MASTER

COMPARATIVE MUTAGENESIS

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II. ABSTRACT: Our major research efforts have been on the mutagenicity of certain ICR-compounds and on studies in environmental mutagenesis. From a comparison of the mutagenicity of 16 ICR-compounds, we concluded that Neurospora crassa is a better predictor than Salmonella typhimurium of anti-tumor and carcinogenic potencies in mice. Streptonigrin induced a high frequency (25%) of multilocus deletions at the ad-3 region of N. crassa. Most of the water concentrates prepared from Lake Bloomington, IL were weakly mutagenic in the Salmonella/microsome test; one concentrate was highly mutagenic in strain TA100. Chemical analysis of the highly mutagenic concentrate revealed the presence of a number of organic compounds that were absent or very low in the weakly mutagenic concentrates. Cigarette smoke condensate and certain fractions of the condensate were mutagenic in N. crassa, Saccharomyces cerevisiae, and Drosophila melanogaster. In D. melanogaster, cigarette smoke also was mutagenic. In N. crassa, cigarette smoke condensate appeared to be direct acting. Sodium azide was not mutagenic in N. crassa at pH values of 3 to 8, and there were no significant differences in the induced reversion frequencies over this pH range in S. typhimurium.

III. PROGRESS REPORT: The Proposed Technical Program of October, 1976¹ indicated that our major research efforts within the area of comparative mutagenesis would be on studies of the mutagenicity of certain ICR-compounds and on studies in environmental mutagenesis. Four specific areas of investigation were proposed. Our results in each of these areas are summarized below.

1. Relationship of the mutagenic activity of 16 ICR-compounds in Neurospora crassa to their mutagenic activity in Salmonella typhimurium and to their antitumor and carcinogenic activities in mice:

Creech and coworkers have synthesized and studied over 80 ICR-compounds for their ability to induce reversion in a histidine-requiring frameshift mutant of S. typhimurium and to possess antitumor activity against ascites tumors in mice². We have studied the ability of 16 of these compounds to induce reversion in an ad-3 mutant of N. crassa that is a presumptive frameshift^{3,4}. Therefore, for these 16 compounds it is possible to compare their mutagenic activity in S. typhimurium, their mutagenic activity in N. crassa, and their antitumor activity in mice. We have done so^{3,4}, and two major conclusions are that there are differences in the relative mutagenic activities of some of the compounds between N. crassa and S. typhimurium and that there is a better association between mutagenic activity in N. crassa and antitumor activity in mice than between mutagenic activity in S. typhimurium and antitumor activity in mice.

Recently, the carcinogenic activity of some of these ICR-compounds has been reported⁵. Thus, the ICR-compounds are a unique set of agents in that they are structurally related compounds for which four kinds of biological activities have been and are being determined -- mutagenic activity in a prokaryote, mutagenic activity in a lower eukaryote, and antitumor activity and carcinogenic activity in mice. The positive association generally found between mutagenic and carcinogenic activities is now well

documented^{6,7}. On the other hand, the mutagenic activity of antineoplastic agents has not been studied extensively^{8,9}. The suspension method we have used in reversion studies of an ad-3 mutant of N. crassa³ is highly quantitative, but it is rather time consuming. A plate-incorporation test for reversion, such as is used routinely in bacteria and yeasts, was not developed for N. crassa until recently¹⁰. We felt that if we could use this plate-incorporation test we would be able to better determine the mutagenic activity of the ICR-compounds in N. crassa and to relate that activity to the other activities known for these agents from the work of others, as mentioned above.

Therefore, we proposed¹ to determine the validity of the plate-incorporation test for detecting reversion induced by ICR-compounds in N. crassa. We proposed to do this by studying the 16 ICR-compounds that we had reversion data on from our suspension test. We proposed to use the strain that we had used mainly in our previous suspension reversion tests with these agents (an ad-3 mutant that is a presumptive frameshift) and the ad-3 mutant that Ong¹⁰ used in his plate-incorporation test, which is also a presumptive frameshift mutant. Because conidia are exposed to a mutagen much differently in a suspension test compared to a plate-incorporation test, we did not expect the reversion frequency induced by a given ICR-compound to be the same in both tests; but what we wished to determine was whether the ranking of the mutagenic activities of the 16 ICR-compounds would be the same in the plate-incorporation test as in the previously studied suspension test. If it was, we would have good evidence that the plate-incorporation test with these mutants is a valid test for determining the relative mutagenicities of the ICR-compounds. We would then be able to use the plate-incorporation test to study the mutagenic activities of a much larger number of this unique group of structurally related compounds, as well as other chemicals. We were

especially interested in determining what association, if any, there is between mutagenic activity in N. crassa and antitumor and carcinogenic activities in mice. Furthermore, by comparing these three activities to the mutagenic activity in S. typhimurium, we would be able to determine whether N. crassa or S. typhimurium is a better predictor of antitumor and carcinogenic activities of these compounds in mice.

In the paper on the antitumor and mutagenic activities of the ICR-compounds that we are studying, the mutagenicity of these compounds was studied only with a spot test in only one strain of S. typhimurium². Since this paper was published in 1972, Ames and coworkers have developed what are now recognized as the standard strains to use in the Salmonella/microsome test¹¹. Therefore, we decided that we also should determine the mutagenicity of the 16 ICR-compounds in six of the standard Ames' strains using the standard plate-incorporation test¹¹. We have completed this extensive analysis, and a preprint has been prepared¹². The Abstract from this paper (see below) summarizes the most important findings. In the Discussion of this paper, we discuss extensively the interesting structure-potency relationships that we discovered among the 16 ICR-compounds.

Sixteen heterocyclic mustards (ICR compounds), which were synthesized for use as possible antitumor agents by Creech and coworkers, were tested for mutagenicity in Salmonella typhimurium strains TA1535, TA1536, TA1537, TA1538, TA98, and TA100. The compounds were incorporated into the top agar at 5 doses--0.5, 1, 2.5, 5, and 10 µg/plate. All of the compounds were negative in TA1535 except ICR 449, which was positive in all 6 strains. The other 15 compounds were positive in the remaining strains with the following exceptions: ICR 371, 355, and 170 were negative in TA100; ICR 445 was negative in TA98 and TA100; and ICR 360 was negative in TA1537, TA1538, TA98, and TA100. Good qualitative agreement was observed between the mutagenic and antitumor potencies of the 16 compounds, and between the mutagenic and carcinogenic potencies of the 5 compounds that have been tested for carcinogenicity by Peck and coworkers. However, no significant correlation was found between mutagenic potency in Salmonella and antitumor potency in mice for the 16 compounds. Also, for the 5 compounds that have been tested for carcinogenicity, no significant correlation

was found between their mutagenic potency in Salmonella and their carcinogenic potency in mice. In Salmonella, the secondary (2°) amines were generally more mutagenic than their tertiary (3°) amine homologs, although the opposite result has been reported in eukaryotes. Structure-potency relationships for the different nuclei of the 16 ICR compounds are discussed. We conclude that the Salmonella test is not as good a predictor as eukaryotic mutagenicity tests of the antitumor and carcinogenic potencies of the ICR compounds.

We also have completed the proposed study on the reversion of two presumptive frameshift mutants of N. crassa with the 16 ICR-compounds. In this study, the frameshift mutant of S. typhimurium used in the original study of Creech et al.² and a standard presumptive base-pair substitution mutant of N. crassa¹⁰ also were included. A thesis and a paper on the results of this study are being prepared; extensive discussions of the associations between mutagenicity in N. crassa, mutagenicity in S. typhimurium, antitumor activity in mice, and carcinogenicity in mice will be included. Therefore, they will not be discussed in detail here. The two most important conclusions are that: (i) there is a positive correlation between the rank of mutagenic potencies in N. crassa in the plate-incorporation test used in the present study and the suspension test used in our previous studies of the 16 ICR-compounds. This means that we can use the plate-incorporation test, which is less time-consuming and complex than the suspension test, in future reversion studies in N. crassa, (ii) Comparisons for the 16 ICR-compounds for their mutagenic potencies in N. crassa and S. typhimurium with their antitumor and carcinogenic activities in mice show that N. crassa is a better predictor than S. typhimurium of antitumor and carcinogenic potencies in mice. The Abstract of a paper¹³ presented at a meeting (see below) summarizes the major findings from the research on N. crassa.

A reverse-mutation system similar to the Ames Salmonella test has been developed using adenine auxotrophs of Neurospora crassa (T. M. Ong, Mutation Res., in press). Sixteen ICR-compounds were tested for mutagenicity at various doses in

two frameshift and one base-pair substitution ad-3 mutants of N. crassa. These 16 compounds were tested at various doses with Salmonella typhimurium strain hisC207 as an internal control for mutagenic potency, because the compounds were tested previously in a spot test with this strain (Creech et al., J. Med. Chem. 15:739, 1972). For each Neurospora and Salmonella mutant, the ICR-compounds were ranked from least to most mutagenic using several parameters obtained from the dose-response curves. Statistical analyses show no significant correlation between the Salmonella and Neurospora rankings; however, there was a significant positive correlation between the rankings of the Neurospora frameshift mutants. Statistical correlations of anti-tumor, carcinogenicity, and mutagenicity rankings for the 16 ICR-compounds will be discussed.

2. Mutagenic activity and specificity of streptonigrin at the ad-3 region of N. crassa:

The antibiotic and antineoplastic agent streptonigrin has been reported to show an anoxia effect for chromosome breakage in Vicia faba¹⁴. We proposed¹ to study various doses of streptonigrin in order to determine whether there is an anoxia effect for forward mutation at the ad-3 region and for killing in N. crassa. To our knowledge, the type(s) of mutations caused by streptonigrin had not been determined. Therefore, we proposed¹ to characterize ad-3 mutants induced by streptonigrin in N. crassa. The relative frequencies of multilocus deletions (ad-3^{IR}) and point mutations (ad-3^R) indicate the chromosome breaking and intragenic mutagenic activities of this agent¹⁵. The frequencies of leakiness, allelic complementation, and non-polarized complementing types indicate the relative frequencies of base-pair substitutions and base-pair additions and deletions¹⁵.

We have completed this study, and a thesis and paper are being prepared. The Abstract of a paper¹⁶ presented at a meeting (see below) summarizes the major findings from this research.

The antibiotic and antineoplastic agent streptonigrin (SN) causes greater lethality in the presence than in the absence of O₂ in bacteria (White and White, 1968, Mol. Pharmacol. 4:549). SN is a potent inducer of chromosome aberrations in cultured human leukocytes (Cohen et al.,

1963, Proc. Nat. Acad. Sci. USA 50:16) and Vicia faba (Kihlman, 1964, Mutation Res. 1:54). In V. faba, SN induced more aberrations under an anoxic condition than when O₂ was present. In the present work, the killing and mutagenic activities of SN were studied by suspending conidia from a two-component heterokaryon (heterokaryon-12) of Neurospora crassa in a 0.1M, pH 7 phosphate buffer and bubbling with O₂ or N₂ gas 2 hr before and 4 hr during exposure to SN (60, 120, 180, 360, and 600 μ M). The killing or mutagenic activities of SN were not appreciably different in the two gases. A sample of ad-3 mutants was analyzed from a dose of SN (360 μ M for 4 hr) in the presence of O₂ that resulted in 29% survival and 135 ad-3 mutants/10⁶ survivors. A high frequency (25%) of the ad-3 mutants were multilocus deletions. Among the ad-3 mutants, 34% exhibited allelic complementation, and among these complementing ad-3B mutants, 79% had nonpolarized complementation patterns.

3. Possible mutagenic activity of water samples from Lake Bloomington:

The major effort of researchers in the area of environmental mutagenesis has been to determine whether individual chemicals that are known or suspected of being in the environment are mutagenic. Certainly this effort is needed and is identifying specific environmental mutagens that may be a hazard to the genetic material of man and other organisms. We indicated in previous proposals that we thought an additional kind of research effort is needed in this area. Man and other species are in an environment that contains a complex mixture of chemicals -- mutagens and carcinogens, some of which may show synergistic effects, as well as activators and inactivators of mutagens and carcinogens. Even if we knew the exact chemical composition of the air we breathe, the food we eat, and the water we drink; we could not predict the total mutagenic or carcinogenic potency of the mixture because of the complex interactions that might occur. Therefore, we have conducted experiments with the objective of being able to assay samples of our environment in order to determine if any of them have mutagenic activity.

We decided to initiate studies of water samples first for three main

reasons. First, there is considerable evidence that carcinogenic chemicals are present in rivers and lakes, and that they are also in the drinking water taken from these water sources (see reference 17 for a 1975 report that has some of the appropriate references). Second, we expected that the lake used for the water supply of Bloomington, Illinois would contain farm chemicals, as discussed below. Third, the work that we did a few years ago on the effect of incubating conidia of N. crassa for extended periods in distilled water provided necessary background information for the study of experimental water samples in N. crassa.

Lake Bloomington, which was created by damming a tributary of the Illinois River, provides the drinking water for Bloomington, Illinois. The Lake is located in a region that is farmed intensively -- corn and soybeans being the two main crops. The application of herbicides, insecticides, and other biocides to this farm land is an established agricultural practice. Runoff from this farm land provides most of the water for Lake Bloomington. Therefore, we proposed in 1975 to test samples of water from this Lake before and after treatment at the Bloomington Treatment Plant for mutagenic activity. We reported in a previous Progress Report¹⁸ on our results during 1976 with resting conidia and growing cultures of N. crassa and to a lesser degree with mitotic gene conversion in Saccharomyces cerevisiae. We proposed to follow the same general procedure as reported in 1976¹⁸ for collecting the water samples. However, some changes in and additions to the preparation of the samples and the assays for mutagenesis of the water samples were proposed¹⁸. The Abstract from a preprint¹⁹ we have prepared on the 1976 and 1977 results (see below) summarizes our most important findings.

We have used four short-term tests to test for the mutagenicity of the drinking water of Bloomington, IL (pop. 44,000). We have tested the water from Lake Bloomington and the tap water made from it. Different protocols were used with unconcentrated and concentrated water samples in the

four different tests in order to determine which combinations are most suitable for detecting mutagens in drinking water. We tested the ability of the water to induce: reverse mutation in a prokaryote, Salmonella typhimurium; forward mutation in a mold, Neurospora crassa; mitotic gene conversion in a yeast, Saccharomyces cerevisiae; and reverse mutation in maize, Zea mays. The results show that: lake and tap water samples collected during 1976 were toxic but not mutagenic in N. crassa, and neither toxic nor genotoxic in S. cerevisiae; lake water collected during 1977 was mutagenic in one line of Z. mays; and neither lake nor tap water collected during 1977 was mutagenic in S. typhimurium in either the presence or absence of rat liver S-9. We conclude that XAD-2 water concentrates tested in S. typhimurium in the presence and absence of rat liver S-9 permits the most rapid and inexpensive procedure by which to monitor municipal water for mutagenicity. Based on these results, we initiated studies in 1979 using water that was concentrated 3000X with XAD-2 resin and assayed in the Salmonella/microsome test.

Based on these studies in 1976 and 1977, we selected XAD-2 resin as the best method of concentrating the organic chemicals in the water, and we selected the Salmonella/microsome test as the best mutation test for assaying a large number of samples. Furthermore, we decided to supplement the mutation test with analytical chemical analyses. These studies were done in 1979, and an Abstract from a preprint²⁰ is included (see below) for completeness.

We have studied the mutagenicity of the water from Lake Bloomington and of the tap water that is made from the lake water. The lake, which is the source of drinking water for Bloomington, IL (pop. 44,000), is surrounded by land that is farmed intensively--being mainly in maize and soybeans. Samples were collected monthly from May through October of 1979 and concentrated 3000X with XAD-2 resin. Nearly all of the lake and tap water concentrates were mutagenic in Salmonella typhimurium TA98 and TA100 in the presence of S-9 mix, and the May tap water concentrate was highly mutagenic. In addition, most of the concentrates were toxic to the bacteria in the absence of S-9 mix. Chemical analysis of the highly mutagenic tap water concentrate from May revealed the presence of a number of organic contaminants that were absent from control concentrates prepared from deionized and distilled treated well water. In addition, unconcentrated lake and tap water was tested in a reverse-mutation test in maize (Zea mays); no mutagenicity was detected. This study indicates that the contamination of drinking water with agricultural and/or industrial chemicals may result in a potential health hazard.

4. Mutagenic activity of cigarette smoker's urine and of cigarette smoke condensates: In section three above, I indicated that although the identification of individual chemicals that are environmental mutagens is necessary, it is also necessary to attempt to determine the total mutagenic activity of the environment that a given population resides in. The approach indicated in section three to this problem is to develop the techniques needed to assay the water, air, and food components of the environment. Another related approach to the same goal of being able to determine the mutagenic activity of the environment that a population resides in is to assay some part, such as urine or blood, from the people or other animals that are in that environment. In this case, one is actually studying the mutagenic agents taken in by the organism through its food, air, and water after they have been subjected to the metabolism of the organism.

We proposed as a first study of human urine that from heavy cigarette smokers versus nonsmokers as a control. The detection of mutagenic activity in the urine of rats^{21,22,23} and humans²⁴ that had received a carcinogen or procarcinogen had been reported; but study of the urine of humans for mutagenic activity had not been reported when we initiated our study. Because it had been reported that cigarette smoke condensates are mutagenic^{25,26} and because cigarette smoking clearly causes cancer, we thought that a study that would determine if the mutagenic activity of the urine from heavy cigarette smokers differed from that of nonsmokers might provide a useful model for future studies utilizing urine from humans. During the course of our studies, a paper was published²⁷ on an extensive study of the mutagenicity of urine from cigarette smokers. Therefore we decided to emphasize our proposed research on the mutagenicity of cigarette smoke condensate (CSC) in eukaryotes. When we proposed this research¹, two laboratories had reported

that CSC was mutagenic in S. typhimurium^{25,26}. We decided that basic studies on the mutagenicity of CSC would be useful. Because the two published papers on the mutagenicity of CSC were with S. typhimurium, we proposed to emphasize the two lower eukaryotes N. crassa and S. cerevisiae. In S. cerevisiae we proposed to study the reversion of selected auxotrophs. In N. crassa, we proposed to study forward mutation at the ad-3 region. This was proposed because of the obvious advantage of being able to detect all the possible types of genetic alterations at this region. During the course of the study on CSC in N. crassa, we decided that a comparative study of CSC in S. typhimurium would be desirable. This proposed research on the mutagenicity of CSC has been completed. It is summarized below by using the Abstract from a paper²⁸ presented at a meeting and the Summaries from two papers^{29,30} that are in press.

The mutagenicity of whole cigarette smoke condensate (WSC) and nine fractions of WSC was studied in Saccharomyces cerevisiae. The condensates were from the University of Kentucky Reference Cigarette IRI; and the fractions tested were WI, I-I₂, I-I₃, W-G₁, W-G_{2a}, W-G_{2b}, W-A_E, N_{NM}, and EPAH. The condensates were investigated for their ability to revert six frameshift and two base-pair substitution auxotrophs of S. cerevisiae. A semi-quantitative spot-test procedure was employed for the reversion analysis. In the absence of hepatic microsomes, none of the condensates were mutagenic up to 500 µg/plate. However, in the presence of Aroclor 1254-induced rat hepatic microsomes, WSC was mutagenic in all eight strains. This indicated that WSC has both frameshift and base-pair substitution mutagenic activity. The fractions of WSC gave inconsistent results which are being further investigated. The dose response of strain S154 to WSC was also studied using an agar overlay method. Cells, microsomes, and various concentrations of WSC were incorporated into a top agar and poured onto a plate containing slightly enriched minimal medium. The dose response was approximately linear up to 250 µg/plate. Also, the number of WSC-induced revertants was increased 2-3 times by increasing the amount of microsomes from 0.1 to 0.5 ml/plate.

The mutagenicity of cigarette smoke condensate (CSC) was investigated in Neurospora crassa in the presence and absence of S-9 mix prepared from Aroclor-1254-induced rat liver. CSC from the University of Kentucky Reference Cigarette IRI was assayed in a forward-mutation test at the adenine-3 (ad-3)

region in resting conidia of two-component heterokaryons. In the absence of S-9 mix, CSC exhibited direct-acting mutagenicity. CSC was also mutagenic in the presence of S-9 mix, but higher doses were required than in the absence of S-9 mix. The dose range, survival curves, and mutation-induction curves were not significantly different when CSC was used in the presence of unheated or heat-inactivated S-9. There was a positive association between killing and mutagenicity, and CSC killed conidia of N. crassa by a cytoplasmic, rather than by a nuclear, mechanism. The mutagenic potency of CSC was similar in a repair-sufficient and a nucleotide excision repair-deficient heterokaryon of N. crassa. CSC did not exhibit a photodynamic effect for killing, and CSC caused more killing at high pH than at low pH. In addition, CSC caused more killing at 37°C than at 25°C and also caused more killing in higher concentrations (20%) of solvent (DMSO) than in low concentrations (1%). This is the first report of the presence of potent direct-acting mutagens in CSC.

The mutagenicities of selected fractions of cigarette smoke condensate (CSC) were studied in Neurospora crassa for the presence of direct-acting mutagens. CSCs from the University of Kentucky Reference Cigarette 1R1 were assayed in a forward-mutation test at the adenine-3 (ad-3) region in resting conidia of a two-component heterokaryon. Direct-acting mutagenic activity was found in an enriched polycyclic aromatic hydrocarbons (EPAH) fraction and in a basic fraction (Swain 5). No direct-acting mutagenic activity was detected in an acidic fraction (Swain 8), although it was highly toxic to resting conidia. The EPAH fraction also was tested in the presence of S-9 mix prepared from Aroclor-1254-induced rat liver. It was found to be mutagenic, but higher doses were required than in the absence of S-9 mix. In addition, the mutagenicities of CSC and 10 fractions of CSC were investigated in Salmonella typhimurium TA1538 by the incorporation and preincubation methods. In general, preincubation did not enhance the mutagenicities of the fractions, and the two rankings of mutagenic potency of the condensates that were obtained by the two methods were not significantly different. This is the first report of the presence of potent direct-acting mutagenicity in the EPAH and Swain 5 fractions of CSC.

In addition, we studied the mutagenicity of CSC and of cigarette smoke in Drosophila melanogaster. This research is summarized in the Abstract (see below) of a paper³¹ presented at a meeting.

The mutagenicity of cigarette smoke condensate (CSC) has been reported in Salmonella typhimurium (Kier et al., 1974, Proc. Nat. Acad. Sci. USA 71:4159; Hutton and Hackney, 1975, Cancer Res. 35:2461) and Saccharomyces cerevisiae (DeMarini, 1978, Mutation Res. 53:84). In the present work, the mutagenicity of CSC and cigarette smoke was studied in Drosophila melanogaster. Wild-type (Oregon-R) males were exposed to the agent and mated to Basc females, and the frequencies of sex-linked recessive lethals (SLRL) were determined in the F₂. The condensate from a popular brand of cigarettes was collected on filter paper with a cigarette smoking machine. The condensate was dissolved in dimethylsulfoxide; this solution is referred to as CSC. Males that were fed a CSC-sucrose solution as adults had a small increase in the frequency of SLRL over the control frequency, but this increase was not significant. In two experiments, males that were fed CSC equivalent to six-hundredths of one cigarette per 10 ml of food as larvae had a significantly higher frequency of SLRL than the control frequency ($p < 0.05$). Adults that were exposed to two to three puffs of cigarette smoke a day for seven days with a smoking machine had a small increase in the frequency of SLRL over the control frequency, but the increase was not significant. In two experiments, there was a significant increase in the frequency of SLRL over the control frequency ($p < 0.05$) when larvae were exposed to two to three puffs of cigarette smoke per day. To my knowledge, this is the first report of the mutagenicity of CSC in Drosophila and of the induction of mutations by cigarette smoke.

5. Effects of pH on the mutagenicity of sodium azide in Neurospora crassa and Salmonella typhimurium: During this contract period, we decided to do a comparative mutagenesis study on an important environmental chemical, sodium azide. This research has been published³²; the Summary (see below) from this paper summarizes the results.

Sodium azide at various pH values did not cause a significant increase in the frequency of forward mutation above the control frequency at the adenine-3 (ad-3) region in resting conidia and in conidia from growing cultures of heterokaryons 12 and 59 of Neurospora crassa. Conidia from ad-3 mutants were plated with sodium azide at various pH values, and no obvious increase in reverse mutation above the controls was observed. Data are presented showing that sodium azide at pH 3 is inactivating conidia by interacting with the cytoplasm rather than the nucleus, and this may be the primary reason that no mutation at the ad-3 region was detected. The dependence of sodium azide mutagenicity on pH was investigated in histidine-requiring mutants of Salmonella typhimurium using

a suspension test. There were no significant differences in the reversion frequencies among the pH values (3-8) tested. Thus, no pH dependence is associated with sodium azide mutagenicity, nor are growth and/or DNA replication required for mutagenicity by sodium azide, in S. typhimurium.

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31. Pescitelli, A. R. 1979. Mutagenicity of cigarette smoke condensate and cigarette smoke in Drosophila melanogaster. *Environmental Mutagenesis* 1:118. (Abstr.)
32. Tomlinson, C. R. 1980. Effects of pH on the mutagenicity of sodium azide in Neurospora crassa and Salmonella typhimurium. *Mutation Res.* 70:179-191.

IV. PUBLICATIONS DURING REPORTING PERIOD: The following papers reporting research performed under this contract were published during the reporting period, are in press, or are in preprint form.

DeMarini, D. M. 1978. The mutagenicity of cigarette smoke condensate in Saccharomyces cerevisiae. *Mutation Res.* 53:84. (Abstr.)

Whong, W., T. Ong, and H. E. Brockman. 1978. Effect of pH on the mutagenic and killing activities of ICR-170 in Neurospora crassa. *Mutation Res.* 53:83. (Abstr.)

Pham, H. N., D. M. DeMarini, M. Babula, and H. E. Brockman. 1978. The mutagenicity of sixteen ICR-compounds in Salmonella typhimurium. *Environmental Mutagen Society Meeting Abstracts*, p 49. (Abstr.)

Babula, M. 1978. The mutagenicity of sixteen ICR-compounds in Neurospora crassa using a simple reverse-mutation test. *Environmental Mutagen Society Meeting Abstracts*, p 49. (Abstr.)

DeMarini, D. M. 1979. The mutagenicity of cigarette smoke condensate in Neurospora crassa. *Environmental Mutagenesis* 1:117. (Abstr.)

Weaver, D. L. 1979. The characterization of streptonigrin-induced mutation at the ad-3 region in a two-component heterokaryon of Neurospora crassa. *Environmental Mutagenesis* 1:133-134. (Abstr.)

Pescitelli, A. R. 1979. Mutagenicity of cigarette smoke condensate and cigarette smoke in Drosophila melanogaster. *Environmental Mutagenesis* 1:118. (Abstr.)

Tomlinson, C. R. 1979. Effects of pH on the mutagenicity of sodium azide in Neurospora crassa and Salmonella typhimurium. *Environmental Mutagenesis* 1:138. (Abstr.)

Tomlinson, C. R. 1980. Effects of pH on the mutagenicity of sodium azide in Neurospora crassa and Salmonella typhimurium. *Mutation Res.* 70:179-191.

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DeMarini, D. M., H. N. Pham, M. J. Babula, A. J. Katz, and H. E. Brockman. 1980. Relationships between structures and mutagenic potencies of 16 heterocyclic nitrogen mustards. Preprint.

DeMarini, D. M., M. J. Plewa, and H. E. Brockman. 1980. Use of four short-term tests to evaluate the mutagenicity of municipal water. Preprint.

Heartlein, M. W., D. M. DeMarini, A. J. Katz, J. C. Means, M. J. Plewa, and H. E. Brockman. 1980. Mutagenicity of municipal water obtained from an agricultural area. Preprint.

DeMarini, D. M. 1981. Mutagenicity of cigarette smoke condensate in Neurospora crassa. *Mutation Res.* in press.

DeMarini, D. M. 1981. Mutagenicity of fractions of cigarette smoke condensate in Neurospora crassa and Salmonella typhimurium. *Mutation Res.* in press.

The following theses and dissertation reporting research performed under this contract were written or completed during the reporting period.

Tomlinson, C. R. 1978. Effects of pH on the mutagenicity of sodium azide in Neurospora crassa and Salmonella typhimurium. M.S. Thesis. Illinois State Univ. 84 pp.

Pescitelli, A. R. 1980. Mutagenicity of cigarette smoke condensate and cigarette smoke in Drosophila melanogaster. M.S. Thesis. Illinois State Univ. 61 pp.

DeMarini, D. M. 1980. Mutagenicity of cigarette smoke condensate in Neurospora crassa and Salmonella typhimurium. Ph.D. Dissertation. Illinois State Univ. 170 pp.

V. APPROXIMATE PERCENTAGE OF TIME OR EFFORT: The approximate percentage of time that the principal investigator has devoted to the project since the beginning of the current term of the agreement is:

25% of time for 9 months: Jan. 1 through May 15 and Aug. 15 through December 31, 1977. 100% of time for 1 summer month: May 15 through Aug. 14, 1977.