Ltd. and the Allied Chemical Corporation.

from the:
New York
Times
5-12-77

OAK RIDGE NATIONAL LABORATORY
OPERATED BY
UNION CARBIDE CORPORATION
NUCLEAR DIVISION

POST OFFICE BOX Y
OAK RIDGE, TENNESSEE 37830

June 3, 1977

Dr. Bruce N. Ames
Biochemistry Department
University of California
Berkeley CA 94720

Dear Dr. Ames:

Enclosed is a group of manuscripts that we have written over the past year. I suspect the nitrosopiperidine work will be of particular interest to you in deriving the relationship between mutagenicity and carcinogenicity.

Thank you for your aid and the strains that made the work possible. If possible, please send us your strains TA92 and TA94 so that we might evaluate their use with crude materials containing inorganics in addition to organics.

We have also had troubles with a background or spontaneous level that varies considerably with TA1535 and TA1977. Reisolation and maintenance of frozen stocks has not helped. Thus, new TA1535 and TA1977 stocks would be useful. Thank you in advance for your courtesy.

Respectfully yours,

James L. Epler
Biology Division
Oak Ridge National Laboratory

JLE:caa

Enclosures

P.S.: Strains requested: TA92, TA94, TA1535, and TA1977

Submitted to:
J. Toxicol. Environ. Health

ANALYTICAL AND BIOLOGICAL ANALYSES OF TEST MATERIALS FROM THE SYNTHETIC FUEL TECHNOLOGIES. I. MUTAGENICITY OF CRUDE OILS DETERMINED BY THE *Salmonella typhimurium*/MICROSOMAL ACTIVATION SYSTEM

J. L. Epler, Jennifer A. Young, A. A. Hardigree, T. K. Rao

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Oak Ridge, Tennessee 37830

Research was sponsored in part by the Environmental Protection Agency (IAG-D5-E681) and in part by the Energy Research and Development Administration under contract with Union Carbide Corporation.

2a

BOSTON UNIVERSITY MEDICAL CENTER

BOSTON UNIVERSITY SCHOOL OF MEDICINE

80 EAST CONCORD STREET, BOSTON, MASSACHUSETTS 02118

June 2, 1977

Department of Pathology
Samuel L. Baker M.D. Chairman
Hugues J.-P. Ryser M.D.
Wilcke Bennett M.D.
Vijay Kumar M.D.

Dr. Bruce N. Ames
Department of Biochemistry
University of California, Berkeley
Berkeley, California 94720

Dear Bruce:

I am just returning from the public hearings staged by the ACS on smoking and health at which I presented your and E. Yamasaki's data on the mutagenicity of urines. The Chairman of the session, David Baltimore, made the thanks of the Blue Ribbon Commission to you a part of the record and was very appreciative of your generosity in making these lovely data available. The testimony drew attention from two reporters who were briefed in more details about your work. If they write anything sensible, I shall send it along to you. Enclosed is a brief written summary of my testimony.

Looking up reference #32 of your preprint, I found a minor typing error. Cancer Res. 35 came out in 1975 (not in 1974). Perhaps there is still time to correct it before it goes to print.

Again, many thanks

Sincerely,

Hugues J.-P. Ryser, M.D.
Professor of Pathology and
Pharmacology

HJPR/mah

PS. Thanks also to D. Yamasaki.

2a

Dr. H. J.-P. Ryser's Testimony
Blue-Ribbon Committee on Tabacco and Health, American Cancer Society
Boston, June 2, 1977

Cigarette Smokers have mutagenic urine: What does it mean?

Data gathered in a number of laboratories in the last 15 years explain how a carcinogen can cause mutations and how mutations caused either by radiation or chemicals can lead to cancer (1). Carcinogens or their close derivatives bind covalently to cellular DNA and lead to errors in DNA replication, which are passed along to daughter cells as somatic mutations. It had long been suspected that the slow process by which a normal cell becomes a cancerous tissue begins with a somatic mutation. To be potentially carcinogenic, mutations must confer a growth advantage to the mutated cell (1). This theory of carcinogenesis has been recently strengthened by the finding that carcinogens are mutagens. Ames and co-workers have found that 157 of 175 carcinogens tested cause mutations in one or several strains of bacteria (2). Among these 157 compounds there is a rough correlation between mutagenic potency in bacteria and carcinogenic potency in animals.

The Ames Test allows one to examine pure compounds as well as complex mixtures such as cigarette smoke condensates. It comes as no surprise that cigarette smoke condensate is strongly mutagenic in two strains of bacteria (3). It has been known for a long time that tobacco tar can cause a variety of cancers in several animal species. There is overwhelming epidemiologic evidence that it causes cancer in man. Heavy smokers present a significantly increased incidence of cancers of the lung, larynx, esophagus, stomach, bladder, kidney and pancreas. The latter three localizations indicate that carcinogens of tobacco tar are absorbed from the lung and enter the general circulation. The most recent data from Ames' laboratory further prove this point. They demonstrate that the urine of heavy smokers is mutagenic while the urine of nonsmokers is not (4).

Sandia Laboratories
Albuquerque, New Mexico 87115

April 7, 1977

Dr. Bruce Ames
Department of Biochemistry
University of California
Berkeley, California 94720

Dear Dr. Ames:

Thank you very much for your prompt response to our sludge mutant problem. We appreciate your taking the time to help us out.

I should also like to request a set of Salmonella tester strains. We shall be using them to test the components of various heat-transfer fluids intended for use in domestic solar heating and cooling (SHAC) systems as part of an environmental study of SHAC for ERDA's Division of Environmental Control Technology.

Thank you for your consideration.

Sincerely yours,

Sieglinde Neuhauser

Sieglinde Neuhauser
Biosystems Studies
Division 5441

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
ARCTIC ENVIRONMENTAL RESEARCH LABORATORY
COLLEGE, ALASKA 99701

January 21, 1977

Dr. Bruce Ames
Biochemistry Department
University of California
Berkeley, CA 94720

Dear Dr. Ames:

Our laboratory is involved in a project entitled, "Mutagenicity of Intermediates of Petroleum Degradation." This project was started at Colorado State University and is being continued at the University of Alaska. The five standard tester strains of Salmonella typhimurium suggested for environmental screening studies were obtained from your laboratory by Colorado State University. Characterization of the strains after transfer to the University of Alaska on soft agar indicates that the R-factor has been lost from strains TA98 and TA100. Both strains are sensitive to 10 mcg. ampicillin sensitivity disks. TA100 is no longer reverted by methyl methanesulfonate. Background counts for the strains without R-factors are difficult to maintain within the established limits.

We have consulted your laboratory by phone and it was suggested that we obtain a new set of the standard tester strains TA98, TA100, TA1535, TA1537, and TA1538. Please send us these strains so we may continue our research project. We have the reprints you sent previously. Thank you for your cooperation in this matter.

Sincerely,

Ruth F. McFadden

Ruth F. McFadden, Chief
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Ford Motor Company

Scientific Research Laboratory
Chemistry Dept.

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February 8, 1977

Prof. Bruce N. Ames
Department of Biochemistry
University of California
Berkeley, California 94720

Dear Prof. Ames:

We would appreciate receiving the Salmonella strains for the mutagenesis test as described in your general letter of May 28, 1976.

The test will be conducted by Ms. Patricia Mucci, a research technician with considerable microbiology experience, using the microbiology laboratory at Mary grove College under the supervision there of Sister Mary Reuter.

This experiment is a senior research project for Ms Mucci, and is also being run to give Scientific Research Laboratory personnel experience with the assay.

Thank you very much for this courtesy. Please send the sample to me at the above mailing address and include in the address: Chemistry Dept., Scientific Research Laboratory.

Yours truly,

Irving Salmeen

Irving Salmeen, Ph.D.
Chemistry Dept.

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March 31, 1977

Professor B.N. Ames
Biochemistry Dept.
University of California
Berkeley, California 94720

Dear Dr. Ames :

I am a Ph.D. candidate at the Seoul National University in Korea and also work as a researcher at the Molecular Biology Laboratory of Korea Atomic Energy Research Institute. For last two years, I have been working on environmental mutagens with your Salmonella TA strains which you had provided to Dr. Se Yong Lee, the head of our Molecular Biology Laboratory.

For my Ph.D. thesis, I work on chemical mutagenesis in S. typhimurium. For this work, I need some additional your Salmonella strains. I would appreciate if you could provide me with hisD3052 TA1534, and TA1975 strains. These strains will be very helpful for my research. Dr. Kwang Woong Lee, an exchange professor from Seoul National University at the Botany Department of Berkeley campus will contact with you for the strains. He is going to send me as soon as he get the straine.

I thank you for your help and time.

Sincerely yours,

Woo-Hyeon Byeon

Woo-Ryeon Byeon
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Molecular Biology Lab.

CHEMICAL INDUSTRY INSTITUTE OF TOXICOLOGY

CIIT

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PRESIDENT, LEON GOLBERG, M.B., D.Sc., D.Phil.

22 March 1976

Dr. Bruce N. Ames
Department of Biochemistry
University of California at Berkeley
Berkeley, California 94720

Dear Dr. Ames,

Just a brief note to thank you most sincerely for sending me copies of reprints of your very interesting publications. These have already proved useful and I am sure will continue to be so.

I looked for you at the meeting on the Environmental Mutagen Society, but unfortunately missed seeing you. In my presentation on Sunday afternoon, I reported the very great value that industry is placing upon the use of your test as a screening procedure. I emphasized that this test was proving a real boon. I can assure you that I shall encourage all members of the Chemical Industry to make use of this procedure.

I should like to congratulate you on the "FASEB Award", which is a just recognition of your splendid contribution in the field of Mutagenesis. I look forward to the time when the world of Chemistry accords you some similar mark of distinction and appreciation.

I do hope that we shall meet again soon.

Yours very sincerely,

Leon Golberg

Leon Golberg, M.B., D.Sc., D.Phil.
President

LG:mkb

Southwest Texas State University
San Marcos, Texas 78666

BIOLOGY DEPARTMENT

March 17, 1977

Dr. Bruce Ames
Biochemistry Department
University of California
Berkeley, California 94720

Dear Dr. Ames:

Would it be possible to obtain samples of your Salmonella typhimurium strains TA 1535, TA 1537, TA 100, and TA 98? I am interested in attempting to apply your mutagenicity test system to water quality analysis. Specifically, I would like to test for the presence of mutagenic activity in concentrated samples from two nearby waterways contaminated with industrial effluents. Concentration of the water samples will initially be carried out by lyophilization. If positive results are obtained, I hope to test other water samples from the refinery areas of the Texas Coastal region. Recently, a similar approach was used to demonstrate the presence of mutagenic activity in Cincinnati water samples (Mut. Res. 38(6): 389, 1976).

I am prepared to check out the strains and commence work immediately. I have all necessary culture supplies and equipment, as well as Aroclor 1254 for induction of rat liver enzymes. I have, in addition, access to a Revco freezer for storage of stock cultures. You may be assured that I will gratefully acknowledge receipt of these strains in any manuscript resulting from this work.

Thank you.

Sincerely yours,

Roger P. Brown

Roger P. Brown
Assistant Professor of Biology

JANSSEN PHARMACEUTICA

naamloze vennootschap

research laboratoria

Bruce N. Ames
Biochemistry Department
University of California
Berkeley
California 94720 (U.S.A.)

2340 Beerse, April 27, 1977.

Dear Mr. Ames,

We are going to integrate the Salmonella/microsome in vitro test into our routine toxicological animal test systems for the evaluation of the potential risk of novel chemical substances and new drugs synthetisized in our laboratories.

We had the opportunity to take benefit from a workshop on "the Ames test" which was held last month at the University of Louvain under the leadership of Dr. Malaveille from Lyon.
From Miss C. Schreiner, McNeil, N.J. we received the principal tester strains: TA 98 - TA 100 - discs in soft agar and lyophilised strains of G 46, TA 1535-1537-1538.

According to your recommendations in Mutation Research 31 (1975) we would very much appreciate if you could forward us with the newly improved strains together with more special recommendations for applying the test in a general screening programme.

Sincerely yours,

Dr. R. Marsboom

TEL. 014/61 14 31 - 613031 - TELEX 32540 - CABLES: JANSSENRESEARCH - BELGIUM

RUHR-UNIVERSITÄT BOCHUM
ABTEILUNG FÜR BIOLOGIE
LEHRSTUHL FÜR BIOLOGIE DER MIKROORGANISMEN

ABTEILUNG FÜR BIOLOGIE
LEHRSTUHL FÜR BIOLOGIE DER MIKROORGANISMEN
POSTFACH 10 21 48, 4630 BOCHUM 1

BOCHUM-QUERENBURG
UNIVERSITÄTSSTRASSE 150
POSTFACH 102148
GEBÄUDE: NDEF-06
TEL.: (0234) 700-
TELEX: 0825860

3100/Wi/Mi

DEN 20.12.1976

AZ:

Prof. Bruce N. Ames
Biochemistry Department
University of California

Berkeley/Calif. 94720

U.S.A.

Dear Colleague:

I would appreciate very much obtaining the following strains for mutagenicity test. You recommended these strains in your paper published in Mutation Research 34, 347-364 (1975):

TA 1535	TA 98
TA 1536	TA 100
TA 1537*	
TA 1538	
TA 1975	
TA 1977	
TA 1978	

non aromatic rings

We are just beginning to work on the microbial oxidation of alicyclic hydrocarbons. We would like to test the chemicals we are dealing with for mutagenicity. If you have any suggestion for using some additional strains for the tests we would appreciate them. Thank you very much for your help.

Furthermore, I am kindly asking you for reprints and to put my name on your mailing list for reprints regarding this subject.

With very best wishes for the Christmas season and for the New Year!

Uli Winkler

(Prof. Dr. U. Winkler)

*or its derivative with the R-factor

THE UNIVERSITY OF TEXAS SYSTEM
CANCER CENTER

Texas Medical Center Houston, Texas 77030

January 24, 1977

Department of Information and Publications

Doctor Bruce N. Ames
Biochemistry Department
University of California
Berkeley, California 94720

Dear Doctor Ames:

We are compiling the Year Book of Cancer 1977, a publication designed to bring both general practicing physicians and specialists comprehensive abstracts of the most significant articles on cancer from the preceding year. It is published in cooperation with Year Book Medical Publishers.

The editors of the section on Biochemistry, under the direction of Doctor Jorge Awapara, have chosen one of your articles for inclusion. It is:

> Ames, B. N., J. McCann, and E. Yamasaki: Methods for detecting carcinogens and mutagens with the salmonella/mammalian-microsome mutagenicity test. Mutat. Res 31(6): 347-363, December, 1975.

We would appreciate your preparing an abstract of this article, no more than 500 words long, and double spaced. Please include a list of definitions of any abbreviations used and three reprints of the original article.

Fig. 1 & Fig. 2 Yes

You may select two pictures to illustrate the abstract, either glossy black and white photographs or the original art work, which will be returned after publication. Captions should be about 25 words long.

We hope this arrangement will meet with your approval. Your prompt attention will assist us in making the Year Book of Cancer of current interest. We should appreciate having the abstract within the next 2 weeks. Would you please let us know if this is agreeable?

Sincerely,
Elaine Tallman
Editor, for
R. W. Cumley, Ph.D.
Executive Editor

Section 26

M. D. ANDERSON HOSPITAL AND TUMOR INSTITUTE Rehabilitation Center
EXTRAMURAL PROGRAMS DIVISION Oncology Council-Biomedical Institutions Collaborative Studies
Substations Environmental Science Park
UNIVERSITY CANCER FOUNDATION The Anderson Mayfair

Ames, B.N., J. McCann, and E. Yamasaki: Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutation Research 31(6): 347-363, December, 1975.

ABSTRACT

We have previously described a very sensitive and simple bacterial test for detecting chemical mutagens which has recently been reviewed (McCann & Ames, in Origins of Human Cancer, eds. H. Hiatt and J.D. Watson, Cold Spring Harbor Laboratory, N.Y.). The compounds are tested on petri plates with several specially constructed mutants of Salmonella typhimurium selected for sensitivity and specificity in being reverted from a histidine requirement back to prototrophy by a wide variety of mutagens.

The test has been adapted for use in detecting chemicals which are potential human carcinogens or mutagens by adding homogenates of rat (or human) liver directly to the petri plates thus incorporating an important aspect of mammalian metabolism into the in vitro test. In this way, a wide variety of carcinogens requiring metabolic activation can be detected easily as mutagens.

There is considerable evidence, much of it obtained using this test that with few exceptions carcinogens are mutagens. This supports the desirability of using this type of rapid and economical test system as a screening technique to pinpoint potentially dangerous chemicals among the thousands of chemicals to which humans are exposed. The test complements the traditional expensive two year rodent cancer tests as it can be used to test thousands of chemicals under development and also complex mixtures, uses that are impractical with the animal tests.

The test has been validated by examining 300 chemicals (Proc. Nat. Acad. Sci. USA 72, 5135; 73, 950) and we have found that 90% fo the carcinogens are detected by the system described in this paper and that few "non-carcinogens" are mutagens.

THE UNIVERSITY OF TENNESSEE
KNOXVILLE, TENNESSEE 37916

AREA CODE 615
TELEPHONE 974-3005

GRADUATE PROGRAM IN ECOLOGY
408 10th Street

January 26, 1977

Dr. Bruce Ames
Biochemistry Department
University of California at Berkeley
Berkeley, California 94720

Dear Dr. Ames:

We are planning investigations into the microbial degradation of polychlorinated bipphenyls (PCB) in the natural environment. One of the aspects we would like to investigate is the mutagenic potential of transformation products of this degradation. We would like to obtain several of your stable Salmonella typhimurium strains for the purpose of screening some of the degradation products for mutagenic agents. We are also interested in receiving any advice and reprints on the experimental procedure itself. Of course, we will be happy to follow any standard procedure for obtaining and using your test strains and methods. We greatly appreciate your cooperation.

Sincerely,

Lisa Lund

Lisa Lund
Research Assistant

LL:tt

cc: Dr. Gary Sayler

PURDUE UNIVERSITY SCHOOL OF CIVIL ENGINEERING

January 18, 1977

Dr. Bruce Ames
Biochemistry Department
University of California
Berkeley, CA 94720

Dear Dr. Ames:

I have followed with great interest your development of tester strains for detection of mutagenic activity of diverse chemical compounds. I am a microbiologist teaching microbiology to environmental engineering students and doing research in the general area of water pollution microbiology at Purdue. My associate, Dr. Leslie Grady and I are codirecting graduate student research related to the quality of wastewater treatment plant effluent prior to and after disinfection and its impact on the aquatic environment. We would like to use the Salmonella reversion test as part of our evaluation of effluent quality and would appreciate obtaining from you the four tester strains TA 1535, TA 100, TA 1537 and TA 98 for this purpose.

Some years ago (about 15) when I worked in the biochemical research section of Lederle Laboratories at Pearl River, NY I used the E. coli SD-4 streptomycin dependent strain to study radiomimetic effects of a variety of antibiotics and other industrial compounds. We recognized the partial correlation between mutagenicity and carcinoginicity but were not wise enough to pursue this relationship and try to improve the correlation as you have done. You and your associates are to be congratulated.

Thank you for your assistance.

Sincerely,

E.J. Kirsch

E.J. Kirsch
Professor
Environmental Engineering

EJK/mk

Civil Engineering Building
West Lafayette, Indiana 47907

מדינת ישראל, משרד ראש הממשלה
State of Israel, Prime Minister's Office

ISRAEL INSTITUTE FOR BIOLOGICAL RESEARCH
TEL AVIV UNIVERSITY MEDICAL SCHOOL
P. O. B. 19, Ness-Ziona

המכון למחקר ביאולוגי בישראל
בית הספר לרפואה, אוניברסיטת תל-אביב
ת. ד. 19, נס-ציונה

8 March , 1977
17/296

Dr. Bruce N Ames
Biochemistry Department
University of California
Berkeley, California 94720
U S A.

Dear Dr. Ames,

We are starting in our laboratory a new program for screening for carcinogens in industrial materials.

Referring to your paper in Mutation Research 31 (1975), we would very much appreciate receiving tester strains from you: (TA 1535, TA1537, TA1538, TA1978, TA100, TA98) and any other you consider appropriate.

We will be happy to communicate to you our results for inclusion in your running compilation of the chemical tested on the strains.

Yours very sincerely,
Renana Ben-Gurion
Dr. Ranana Ben-Gurion

PULP AND PAPER RESEARCH INSTITUTE OF CANADA
570 ST. JOHN'S BOULEVARD, POINTE CLAIRE, PQ, CANADA H9R 3J9
TEL. (514) 697-4110 CABLE: PAPRICAN TELEX 05-821541

April 18, 1977.

Dr. Bruce N. Ames,
Biochemistry Department,
University of California,
Berkeley, California.

Dear Dr. Ames:

I listened to your paper at the last FASEB meeting in Chicago and became interested in applying your method of detection of carcinogen suspects to pulp mill effluents and to some chemicals used in the pulp and paper industry.

I would appreciate it if you could send me reprints of your recent papers describing the methods you use for determination of chemical mutagenicity.

Yours sincerely,
L. Jurasek
L. Jurasek
Supervisor
Microbiology Group
Process Chemistry Division

LJ:bmd

MAIN LIST
SENT
4/27/77
GJH

New York State Department of Environmental Conservation
50 Wolf Road, Albany, New York 12233

Peter A. A. Berle
Commissioner

March 3, 1977

Dr. Bruce N. Ames
Biochemistry Department
University of California
Berkley, California 94720

Dear Dr. Ames:

Our office is currently preparing a technical handbook for use by our field air pollution control staff in regulating toxic air contaminants. We have found your article "Detection of Carcinogens as Mutagens in the Salmonella/microsome Test: Assay of 300 Chemicals, December 1975 Proceedings of the National Academy of Science", useful in identifying chemical substances as carcinogens.

With your permission, we would like to reprint Table 1 of your article in our Air Pollution Control Handbook. This handbook is intended solely for the purpose of providing technical information and guidance for our air pollution control staff.

If available, we would appreciate receiving an up-to-date copy of your latest version of Table 1 if it has been revised. We would perfer a copy having larger print than the original article for reproduction.

Thank you for consideration of this request.

Sincerely,

Jack D. Lauber

Jack D. Lauber
Chief-Toxic Materials and Effects Section

JDL:ng

Susan
OK with me
Could you handle this

Central Toxicology Laboratory

Alderley Park
Nr. Macclesfield Cheshire
SK10 4TJ

PLEASE NOTE NEW TELEPHONE NUMBER AND CODES
FROM 4th SEPTEMBER 1976
(0625) 582711
for local codes see dialling instruction book

Imperial Chemical Industries Limited

Professor B Ames,
University of California,
Department of Biochemistry,
Berkley,
California 94720,
USA.

Your ref	Our ref	Tel ext	Date
	EL/AJD	141	3 Feb 77.

Dear Bruce,

Just a few lines to say how much I enjoyed meeting you at Wilmington and hope you found our discussions concerning testing of gases as interesting and stimulating as I did.

Now that we have confirmed the data generated with R-22 and that you approve of the protocol used I shall try and publish a short note somewhere describing the findings.

Please let me know how your colleagues at Berkley feel about the significance of the work in regard to human risk.

With kind regards,

Yours sincerely,

E Longstaff,
PhD.

GENERAL ELECTRIC

GENERAL ELECTRIC COMPANY, RESEARCH AND DEVELOPMENT CENTER, P.O. BOX 8
SCHENECTADY, NEW YORK 12301, Phone (518) 385-2211

CORPORATE
RESEARCH AND
DEVELOPMENT

Building K-1, Room 7B46
May 18, 1977

Prof. Bruce N. Ames
Biochemistry Department
University of California
BERKELEY, CA 94720

Dear Dr. Ames:

We are planning to initiate some studies regarding an evaluation of carcinogenic potential of various industrial compounds (PCBs, monomers of plastics, etc) at the General Electric Research & Development Center.

We would appreciate it very much if you could send us the following Salmonella typhimurium strains: TA 1535, TA 1537 TA 1538, TA 98, TA 100. We would, of course, be very happy to pay for any charges for these cultures.

Thank you.

Sincerely yours,

A. M. Chakrabarty

A. M. Chakrabarty
PHYSICAL CHEMISTRY LABORATORY

AMC:dc

UNIROYAL

UNIROYAL Ltd.
Research Laboratories

120 Huron Street,
Guelph, Ontario

Telephone: (519) 822-3790

April 18, 1977.

Dr. Bruce N. Ames,
Department of Biochemistry,
University of California at Berkeley,
BERKELEY, California 94720,
U. S. A.

Dear Dr. Ames:

I would very much appreciate cultures of the four Salmonella Typhimurium strains (TA1535, TA100, TA98 and TA1537) which I understand make up the current standard tester set for mutagen detection. If the R factor containing replacement strain for TA1537 is available (indicated in your paper in Mutation Research 31 347 (1975) as being under development) I would appreciate receiving it as well.

We will be using the above tester set for in-house testing of promising agricultural chemicals in the early stages of development.

Thank you for your consideration of this request.

Respectfully yours,

C Hugh Drennan

C. Hugh Drennan, Ph.D.
R/D Biochemist.

CHD:mf

c.c. BAG