AN ABSTRACT OF THE THESIS OF

Vannin Marie Hutchinson McNair for the degree of Master of Science

in General Science presented on Oct 22, 1988

Title: Development of a System to Test for the Toxicity of Solar-Irradiated Polynuclear Aromatic Hydrocarbons and Preliminary Data Using Mammalian Cell Cultures

Redacted for Privacy

Abstract approved: _____

Dr. Rick Jostes

Redacted for Privacy

Dr. Michael Mix

Polynuclear aromatic hydrocarbons (PNAH) classified as non-carcinogens may be photooxidized to mutagenic or toxic products by near-ultraviolet (NUV) wavelengths of sunlight (290-320 nm). The possibility that this represents a major degredation pathway in the aquatic environment is of increasing concern. Large quantities of PNAH from accidental oil spills, anthropogenic and natural combustion, surface run-offs, and industrial discharges are being released into the aquatic ecosystem. In addition there is growing evidence that the stratospheric ozone layer may be diminishing. As a result, more NUV wavelengths from sunlight will reach the earth's surface.

The purpose of this study was to design a system that can measure the effects of simulated sunlight (290 nm) irradiated

PNAH on mammalian cells. A second observation was to utilize the system developed, to collect preliminary data on the effects of two PNAH parent compounds, fluoranthene and phenanthrene, on mammalian cells.

The first set of experiments, conducted during development of the system, involved the selection of methanol as the most suitable PNAH solvent. Determination of the rate of PNAH degradation was also determined during the initial experiments. Other system-related experiments included an analysis of the effects of (a) plastic tissue-culture dishes, (b) methanol, (c) phosphate buffered saline (PBS), and (d) 5-bromodeoxyuridine (BrdUrd) on the induction of sister chromatid exchanges (SCEs) and/or cell survivals of Chinese hamster ovary (CHO) cells. The experimental results indicated that none of these factors significantly affected either the survival or the number of induced SCEs in the CHO cells.

Preliminary data indicated that in the absence of simulated sunlight, PNAH compounds did not cause an increase in SCEs, nor a decrease in cell survival. However, CHO cells exposed to super-saturated concentrations of fluoranthene and a simulated sunlight (290 nm) light dose of 2000 or 3000 j-sec/m^2 did not survive. At similar light doses and lower fluoranthene concentrations, the induction of SCEs and mutation frequency were increased.

The data from phenanthrene studies indicated there was a decrease in survival in cells exposed to super-saturated concentrations and a simulated sunlight (290 nm) dose of 6000 j-sec/m^2.

The number of SCEs was not increased in cells exposed to lower phenanthrene concentrations and light doses of 2000 or 3000 j-sec/m^2.

Development of a System to Test for the Toxicity of Solar Irradiated Polynuclear Aromatic Hydrocarbons and Preliminary Data Using Mammalian Cell Cultures

by

Vannin Marie Hutchinson McNair

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Commencement June 1983

APPROVED:

Redacted for Privacy

Assistant Professor of General Science in charge of major

Redacted for Privacy

Professor of General Science in charge of major

Redacted for Privacy

Head of Department of General Science

Redacted for Privacy

Dean of Graduate School

Date thesis is presented October 22, 1982

Typed by Donna Lee Norvell-Race for Vannin Marie Hutchinson McNair

ACKNOWLEDGMENTS

I wish to thank my major professors, Dr. Rick Jostes and Dr. Michael Mix, for their time, patience and continued encouragement and good humor throughout this project. Dr. Jostes taught me the tissue-culture technique (in case I get desperate for a job), the sense of humor and patience required in research and how to save beanery cups to get a reduced price on the next cup of coffee. Dr. Mix suggested this project and acted as a good listener through the rough spots and helped me to write a clear (?) and grammatically correct thesis.

My appreciation to Dr. Robert Worrest for taking the time to make the spectroradiometer readings. In addition, a special thank you to Dr. Rick Jostes and Mike McKenzie-Carter for their work on determining the mutation frequency expression time period for CHO cells exposed to simulated sunlight, and to Randy Schaffer for the many hours he spent working with me on the HPLC and HP-85 and for the always-open door that serves as a gathering point for many.

Other members in the General Science Department have helped me in many special ways. Thank you ...

... Dr. Willis, for your continuing encouragement and help and, most importantly, for the opportunity to enter this program;

... Karla Russell, Dorothy Sheler and Jackie Mattson, for your quick smiles, interest and help during this time--I couldn't have done it without your help with the phone, mail, and those extra supplies and special favors;

... Keith King, for your photography advice and suggestions; and

... Dr. Henry Van Dyke, for joining me as the only other early bird in the group.

Thanks also to Tim Hughes for the many long hours we put in together in physiology classes.

Finally, for my family--Mom and Dad and Juanita and Mac--how can I thank all of you enough? Your help with moving us (twice in fact), filling in with those extra dinners (not to mention the loads of ice cream and hot fudge sundaes!!), and, most especially, caring for Breanna and believing in me were all greatly appreciated.

Thank you, also, Breanna, for putting up with your mother's typing when you tried to sleep and giving up a lot of your time with me so I could finish.

Gary, loving thanks to you most of all for your wealth of PATIENCE and wit, through the ups and downs of these two years. They have been about the happiest two years of my life, thanks to you.

This research was supported in part by Cooperative Agreement (#CR808000-01-0) in the NCI/EPA Collaborative Program Project No. 3, "Effects of Carcinogens, Mutagens and Teratogens in Non-human Species (Aquatic Animals)," administered by the Gulf Breeze, FL laboratory.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Development of a System to Test for the Toxicity of Solar Irradiated Polynuclear Aromatic Hydrocarbons and Preliminary Data Using Mammalian Cell Cultures

INTRODUCTION

Nonmetabolic activation of unsubstituted polynuclear hydrocarbons (PNAH) by photooxidation processes and the subsequent effects of these products in mammalian cells have been only slightly addressed by researchers. As Barnhart and Cox (1980) suggested, an investigation of the near-ultraviolet (NUV) activation effects on PNAH compounds in polluted air or water, may result in the discovery of another mechanism by which mutations can be induced; such mutations may, in turn, lead to skin carcinogensis.

There are many studies (Koller, 1965; Jagger, 1967; Setlow, 1974; Hsie, et al., 1977) which may have previously identified direct effects of ultraviolet light radiation that are detrimental to living organisms, including man. Ultraviolet light has been implicated as a major causal factor of skin cancer in the United States (Hsie, et al., 1977). Zelle, et al., (1980) suggest that ultraviolet wavelengths emitted by the sun which reach the earth's surface (290-320 nm), induce cytotoxic and mutagenic effects by damaging the cellular DNA.

That particular ultraviolet wavelength region (290-320 nm) is also of importance in the conversion of PNAH. Tricyclic or larger PNAHs absorb strongly at wavelengths of 300 nm and longer. In addition, most PNAHs are readily photoxidized. According to Gibson

et al., (1978) certain PNAHs not previously recognized as being mutagenic were transformed by relatively mild conditions into compounds that were mutagenic when tested against selected strains of S. typhimurium.

The increased presence of PNAHs in aquatic environments, together with Gibson et al., (1978) results, suggests there is a need for a study on the combined effects of ultraviolet irradiation of PNAH compounds on mammalian cells. One of the purposes of my research was to conduct such a study. A discussion of the rationales for my research follows.

The relevance of results obtained with germicidal lamps (Gibson et al., 1978) in understanding the effects of NUV and PNAH interactions in polluted air or water environments can be questioned. The germicidal ultraviolet wavelength, 254 nm, is filtered out by the stratospheric ozone layer and hence does not reach the earth's surface where the NUV-PNAH interactions occur (Parrish et al., 1978). In addition, while a bacterial system such as that developed by Ames et al., (1973) has been well-established with respect to detecting mutagens, a mammalian cell system is more relevant for determining mutagenic (and possibly carcinogenic) effects that may occur in humans. Chinese hamster ovary (CHO) cells were selected for use in my research because their complex genetic apparatus more closely resembles that of man (Barnhart and Cox, 1980). Also, carcinogenesis can be demonstrated in the animals from which these cells

were derived (Barnhart and Cox, 1980). It also seems important, for a project designed to model certain environmental conditions (i.e., simulated sunlight (290 nm) and PNAH interaction in the aqueous environment), that the mode of activiation of the parent PNAH compound be related to the available environmental conditions (Barnhart and Cox, 1980).

Sister-chromatid exchange, survival and the hypoxanthine guanine phosphoribosyl transferase mutation frequency assays were utilized as measures of the effects on the CHO cells in this research.

Polynuclear Aromatic Hydrocarbons (PNAHs)

There is considerable interest about the possible role of PNAHs as carcinogens in indigenous species which inhabit contaminated aqueous environments (Kraybill et al., 1977). Polynuclear aromatic hydrocarbons are an important class of pollutants that enter the sea; they are known to be contaminants of marine ecosystems (Neff, 1979). Polunuclear aromatic hydorcarbons enter the aquatic environment primarily in surface runoff from land, in industrial and domestic sewage, by petroleum spillage and by the deposition of airborne particulates (Neff, 1979). Not only are the parent PNAH compounds entering the aqueous environment through these pathways considered important, but so are certain PNAH degradation products.

Polynuclear aromatic hydrocarbon airborne particulates and PNAHs present in the surface water are subject to degradation by photo-oxidation reactions with atmospheric oxidants or by reaction with sulfur oxides (Neff, 1979). These oxygenated compounds, including quinones which may be carcinogenic, are generally the products of these reactions (Neff, 1979). According to Zafiriou (1977), photo-oxidation by singlet oxygen appears to be the dominant chemical process for degradation of PNAHs results in half-lives of a few minutes to a few hours (Smith and Tyler, 1976; Neff 1979; Zepp and Scholtzhauer, 1979). Most PNAHs absorb sunlight in spectral regions ranging from UB-B to visible (Zepp, 1980). Because of the ability to absorb in the NUV region (290-320 nm), PNAHs may be subject to NUV activation to promutagens (Barnhart and Cox, 1980).

A study by Jones and Leber (1979) indicated that benzo(a)-pyrene (BAP) is oxidized rapidly by simulated sunlight, in air. Also, among the resultant products were BAP quinones which are direct acting mutagens (Jones and Leber, 1979).

Two unsubstituted PNAHs were selected for use in my study. Fluoranthene and phenanthrene were chosen as a result of a study by Mix and Schaffer (in press) which showed these two compounds were present in the greatest concentrations in Mytilus edulis from Yaquina Bay, Newport, Oregon. The ability of mussels to concentrate those two particular PNAHs to levels greater than those of other PNAHs suggests that they may be present in measurable quantities

in the water where they may be subject to photooxidation processes by the NUV wavelengths from sunlight. The resultant products of these processes and their effects on CHO cells were of interest in my research.

Fluoranthene

Fluoranthene (Appendix A) has a molecular weight of 202 g/mole and a maximum solubility of 0.2 ug/ml in water (May et al., 1978). The absorption spectrum of fluoranthene (Appendix A) shows an absorption peak near 290 nm and subsequent smaller peaks in the 300-373 nm range (Roe, 1966).

Fluoranthene is listed as both a priority and a toxic pollutant by the EPA (Keith and Telliard, 1979). It is not classified as a carcinogen, but according to the NCI-SRI-Mitre-DOE report (NCI et al., 1981), it is listed as a tumor promoter or co-carcinogen and tests as a mutagen in the Ames test (Ames et al., 1973).

Little information is available on the possible NUV activation of fluoranthene from a nonmutagen to a mutagen. Barnhart and Cox (1980) has provided the experimental framework by which PNAHs can be tested for such a conversion.

Phenanthrene

Phenanthrene (Appendix A) has a molecular weight of 178 g/mole and a maximum solubility of 1.0 ug/ml in water (May et al., 1978).

The absorption spectrum of phenanthrene (Appendix A) shows a maximum aborption peak at 250 nm with lesser peaks appearing at 280-320 nm (Roe, 1966).

Phenanthrene is not classified as a carcinogen, but it is a priority pollutant (Keith and Telliard, 1979).

Phenanthrene has been studied extensively in regards to the activation (enzymatic) of its K-region into suspected carcinogenic epoxide agents. Boyland and Sims (1963, 1964, 1965a,b) have demonstrated epoxide formation as the result of the metabolism of phenanthrene. Grover and Sims (1970) have extended those results and demonstrated the reactivity of K-region epoxides of phenanthrene and dibenz(a,h)anthracene with DNA, RNA and histones in vitro. The parent hydrocarbons and their respective K-region dihydrodiols did not react (Grover and Sims, 1970). It has been shown that the phenanthrene K-region oxide and its isomeric phenols are mutagenic and preferentially mutate TA1537 in the Ames test (Bucker et al., 1979).

As a result of those studies, it has been suggested that the metabolites of PNAHs may be the ultimate reactive (and carcinogenic?) derivatives of hydrocarbons, in vivo (Miller and Miller, 1971). However, as with fluoranthene, there is little evidence of phenanthrene being activated by NUV into carcinogenic or mutagenic products.

According to Huberman and Sachs (1976) and others, procarcinogens must be metabolized by cellular enzymes into reactive metabolites which are then responsible for biological effects, including

carcinogenicity and mutagenicity. Chinese hamster ovary cells do not have the ability to metabolically activate procarcinogens/promutagens to a reactive state. Therefore, any activation of phenanthrene or fluoranthene by NUV that produced mutagenic or carcinogenic agents could be attributed solely to a nonmetabolic conversion. Such a conversion would be similar to that which could occur in the environment and hence would provide important information.

Near-Ultraviolet Light (NUV)

The biological effects of NUV, particularly those wavelengths contained in the natural sunlight spectrum (290-320 nm), have been studied extensively. The NUV spectrum causes pyrimidine dimer induction in DNA and induces other lesions of biological significance (Zelle et al., 1980). When mutation frequency is expressed as a function of survival, the three types of UV radiation--far UV, NUV 290, NUV 310--are comparable, indicating that lesions which contribute to lethality are equally important for the induction of mutations (Zelle et al., 1980. In a set of experiments by Bradley et al., (1979), wavelenths from a filtered sunlamp which were greater than 305 nm, caused mutagenicity,tosicity, DNA-protein cross-links and DNA single-strand breaks. However, when a second filter was used to block out any wavelengths below 345 nm, the radiation was no longer mutagenic or toxic, but still caused sister chromatid exchanges. Hsie et al. (1977) examined the effects of

sunlamps and sunlight on CHO cells and found a high level of toxicity caused by each, as well as a significant level of mutation induction. The cell survival mutation induction curves of both sunlamp and sunlight irradiation were similar in shape to those of standard UV light (Hsie et al., 1977).

Given the information from these and other studies, it is clear that the mutagenic and cytotoxic effects of simulated sunlight alone must be considered in developing and evaluating a non-metabolic activation system for PNAHs by simulated sunlight.

Sister-Chromatid Exchange (SCE)

The sister chromatid exchange technique has been correlated with mutagenic damage in some studies and disregarded as such in others. The latter view is summarized by the observation that, despite the use of the SCE test for years, it is still unknown whether SCEs represent mutagenic damage (Roszinsky-Kocher et al., 1979). Nevertheless, Bauknecht et al. (1977) demonstrated a good in vivo correlation between the potency of chemical compounds to induce SCEs and structural chromosomal aberrations. Further, it was reported that there were correlations between SCEs and mutations induced by various chemical agents in CHO cells, however, the relative efficiency of SCE and mutation induction varied with the particular agent used (Carrano et al., 1978). By contrast, Bradley et al. (1979) showed that, although SCEs were induced with wavelengths of greater than 345 nm, no

evidence of either mutagenicity or toxicity was determined with that level of radiation.

For my research, the SCE technique was selected as a rapid, convenient method by which a potentially mutagenic PNAH photoproduct could be revealed. In most of my studies, an increase in SCE induction following an exposure to NUV irradiation in the presence of a PNAH was followed by a confirmatory mutation frequency experiment performed under similar conditions.

The SCE technique was discovered by Taylor (1958) in plant cells that had been labeled with tritiated thymidine through the first cell division. Cells were then allowed to replicate once again in the absence of the radioisotope (Wolff and Perry, 1974). Autoradiography revealed chromosomes that contained only one of the two sister chromatids labeled at any point along their length. These observations were interpreted as to mean that switches had occurred in the radioactive label from one chromatid to its sister, hence, "sister chromatid exchange" (Wolff and Perry, 1974). The technique has since been further refined to make it possible to distinguish sister chromatids from one another without using radioisotopes and autoradiography (Zakharov and Egolina, 1972; Latt, 1973; Ikushima and Wolff, 1974; Perry and Wolff, 1974). The basis for newer techniques was the finding that after two rounds of replication in the presence of 5-bromodeoxyuridine (BrdUrd), the chromosomes contain one chromatid in which the DNA is unifilarly substituted with BrdUrd, and one chromatid in which the DNA is

bifilarly substituted (Wolff and Perry, 1974).

After the incorporation of BrdUrd, Latt (1973) found a dramatic difference in the intensity of fluorescence between sister chromatids of human chromosomes which were stained with the fluorescent dye, Hoechst 33258. Permanently stained cells were then produced by a technique developed by Perry and Wolff (1974). This technique utilized both fluorescent dyes and Giemsa stain after light exposure. The unifilarly substituted chromatid stains darkly, whereas the bifilarly substituted chromatid stains lightly, thus giving the chromosomes a "Harlequin-like" appearance which characterizes the SCE technique (Wolff and Perry, 1974).

One problem associated with this technique is that BrdUrd can induce SCEs. It was found that the frequency of SCEs increased as concentrations of BrdUrd increased from .25 uM to 1.0 uM (Wolff and Perry, 1974). Further, the frequency of SCEs was increased by exposure of BrdUrd-labeled cells to fluorescent lights because of photolysis of the BrdUrd-containing DNA (Ikushima and Wolff, 1974; Perry and Wolff, 1974). To overcome the SCE induction by BrdUrd, investigators now use the minimum concentration of BrdUrd that is required to give the optimum differential staining of sister chromatids (Perry, 1980). Also, Wolff and Perry (1974) have shown that the number of SCEs per chromosome, induced by BrdUrd, can be decreased by handling the BrdUrd-containing cells under a dim incandescent light or a photographic safe light. The number of SCEs induced under such lighting conditions is only slightly greater

than that induced in cells handled exclusively in the dark (Wolff and Perry, 1974).

In addition to SCEs, I used survival experiments to provide information on cell toxicity under certain exposure conditions.

Survival

The survival ability of a particular cell line subjected to a specified treatment gives the researcher an indication of the cytotoxic effects of the agents in question. During the course of my research, one factor was taken into consideration that influences the survival of the CHO cells. Stoien and Wang (1974) found that NUV exposure of the medium produced photoproducts that were toxic to Chinese hamster cells. The toxic photoproducts were thought to include tryptophan, riboflavin and tyrosine (Stoien and Wang 1974). Wan et al. (1974) also felt that the cell-killing effect was dependent because when more cells are present, the amount of photoproducts absorbed by each cell would be correspondingly less. As a result, I washed the cells with phosphate buffered saline (PBS) before and after each exposure; also, the cells were covered with PBS during exposures to simulated sunlight.

I used one other method in my studies, the hypoxanthine-guanine phosporibosyl transferase (HGPRT) mutation frequency assay (O'Neill et al., 1977).

Hypoxanthine-guanine phosphoribosyl transferase (HGPRT) Mutation Frequency Assay

The HGPRT mutation assay measures mutation induction at the HGPRT locus in Chinese hamster ovary cells by determining resistance to 6-thioguanine (TG) (O'Neill et al., 1977). Chinese hamster ovary cells were selected for this assay because they had been better characterized genetically than other mammalian cell lines. Also, they were well-suited for mutagenesis studies because they have a stable karyotype of 20 or 21 chromosomes and are easily maintained in a defined medium in suspension or on glass or plastic tissue culture dishes (Puck et al., 1958; Kao and Puck, 1969; Sharp et al., 1973). Thiguanine is lethal to mammalian cells and so, any resistance to this purine analogue will occur only from an alternation or loss of the enzyme, HGPRT (Chasin, 1973; Sharp et al., 1973), the expression time being eight days (O'Neill et al., 1977).

Conclusion

To conclude this introduction the primary objectives of my research were: (1) to develop a system that would simulate the interactions of sunlight and PNAHs as they occur in the aqueous environment and (2) to subsequently measure the effects of simulated sunlight-PNAH interactions on mammalian cells. Specifically, three effects on CHO cells were to be measured: first, the effects of those NUV wavelengths which are part of the natural sunlight

spectrum on the earth's surface; second, the effects of non-activated fluoranthene and phenanthrene, PNAH parent compounds; and third, the effects of interaction between a specific simulated sunlight dose and one of the PNAH parent compounds.

MATERIALS AND METHODS

Cells, Media, Culture Conditions

Chinese hamster ovary (CHO) cells were grown in Gibco (3175 Staley Road, Grand Island, New York) minimum essential medium (MEM) supplemented with 10% Kansas City fetal calf serum. The medium was further supplemented with Gibco 2% L-glutamine, 2% non-essential amino acids, 2% penicillin-streptomycin antibiotics, and 3% sodium bicarbonate (7.5%). Cells were incubated at 37°C under 5% CO_2 in tissue-culture containers. Cells were maintained in an exponential growth phase prior to each experiment.

Exposure Conditions

Solar Ultraviolet Radiation

Four Westinghouse FS40 (40 watt) sunlamps provided the source of simulated solar radiation. The lamps were suspended 0.5 meters above the cell exposure surface in a laboratory hood and the exposure surface was covered with black plastic to minimize reflected light (Figure 1). Radiation from the lamps was filtered through an aged (15 hours) cellulose acetate (10 mil) sheet which removed wavelengths shorter than 290 nm from the spectrum. The sunlamps were also filtered to a lesser extent, by the tissue culture plastic covers which decreased the intensity of the light at

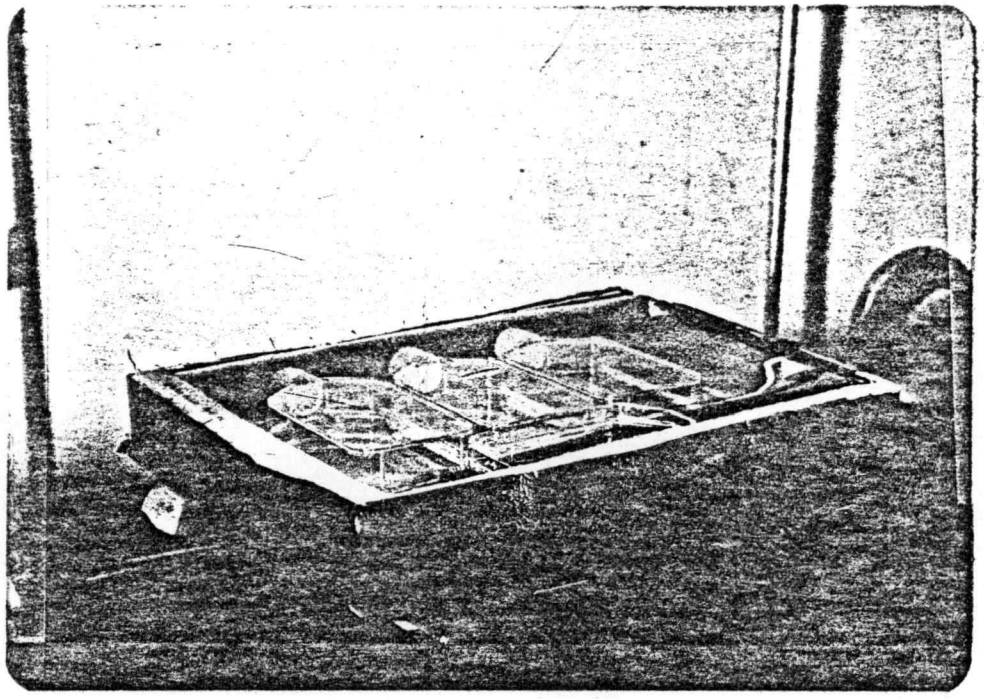


FIGURE 1. Experimental set-up during simulated sunlight-irradiated PNAH exposures

wavelengths less than 315 nm (Elkind et al., 1977; Hsie et al., 1977) (Appendix B). Both of these filtration systems combined to give results which more closely represent the sunlight spectrum at the earth's surface (Elkind et al., 1977). An Optronic Laboratories model 742 spectroradiometer interfaced with a model 755 data reduction system was used to determine the dose rates and specific wavelength profiles for the solar radiation exposures (Appendix B). A Robertson-Berger sunburn meter unit was used to verify that the dose rate distribution did not vary more than 5% over the exposure field.

Polynuclear Aromatic Hydrocarbons (PNAHs)

Two unsubstituted PNAHs, fluoranthene and phenanthrene, were subjected to solar irradiation; they were obtained from Ultra Scientific (Rhode Island). Each compound was dissolved in methanol at a concentration of 0.1% (phenanthrene) or 0.2% (fluoranthene). From these stock solutions, the compounds were further dissolved in phosphate buffered saline (PBS) for final concentrations of 1.0 or 10.0 ug/ml phenanthrene and 0.2, 2.0, or 4.0 ug/ml fluoranthene. High performance liquid chromatography (HPLC) analysis revealed that the concentrations of the compounds change with time. Thus, solutions were prepared fresh for each experiment to minimize such changes.

Sister-chromatid Exchange, Survival, Mutation Frequency Assays

Sister-chromatid Exchange

Exponentially growing CHO cells with a doubling time of 16 hours were plated (2×10^6 cells per flask) into T-75 tissue-culture flasks containing 10 mls of complete minimum essential medium (MEM) supplemented with 10% fetal calf serum and incubated at 37°C two to three hours prior to an exposure. The cells were washed once with sterile PBS and covered with 10 mls of a PNAH-containing PBS solution. Each flask was placed on the exposure surface and covered with a cellulose acetate sheet. A dose of 2000 joules-sec/m^2 was administered to the cells for a period of 14 minutes 49 seconds (Appendix B). Following the exposure, cells were washed once in sterile PBS solution and covered with 10 mls of MEM that had been supplemented with 0.1% 5-bromodeoxyuridine (BrdUrd). Each flask was covered with aluminum foil and replaced in a 37°C humidified incubator, under 5% CO_2, for 24 hours. Colcemid (2×10^{-7}M final concentration) was added at 24 hours if the majority of cells had passed the first division stage, as determined from an accompanying multiplicity T-25 flask of similarly treated CHO cells. For determination of multiplicity, T-25 tissue-culture flasks were plated with 1000 cells and treated in the same manner as the corresponding T-75 flasks. Twenty-four hours following the exposures, each flask was checked to determine if the cells had passed the first division stage. That was done

by counting the number of cells per colony in forty different groups of cells and determining an average number of cells per colony forming unit. A value of 2.5 in approximately 24 hours for untreated controls is appropriate. Two to three hours after the addition of colcemid, the cells were swelled in .075M KCL for 15 minutes and then fixed in 3:1 methanol, glacial acetic acid. Slides were prepared by dropping the cells from a height of 6-12 inches onto labeled microscope slides. The slides were allowed to dry overnight and were stained the following day according to the technique described by Wolff and Perry (1974). Since the staining techniques vary between laboratories due to different environmental and equipment conditions, the steps followed in this research will be outlined.

The slides were first placed in a coplin jar containing 50 ml of Hoechst's dye (5 ml Hoechst's [5 mg/100 ml stock solution, refrigerated] diluted in 45 ml Sörensen's buffer solution formula [.067M, pH 6.8]--prepared fresh weekly and refrigerated) for 15 minutes. They were then washed in distilled water, dried thoroughly and covered with 4-5 drops of Sörensen's buffer and a coverslip. Slides were then exposed to a black light (2 Sylvania F15T8-BLB bulbs) suspended in a desk lamp one-half to one inch above the slide warming tray (56°C) (Figure 2) for 3 or 4 minutes, depending on which time period produced the best sister-chromatid contrasts. Chromosomes with one darkly stained chromatid and one lightly stained sister-chromatid are from second-division cells and are

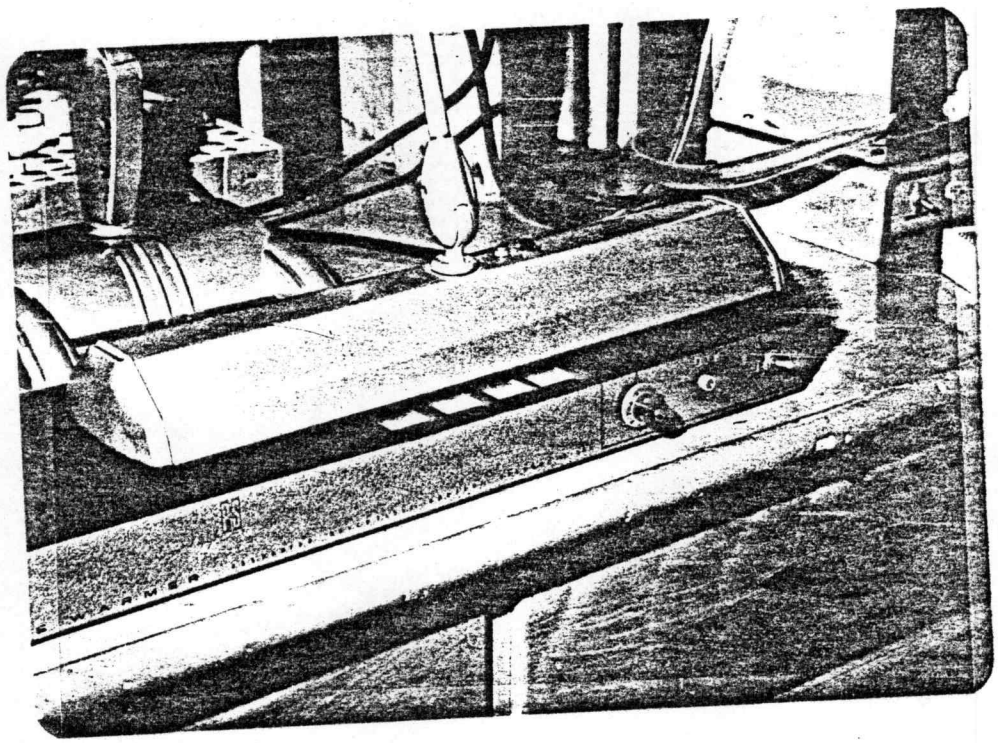


FIGURE 2. Chinese hamster ovary cells exposed to black light for sister-chromatid exchange development

the cells where the exchanges are readily apparent (Figures 3 and 4). First division cells contain chromosomes with both sister-chromatids stained uniformly dark, while third-division cells contain some chromosomes with one chromatid darkly stained and its sister lightly stained, but most chromosomes are uniformly lightly stained (Schwartz et al., 1982). Following the light exposure, the coverslips were rinsed with distilled water and the slides were placed in 12% Giemsa for approximately 8 minutes. At the end of this time, the slides were rinsed in distilled water, covered with a coverslip and examined under the microscope.

Survival

Depending on the treatment conditions, 150 or 1500 CHO cells were plated per 60 mm^2 in a tissue-culture petri dish containing 3 mls of MEM, two to three hours prior to an exposure. Cells were washed once with sterile PBS and exposed to one of the following three conditions in each experiment: (1) light (2000 joules-sec/m^2) and a PNAH; (2) light and no PNAH; and (3) a PNAH and no light. After treatment, the cells were washed once more in PBS and incubated in 5 mls MEM for 8-9 days at 37°C. When the colonies had developed enough to be easily counted (greater than 50 cells per colony), they were fixed in 3:1 methanol, glacial acetic acid. The colonies were rinsed once more in the methanol, glacial acetic acid solution and allowed to dry overnight. Two to three mls of

FIGURE 3. Sister-chromatid exchanges in CHO chromosomes exposed to no light and no PNAHs

FIGURE 4. Sister-chromatid exchanges in CHO chromosomes exposed to a simulated sunlight dose of 6000 j-sec/m^2

Giemsa stain applied to each dish stained the colonies a deep purple color.

Mutation Frequency

The HGPRT mutation assay as described by Van Zeeland and Simons (1976) was used in these experiments. Initially, this assay was used to determine the mutation frequency plateau for certain concentrations of 6-thioguanine using the Gibco serum. The results indicated that 5 ug/ml 6-thioguanine added to medium containing the Gibco serum would be adequate for any mutation assays done using this serum (Figure 5). In other experiments, CHO cells exposed to 2000 and/or 8000 joules-sec/m^2 doses of simulated sunlight and/or 0.2 ug/ml fluoranthene concentration were allowed expression times of 8 days before being replated into MEM medium containing 6-thioguanine (5 ug/ml). Prior to each treatment, a T-75 tissue-culture flask was plated with 1 x 10^6 cells. The day following an exposure, the cells were trypsinized and 1 x 10^6 cells were transferred to and divided among three non-tissue culture 60 mm^2 petri dishes. The cells remained in these dishes in MEM plus 5% Kansas City serum for 8 days. Mutant colonies were counted 7 days after the cells had been redistributed into ten 60 mm^2 tissue-culture petri (5 x 10^4 cells per petri dishes) (5 x 10^4 cells per petri) containing 5 mls selective medium (MEM plus 10/5 Gibco calf/fetal calf serum supplemented with 6-thioguanine). In addition, following the expression time period, a

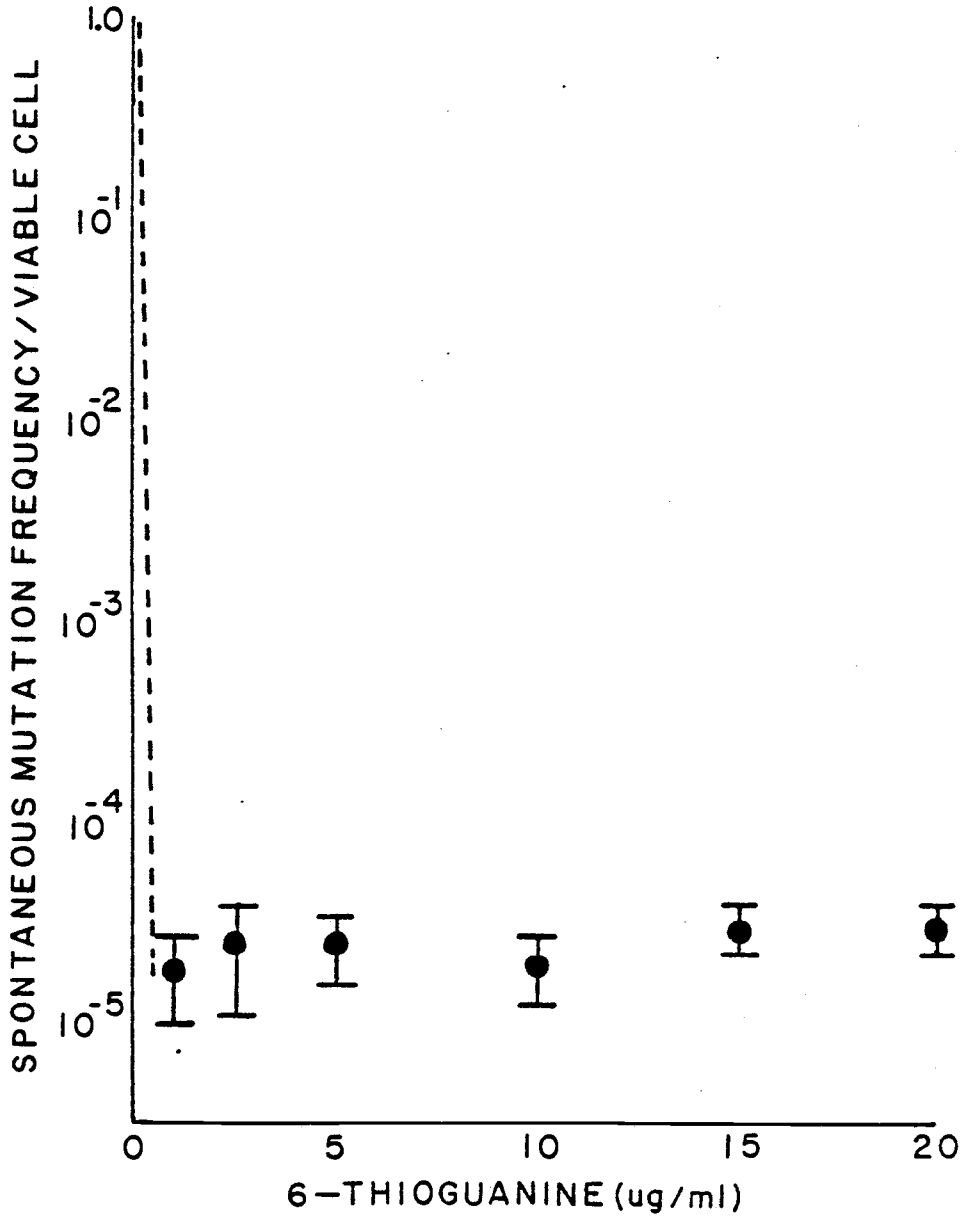


FIGURE 5. Determination of the 6-thioguanine concentration for use in the hypoxanthine-guanine phosphoribosyl transferase mutation frequency assay

portion of the cells (150 cells per 60 mm^2 tissue-culture petri, 6 petris per treatment) were plated into 5 mls non-selective medium (no 6-thioguanine) for the purpose of determining plating efficiencies. The mutant as well as the plating efficiency colonies were fixed and stained as described in the survival section of the materials and methods chapter.

All diluting and plating manipulations were performed in fluorescent light-free conditions to limit any possible contribution to SCE induction or survival and mutation effects.

RESULTS

Development of the System

Prior to performing any solar irradiated PNAH studies, several problems were identified and evaluated. Solution of each of these problems eventually led to the development of the final system utilized in my research. These problems and their solutions are described in chronological order in the following paragraphs.

Solvent Selection

The first question addressed in this research was which solvent should be used for dissolving particular PNAHs. Dimethylsulfoxide (DMSO) is cited in much of the current literature as a solvent that will readily dissolve PNAHs and subsequently transport them into the cells (Huberman and Sachs, 1976; Barnhart and Cox, 1980; Oesch et al., 1981). However, 20% of the DMSO absorption spectrum is in the 290-310 nm range (Przybytek, 1980) which includes wavelengths of the simulated sunlight spectrum that are of particular interest in my study (Appendix A). The use of DMSO would therefore limit the amount of simulated sunlight that would reach the cells at those specific wavelengths. Due to the finding that DMSO interferes with the absorption spectrum of interest, another solvent, methanol, was ultimately selected.

The use of methanol satisfied three important criteria. First,

methanol has the ability to dissolve PNAHs; second, it has an absorption spectrum that does not interfere with the wavelengths of interest in this study (Appendix A); and third, methanol does not affect the CHO cells in terms of increasing the number of SCEs, decreasing survival or affecting the mutation frequencies (Tables 1, 2, and 3).

The PNAHs were dissolved in methanol and then in either PBS or medium as long as the maximum solubility level of the particular PNAH compound was not exceeded. Much of the compound remained in a crystalline form if solubility levels were exceeded. The maximum solubility level of fluoranthene in water is 0.2 ug/ml and for phenathrene, 1.0 ug/ml (May, 1980). These maximum solubility levels were confirmed by HPLC tests that compared PNAH concentrations from filtered (#30 S&S glass filters .3 um) and non-filtered samples. Although a saturated solution had been prepared for a particular compound once it was filtered, only the amount in solution (the maximum solubility level) was detected by the HPLC. Non-filtered samples, however, maintained the initial saturated PNAH concentration levels (Table 4).

The absorption spectrum of methanol, as noted in Appendix A, indicates a maximum absorption peak at 205 nm. The absorption spectrum decreases to about 0.5% of the total spectrum at 300 nm, thereby making methanol a preferred choice as a solvent in these studies.

The effects of methanol on SCE induction, survival and

TABLE 1. Induced sister-chromatid exchanges (SCEs) in CHO cells exposed to 0.1% methanol.

Experiment number	Treatment	Medium	Light Dose (j-sec/m^2)	Exposure time (hours)	No. SCEs/chromosome[1] ± standard error	t-test
18	Control	McCoy's+10% K.C.[2]	None	26.5	.3619 ± .03	
18	0.1% methanol	McCoy's+10% K.C.	None	26.5	.4632 ± .03	$p \leq 0.01$[6]
18	0.1% methanol	McCoy's+10% K.C.	None	26.5	.4169 ± .03	N.S.[7]
21	Control	MEM + 5/5[3] Gibco calf/fetal calf	None	24.0	.4028 ± .02	
21	0.1% methanol	MEM + 5/5 Gibco calf/fetal calf	None	24.0	.3696 ± .03	N.S.
41	Control	PBS[4]	None	0.25	.3691 ± .02	
41	0.1% methanol	PBS	None	0.25	.3713 ± .02	N.S.
41	Control	PBS	2000-C.A.[5]	0.25	.6418 ± .04	
41	0.1% methanol	PBS	2000-C.A.	0.25	.6103 ± .03	N.S.

[1]The SCEs were counted in 40 different cells.

[2]McCoy's + 10% Kansas City serum, growth medium.

[3]Minimum Essential Medium + 5% Gibco calf serum and 5% Gibco fetal calf serum, growth medium.

[4]Phosphate buffered saline.

[5]Cellulose acetate.

[6]Significantly different from the control at the 1% level.

[7]No significant difference compared to the control.

TABLE 2. Surviving fraction of CHO cells exposed to 0.1% methanol.

Experiment number	Treatment	Medium	Light Dose (j-sec/m^2)	Exposure time (minutes)	Surviving fraction[1] ± standard error	t-test
18	Control	McCoy's+10% K.C.[2]	None	26.5 (hours)	.5920 ± .02	
18	0.1% methanol	McCoy's+10% K.C.	None	26.5 (hours)	.6893 ± .02	$p \leq 0.01$[5]
29	Control	PBS[3]	None	13:40	.8483 ± .02	
29	0.1% methanol	PBS	None	13:40	.6300 ± .02	$p \leq 0.001$
29	Control	PBS	2000-C.A.[4]	13:40	.7800 ± .01	
29	0.1% methanol	PBS	2000-C.A.	13:40	.6580 ± .03	$p \leq 0.001$
35	Control	PBS	None	13:40	.5850 ± .02	
35	0.1% methanol	PBS	None	13:40	.5689 ± .02	N.S.[6]
35	Control	PBS	2000-C.A.	13:40	.6562 ± .02	
35	0.1% methanol	PBS	2000-C.A.	13:40	.5967 ± .01	$p \leq 0.05$
40	Control	PBS	None	13:40	.4373 ± .01	
40	0.1% methanol	PBS	None	13:40	.4220 ± .01	N.S.
40	Control	PBS	2000-C.A.	13:40	.4193 ± .01	
40	0.1% methanol	PBS	2000-C.A.	13:40	.4013 ± .02	N.S.
40	Control	PBS	None	20:29	.4293 ± .02	
40	0.1% methanol	PBS	None	20:29	.4267 ± .02	N.S.
40	Control	PBS	3000-C.A.	20:29	.3967 ± .01	
40	0.1% methanol	PBS	3000-C.A.	20:29	.4242 ± .03	N.S.

[1]The number of surviving cells divided by the number of cells plated.

[2]McCoy's + 10% Kansas City serum, growth medium.

[3]Phosphate buffered saline.

[4]Cellulose acetate.

[5]Significantly different from the control at the 1% level.

[6]No significant difference compared to the control.

TABLE 3. Induced mutation frequency of CHO cells exposed to 0.1% methanol.

Treatment	Light Dose ($j\text{-}sec/m^2$)	Induced mutation frequency[1]
Control	None	9.046×10^{-6}
0.1% methanol	None	1.005×10^{-5}
Control	2000-C.A.[2]	1.392×10^{-5}
0.1% methanol	2000-C.A.	1.724×10^{-5}

[1]Mutation frequency (induced) is determined by this formula:

$$\frac{\text{number of mutant colonies counted}}{\text{number of cells plated x plating efficiency (PE)}}$$

[2]Cellulose acetate.

TABLE 4. Comparison of filtered and unfiltered fluoranthene concentrations as determined by the HPLC.[1]

Filtered[2]	Unfiltered	Expected concentration (ug/ml)[3]	Measured concentration (ug/ml)[4]
+	-	1.0	0.203[5]
-	+	1.0	0.650
-	+	10.0	10.000

[1]High performance liquid chromatography.

[2]Fluoranthene solution filtered through two, #30 glass filters.

[3]Expected concentration refers to the fluoranthene concentration that was to have been prepared.

[4]Measured, refers to the concentration of the fluoranthene solution as measured by the HPLC.

[5]This value is an average of the U.V. and fluorescence detector HPLC readings.

mutation frequency are summarized in Tables 1, 2, and 3. The results indicate that methanol does not significantly contribute to the effects on CHO cells.

Possible Photooxidation Products

Once a suitable solvent (methanol) had been selected, the next question was to determine if any photooxidation products appeared as a result of exposing a parent PNAH to solar radiation in the absence of CHO cells. Analysis of PNAH samples measured during exposures to high (.25 meters from the sunlamps, 1100 kj-sec/m^2 dose), low (1 meter from the sunlamps, 280 kj-sec/m^2 dose) and no light conditions revealed a contaminant peak at 185 nm which increased in concentration as the light dose increased. At the same time, the concentration of the parent compound (fluoranthene) decreased. Because the unidentified peak did not appear after the fluoranthene peak as indicated by the HPLC analysis, it was considered to be a contaminant and not a photooxidation product of fluoranthene. The source of the contaminant was suspected to be the plastic tissue-culture dishes (T-75). As a result, an experiment was designed to test this possibility.

Glass and plastic tissue-culture flasks which contained an equal concentration of fluoranthene dissolved in PBS were subjected to dark or high light conditions. Results of this experiment indicated that there was a difference between the rate of

fluoranthene disappearance in the two types of flasks. Fluoranthene (0.5 ug/ml) exposed to high light conditions in the plastic tissue-culture flasks disappeared more rapidly than that in the glass flasks (Table 5). Also, no contaminant peak at 185 nm appeared in the HPLC measurements of samples taken from the glass flasks. Hence, it was concluded that the contaminant contribution, and possibly the fluoranthene absorption, was due to the plastic tissue-culture flasks. It became apparent in similar studies with phenanthrene that absorption to the plastic containers was a characteristic confined to fluoranthene, since phenanthrene did not disappear from the PBS solution as quickly as fluoranthene (Table 6). As a result in subsequent experiments, PNAH solutions were prepared fresh each time to insure that the desired concentration levels were obtained. Also, the exposure times had to be limited to also insure the maintenance of the correct concentration levels.

An additional experiment was conducted to determine if the presence of the unidentified contaminant affected the induction of SCEs. There was no significant difference in the number of SCEs associated with plastic flasks compared to the number of SCEs induced in glass flasks (Table 7). Therefore, based on this information, together with the greater handling convenience, plastic tissue-culture flasks were selected for use in my research.

TABLE 5. Comparison of the fluoranthene degradation rate between glass and plastic tissue-culture flasks (T-75s).

Glass or plastic	Light conditions	Expected concentration[1] (ug/ml)	Exposure time (minutes)	Measured concentration[2] (ug/ml)	Degradation rate (ug/min)
Glass	Dark[3]	0.5	15	.179	.00087
			70	.131	
Plastic	Dark	0.5	15	.123	.00082
			70	.078	
Glass	High[4]	0.5	15	.226	.00018
			70	.216	
Plastic	High	0.5	15	.158	.00085
			70	.111	

[1]The fluoranthene concentration that was to have been prepared.

[2]The concentration of the fluoranthene solution as measured by the HPLC.

[3]T-75 flasks were wrapped in foil and placed in the dark.

[4]T-75 flasks were exposed to 4-FW40 sunlamps at a distance of .25 meters, and covered with both a cellulose acetate and a mylar filter.

TABLE 6. Comparison of fluoranthene and phenanthrene degradation rates.

PNAH[1]	Expected concentration[2] (ug/ml)	Exposure time (minutes)	Measured concentration[3] (ug/ml)	Degradation rate (ug/min)
Fluoranthene	0.2	0	0.7780	.0130
		44	0.2069	
Phenanthrene	1.0	0	1.1160	.0100
		60	0.5191	

[1]Polynuclear aromatic hydrocarbon.

[2]The fluoranthene or phenanthrene concentration that was to have been prepared.

[3]The fluoranthene or phenanthrene concentration as measured by the HPCL.

FIGURE 7. Comparison of induced sister-chromatid exchanges (SCEs) in CHO cells between glass and plastic tissue-culture flasks.

Experiment number	Glass or plastic	Medium	Exposure time (hours)	No. SCEs/chromosome[1] ± standard error	t-test
16	Plastic	McCoy's + 10% K.C.[2]	26.5	.4547 ± .02	
16	Glass	McCoy's + 10% K.C.	26.5	.4305 ± .03	N.C.[3]

[1]The SCEs were counted in 30 different cells.

[2]McCoy's + 10% Kansas City serum, growth medium.

[3]No significant difference compared to the control.

Chinese Hamster Ovary Cell Exposure to PNAHs

At this point in my research, attention was directed towards gathering baseline data by exposing CHO cells to PNAH-containing PBS solution for short time periods (1, 2, 3, 5 hours) since PBS solution cannot sustain the cells for 24 hours as well as medium. The baseline data revealed that there was no significant difference in SCE induction between PNAH-containing medium and PNAH-containing PBS solution (Table 8).

During these baseline studies, another study was initiated: 5-bromodeoxyuridine (BrdUrd) was tested to see if its presence during the 24 hours required for SCE development affected the induction of SCEs; comparisons were made with cells contained in a flask with .1% Brd Urd and 0.2 ug/m/fluoranthene. Because the BrdUrd must be present through 2 cell divisions (approximately 24 hours), and because the 0.2 ug/ml fluoranthene had already been shown not to induce SCEs or affect survival in the absence of light, this comparison was made. The results indicated that the presence of BrdUrd did not affect the induction of SCEs (Table 9).

Dosimetry

The light exposure area was confined to a laboratory hood in which the base was covered with black plastic to minimize any reflected light. The exposure surface was a 28 cm by 35.5 cm plywood sheet (1 cm thickness) set on an adjustable device .5 meters

TABLE 8. Comparison of induced sister-chromatid exchanges (SCEs) in CHO cells exposed to medium or phosphate buffered saline.

Experiment number	Treatment	Medium	Exposure time (hours)	No. SCEs/chromosome[1] ± standard error	t-test
12	Control	PBS[2]	5	.4818 ± .03	
16	Control	McCoy's[3] + 10% K.C.	24	.4547 ± .02	N.S.[4]

[1]The SCEs were counted in 30 different cells.

[2]Phosphate buffered saline.

[3]McCoy's + 10% Kansas City serum, growth medium.

[4]No significant difference compared to the control in Experiment 12.

TABLE 9. Induced sister-chromatid exchanges (SCEs) in CHO cells exposed to 5-bromodeoxyuridine (BrdUrd) or BrdUrd and 0.2 ug/ml fluoranthene.

Experiment number	Treatment	Medium	Exposure time (hours)	No. SCEs/chromosome[1] ± standard error	t-test
14	0.1% BrdUrd	McCoy's + 10% K.C.[2]	24.0	.4504 ± .03	N.S.[3]
14	0.2 ug/ml fluoranthene + 0.1% BrdUrd	McCoy's + 10% K.C.	24.0	.4039 ± .02	
16	0.1% BrdUrd	McCoy's + 10% K.C.	26.5	.4547 ± .02	N.S.
16	0.2 ug/ml fluoranthene + 0,k% BrdUrd	McCoy's + 10% K.C.	26.5	.4439 ± .03	

[1]The SCEs were counted in 30 different cells.

[2]McCoy's + 10% Kansas City serum, growth medium.

[3]No significant difference compared to the BrdUrd + 0.2 ug/ml fluoranthene-treated cells.

from the Westinghouse FS40 (40 watt) sunlamps. The surface was covered with black plastic and could be lowered or raised over a range of 23 cm. This assembly was placed on another plywood sheet suspended 20.5 cm from the floor of the hood by a series of clamps. It also was covered with black plastic (Figure 1).

Once the exposure area had been prepared, a series of radiometric readings were taken to determine the fluence rates (i.e., 2.25 joules-sec/m^2) of the four sunlamps for a variety of tissue-culture conditions. An Optronic Laboratories model 742 spectra-radiometer was interfaced with a model 755 data reduction system to determine these rates and also to develop specific wavelength profiles for the solar radiation exposures using both mylar (7 mil) and cellulose acetate (10 mil-aged 15 hours) filters. The results for each particular condition are summarized in Appendix B.

Wavelength spectra of these lamps through cellulose acetate and mylar are shown in Appendix B. The mylar spectrum was noted to be contained within the cellulose acetate spectrum and did not include the wavelengths of particular interest (i.e., 290-315 nm). Originally, the intent had been to conduct experiments with cellulose acetate and mylar concurrently and then any significant difference in the results could be attributed to the wavelengths (290-315 nm) present in the cellulose acetate filtered system but not in the mylar filtered systems. However, the results of experiment 26 showed no significant increase in SCEs of mylar filtered cells over non-light exposed cells (Figure 6), and subsequently caused

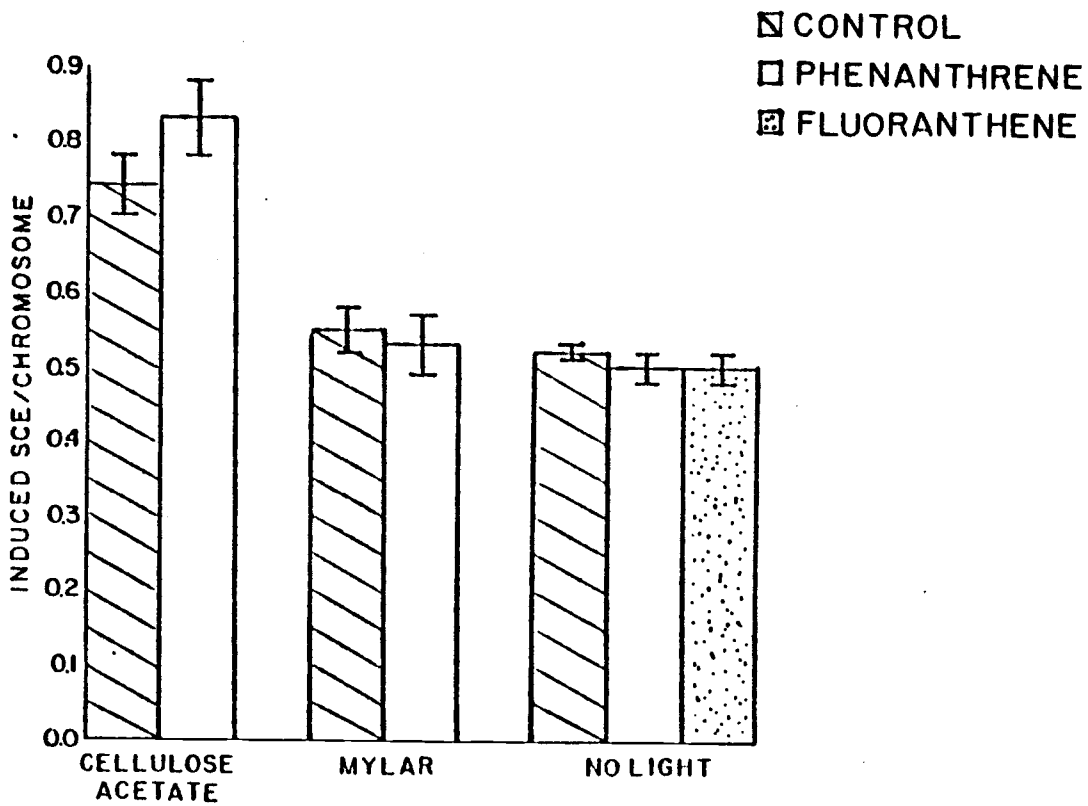


FIGURE 6. Comparison of the number of sister-chromatid exchanges induced by exposure to 4.0 ug/ml fluoranthene or 10 ug/ml phenanthrene and a light dose of 3000 j-sec/m^2 filtered with cellulose acetate or mylar

Note: The fluoranthene, light exposed cells died.

further use of mylar to be terminated. Most of the experiments which involved light exposures were performed using a cellulose acetate filter.

A Robertson-Berger sunburn ultraviolet meter was employed to assess the dose distribution across the exposure surface (Berger, 1976). The results indicated there was less than 5% variation in dose across the entire surface. However, beyond the limited exposure surface (28 cm by 35.5 cm) the dose decreased significantly. Thus, the exposures were confined to the limited exposure surface area described above.

CHO Cell Exposure to Simulated Sunlight

Once the fluence rates for tissue-culture flasks (T-75) and 60 mm^2 petri dishes containing PBS had been established (Appendix B), the next step was to ascertain the effects (SCE induction, survival and mutation frequency) of light only exposures on the CHO cells.

The affects of varying light doses (1000, 2000, 3000, 4500 and 6000 j-sec/m^2) on the induction of SCEs revealed a linear relationship (Figure 7). The induction of SCEs increased proportionally as the light dose increased. Therefore, the amount of SCEs expected at a particular light (only) exposure must be taken into account in considering the number of SCEs induced in those experiments where a PNAH and a light dose were combined in a treatment.

In an independent study by Dr. Rick Jostes, a survival curve

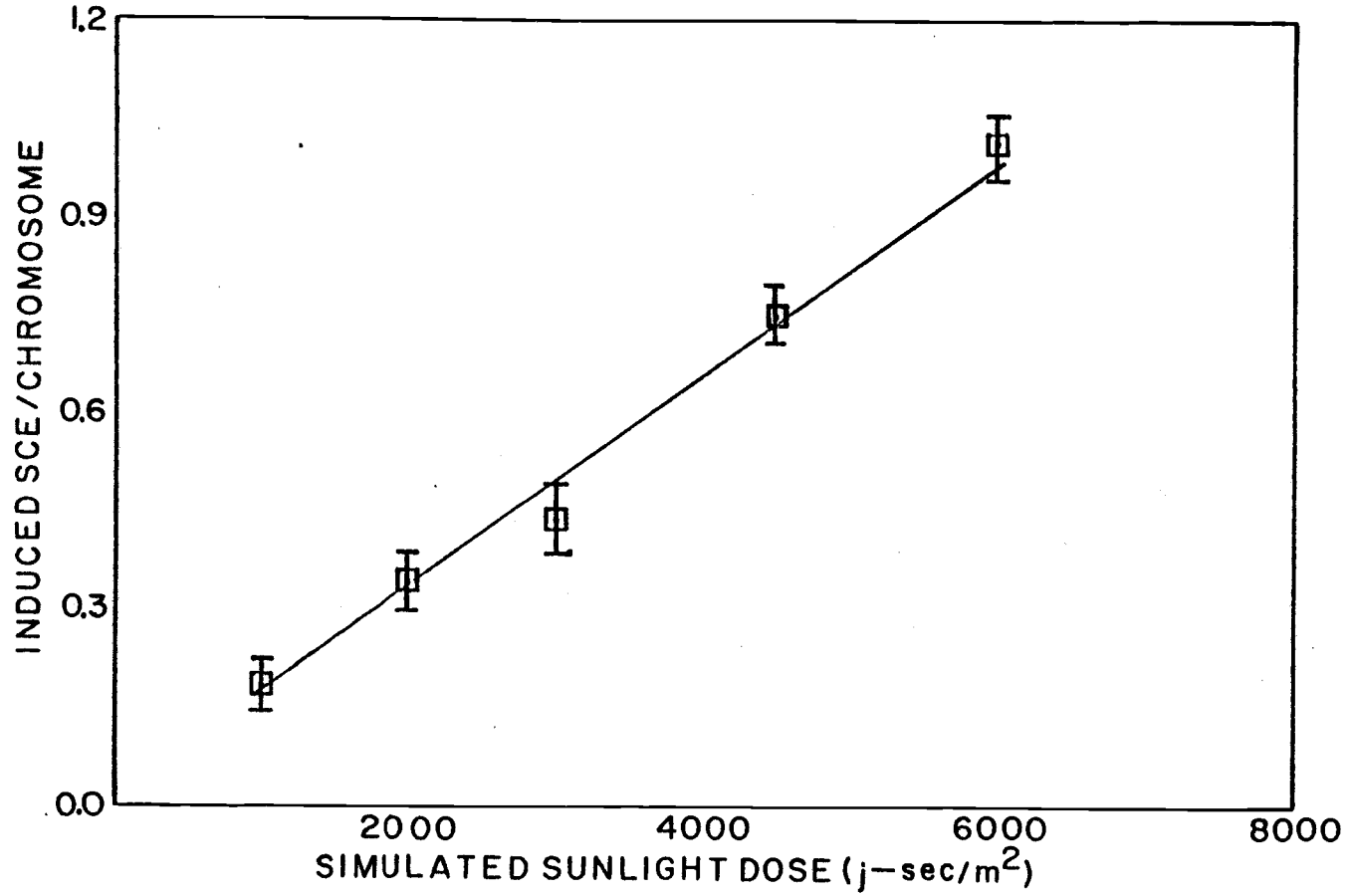


FIGURE 7. Sister-chromatid exchanges induced per chromosome after CHO cell exposure to several simulated sunlight doses

was determined for CHO cells exposed to light doses ranging from 0-12,000 j-sec/m^2. As the shoulder region in Figure 8 indicates, light doses of 0-3500 j-sec/m^2 did not alter the survival of the cells compared to a controls.

On the basis of these results, subsequent experiments were conducted with light doses of 2000 j-sec/m^2 when a PNAH compound was also present. Thus, any alterations in survival would not be attributed to an affect by light alone, but to a combination of the PNAH and the light.

In HGPRT mutation studies, it was determined that an expression time of eight days was appropriate for CHO cells that had been treated with a light dose of 8000 j-sec/m^2. The eight-day expression period was used for other combination PNAH and light exposure treatments.

CHO Cell Exposure to Light and a PNAH

Experiments, in which CHO cells were exposed to light in the presence of a PNAH, required that the manipulations be done in a fluorescent light-free area. In some cases a yellow bulb was placed over a desk-top surface while for most experiments the preparation of the flasks and dishes for a specific exposure was done in darkness. Prior to placing the prepared tissue-culture petri dishes under the lights, the lids were checked for the presence of any condensation. Condensation on the lid contributed to

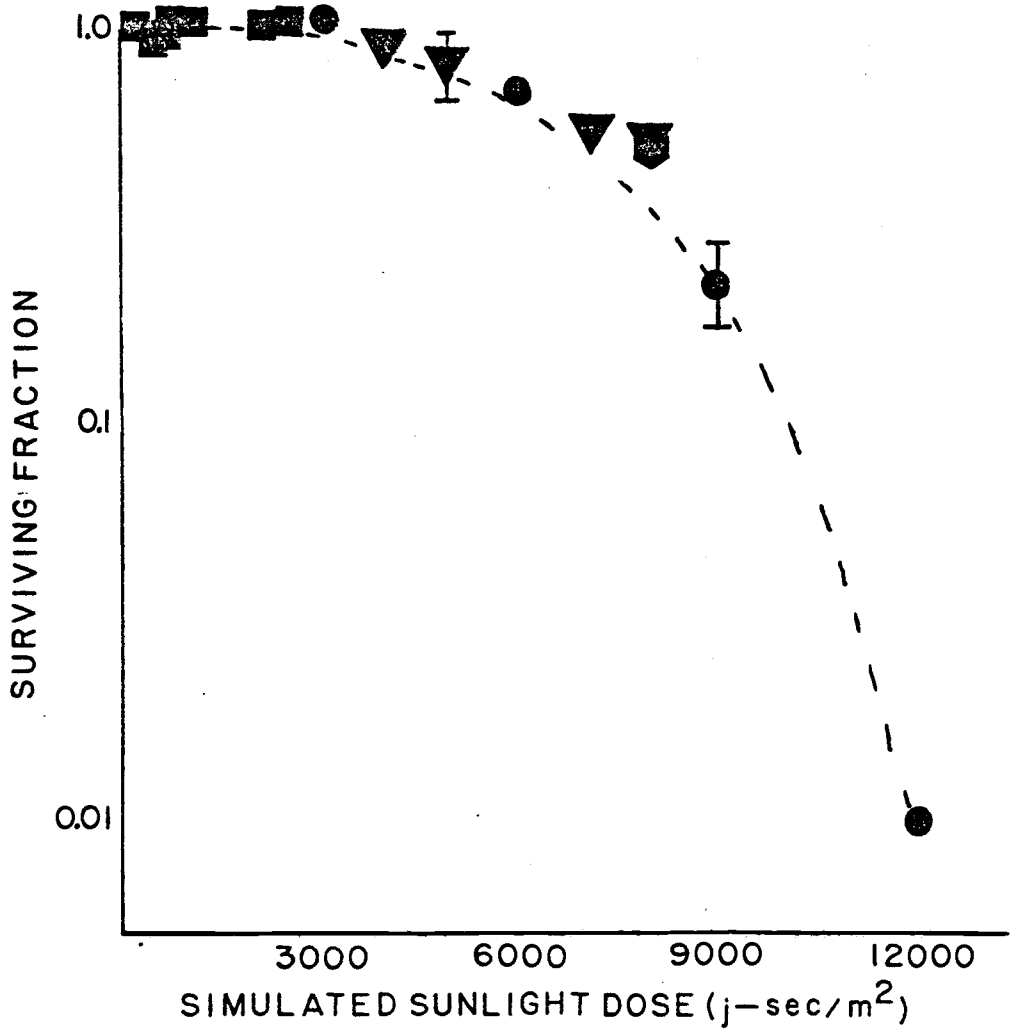


FIGURE 8. Chinese hamster ovary cell survival after irradiation with various simulated sunlight doses. The various symbols represent data obtained from independent experiments

some light attenuation thereby reducing the dose to the cells and consequently affecting the survival results. Any lids which showed any condensation were replaced with other sterile petri dish covers. The problem of condensation in the T-75 flasks was solved by inverting the flask to allow the PBS solution to absorb the condensation. The flask was then returned to its exposure position (PBS over the CHO cells).

Another problem that developed in the system was that many surviving colonies seemed to collect around the edge of the petri dish bottoms. In considering this, it was thought that the sides of the dish may have attenuated the light to an extent that cells closest to the outer edge of the colony had a better survival chance than those plated in the middle. To alleviate this problem, no colonies were counted as a survival colony that adhered to the very edge of the bottom of the dish (i.e., closest to the walls of the dish).

In presenting this section in the results, the number and variety of problems associated with the completion of the primary research objectives can be more fully understood. Solvent selection, the presence of possible photooxidation products, appropriate exposure solutions, dosimetry, and tissue-culture dishes were only a few of the problems requiring solutions before definitive experiments with PNAH-NUV effects on CHO cells could be undertaken.

Simulated Sunlight-irradiated Polynuclear Aromatic Hydrocarbons

Fluoranthene

Table 10 is a summary of the results of SCEs incurred in Chinese hamster ovary (CHO) chromosomes subjected only to fluoranthene at concentrations of 0.2, 2.0 or 4.0 ug/ml in phosphate buffered saline (PBS) or medium for various time periods; these experiments were all performed in the absence of the sunlamps. As can be determined from Table 11, with the exception of one result (4.0 ug/ml in Experiment 21), none of the treatments contributed to a statistically significant increase in SCEs when compared to a statistically significant increase in SCEs when compared to the control values. While Experiment 21 showed a correlation of increasing SCEs with increasing fluoranthene concentration, even to the point of being significantly different at the 4.0 ug/ml concentration level, the results of Experiment 26, using similar concentrations, did not offer confirmation for the results of Experiment 21. Also, though the results of experiments 31 and 41 are confined to 0.2 ug/ml concentrations, the number of SCEs was only slightly, but not significantly, increased in the PNAH-exposed cells.

The results in Table 11 indicate that at super-saturated fluoranthene concentrations (4.0 ug/ml), CHO cells exposed to a sunlamp dose of 3000 j-sec/m^2 filtered with either mylar (>320 nm)

TABLE 10. Induced sister-chromatid exchanges (SCEs) in CHO cells exposed only to 0.2, 2.0 or 4.0 ug/ml fluoranthene concentrations.

Experiment number	Treatment	Medium	Exposure time (hours)	No. SCEs/chromosome[1] ± standard error	t-test
12	Control (0.0)	PBS	5.00	.4818 ± .03	
12	0.2 ug/ml	PBS	5.00	.4412 ± .03	N.S.[5]
16	Control (0.0)	McCoy's + 10% K.C.[2]	24.00	.4547 ± .02	
16	0.2 ug/ml	McCoy's + 10% K.C.	24.00	.4439 ± .03	N.S.
21	Control 90.0)	MEM + 5/5 Gibco calf/ fetal calf[3]	24.00	.4028 ± .02	
21	0.2 ug/ml	MEM + 5/5 Gibco calf/ fetal calf	24.00	.4060 ± .03	N.S.
21	2.0 ug/ml	MEM + 5/5 Gibco calf/ fetal calf	24.00	.4287 ± .03	N.S.
21	4.0 ug/ml	MEM + 5/5 Gibco calf/ fetal calf	24.00	.4759 ± .03	$p \leq 0.05$[6]
26	Control (0.0)	PBS[4]	0.37	.5220 ± .02	
26	4.0 ug/ml	PBS	0.37	.4882 ± .02	N.S.
31	Control (0.0)	PBS	0.25	.3324 ± .02	
31	0.2 ug/ml	PBS	0.25	.3336 ± .02	N.S.
41	Control (0.0)	PBS	0.25	.3691 ± .02	
41	0.1% methanol	PBS	0.25	.3713 ± .02	N.S.
41	0.2 ug/ml	PBS	0.25	.3541 ± .02	N.S.

[1]The SCEs were counted in 40 different cells.

[2]McCoy's + 10% Kansas City serum, growth medium.

[3]Minimum Essential Medium + 5% Gibco calf and 5% Gibco fetal calf serum.

[4]Phosphate buffered saline.

[5]No significant difference compared to the control.

[6]Significantly different from the control at the 5% level.

TABLE 11. Induced sister-chromatid exchange (SCEs) in CHO cells exposed to 0.2 or 4.0 ug/ml fluoranthene and simulated sunlight doses of 2000 or 3000 j-sec/m^2.

Experiment number	Treatment	Medium	Light Dose (j-sec/m^2)	Exposure time (minutes)	No. SCEs/chromosome[1] ± standard error	t-test
26	Control (0.0)	PBS[2]	3000-mylar	22:13	.5513 ± .02	
26	4.0 ug/ml	PBS	3000-mylar	22:13	No cells	
26	Control (0.0)	PBS	3000-C.A.[3]	22:13	.7395 ± .04	
26	4.0 ug/ml	PBS	3000-C.A.	22:13	No cells	
31	Control (0.0)	PBS	2000-C.A.	14:49	.5502 ± .02	
31	0.2 ug/ml	PBS	2000-C.A.	14:49	.5672 ± .03	N.S.[4]
41	Control (0.0)	PBS	2000-C.A.	14:49	.6418 ± .04	
41	0.1% methanol (0.0)	PBS	2000-C.A.	14:49	.6103 ± .03	N.S.
41	0.2 ug/ml	PBS	2000-C.A.	14:49	.7714 ± .04	$p \leq 0.05$[5]

[1]The SCEs were counted in 40 different cells.

[2]Phosphate buffered saline.

[3]Cellulose acetate.

[4]No significant difference compared to the control.

[5]Significantly different from the control at the 5% level.

or cellulose acetate (>290 nm), did not survive. When compared to the results from exposures to dark conditions in the same experiment (Table 10, Experiment 26), CHO cells exposed to 4.0 ug/ml fluoranthene in the absence of light did survive and showed no statistically significant increase in SCEs. A comparison of these two light conditions is shown in Figure 6.

Other experimental data listed in Table 11 indicate there were contrasting results in some of the experiments. At a fluoranthene concentration of 0.2 ug/ml, CHO chromosomes exposed to a light dose of 2000 j-sec/m^2 did not exhibit a significant increase in the number of SCEs as shown in Experiment 31. However, in Experiment 41, conducted under similar conditions, there was a significant increase in SCEs. Figure 9 compares SCE results of exposures under dark and light conditions.

The data of several survival experiments, in which CHO cells were exposed to different fluoranthene concentrations, periods of time, and mediums, in the absence of light, are listed in Table 12. The results of Experiments 16 and 40 indicate there were no significant decreases in survival due to the presence of fluoranthene when compared to the survival of untreated cells. However, in Experiment 29, the survival of every treated sample was signifcantly lower than the survival of the control cells. When the surviving fractions from the treated samples are compared to those of the control that contained 0.1% methanol, there were no significant decreases in survival of any of the treated samples. Too, there

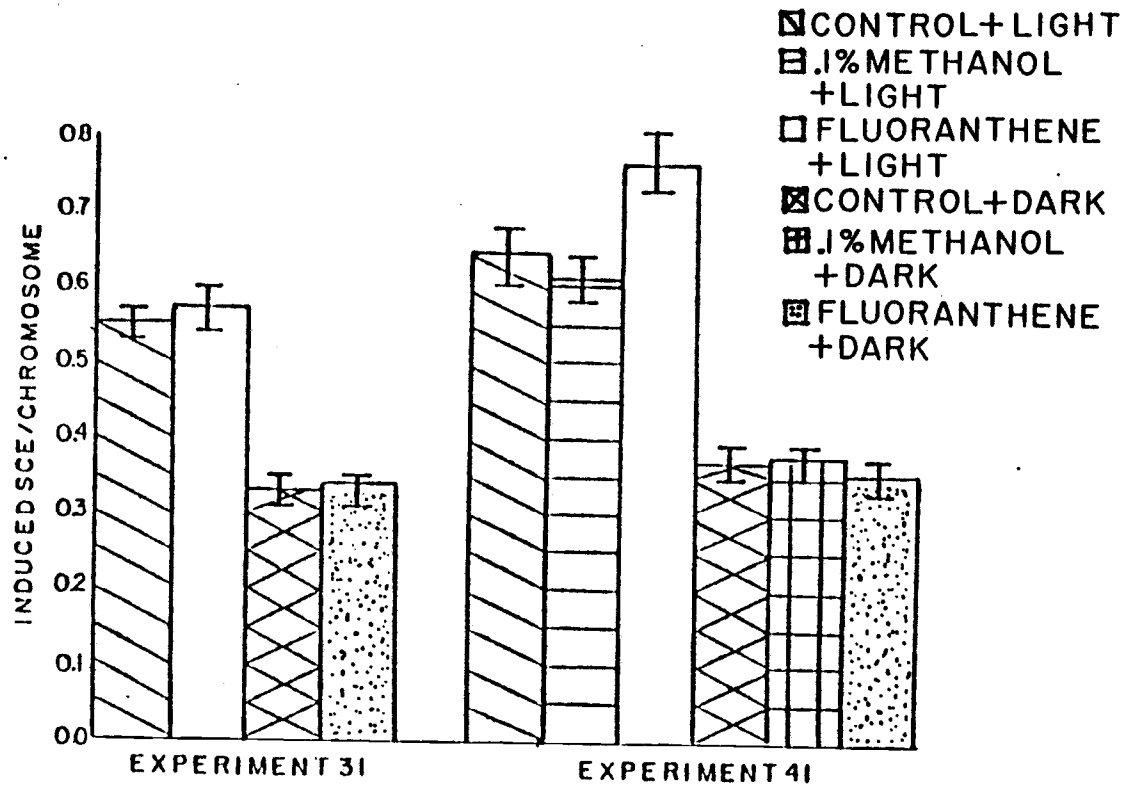


FIGURE 9. Comparison of the number of sister-chromatid exchanges induced by exposure to a simulated sunlight dose of 2000 j-sec/m^2 and no light. The concentration of fluoranthene in both experiment 31 and 41 was 0.2 ug/ml

TABLE 12. Surviving fraction of CHO cells exposed only to 0.002-1.0 ug/ml fluoranthene concentrations.

Experiment number	Treatment	Medium	Exposure time (hours)	Surviving Fraction[1] ± standard error	t-test
12	Control (0.0)	PBS[2]	5.00	.5920 ± .02	
12	0.2 ug/ml	PBS	5.00	.5390 ± .02	N.S.[4]
16	Control (0.0)	McCoy's + 10% K.C.[3]	24.00	.6400 1 .03	
29	0.2 ug/ml	McCoy's + 10% K.C.	24.00	.6156 ± .03	N.S.
29	Control (0.0)	PBS	0.23	.8483 ± .02	
29	0.1% methanol (0.0)	PBS	0.23	.6300 ± .02	$p \leq 0.001$[5]
29	0.002 ug/ml	PBS	0.23	.5822 ± .02	$p \leq 0.001$; N.S.
29	0.05 ug/ml	PBS	0.23	.6278 ± .02	$p \leq 0.001$; N.S.
29	0.1 ug/ml	PBS	0.23	.6056 ± .04	$p \leq 0.005$; N.S.[6]
29	0.2 ug/ml	PBS	0.23	.6511 ± .03	$p \leq 0.001$; N.S.
29	1.0 ug/ml	PBS	0.23	.5840 ± .02	$p \leq 0.001$; N.S.
40	Control (0.0)	PBS	0.23	.4373 ± .01	
40	0.1% methanol (0.0)	PBS	0.23	.4220 ± .01	N.S.
40	0.2 ug/ml	PBS	0.23	.4360 ± .01	N.S.
40	Control (0.0)	PBS	0.34	.4293 ± .02	
40	0.1% methanol (0.0)	PBS	0.34	.4267 ± .02	N.S.

[1]The number of surviving cells divided by the number of cells plated.

[2]Phosphate buffered saline.

[3]McCoy's + 10% Kansas City serum, growth medium.

[4]No significant difference compared to the control.

[5]Significantly different from the control at the .1% level.

[6]Significantly different from the control at the .5% level; no significant difference compared to the 0.1% methanol control.

were significant differences between the surviving fraction in the control and 0.1% methanol control in Experiment 29, but in Experiment 40 there was no significant difference between the two controls. This suggests that either the survival value for the control in Experiment 29 is too high or the survival value for the same control in Experiment 40 is too low, when compared to the survival values of the methanol and PNAH treated cells.

Table 13 summarized the data collected from two experiments. Cells were exposed to light doses of 2000 or 3000 j-sec/m^2 (Experiment 40 only), and fluoranthene concentrations of 1.0, 0.1, 0.05, 0.002 (Experiment 29 only) or 0.2 ug/ml (both experiments). In each experiment, there was a significant decrease in survival of CHO cells exposed to 2000 j-sec/m^2, in the presence of 0.2 ug/ml fluoranthene. In addition, in Experiment 29, there were significant decreases in survival at all fluoranthene concentrations, when the surviving fraction of the treated cells were compared to those of the untreated control cells. When the survival of treated cells is compared to the 0.1% methanol control value, there was no significant difference with the exception of the 0.1 ug/ml concentration. Again, however, the surviving fraction of the 0.1% control in light was also significantly decreased when compared to the control value.

The results of Experiment 42 (Table 14) indicated there was a slight increase in mutation frequency of cells exposed to simulated sunlight-irradiated fluoranthene (0.2 ug/ml) compared to

TABLE 13. Surviving fraction of CHO cells exposed to 0.002-1.0 ug/ml fluoranthene and simulated sunlight doses of 2000 or 3000 j-sec/m^2.

Experiment number	Treatment	Medium	Light Dose (j-sec/m^2)	Exposure time (minutes)	Surviving fraction[1] ± standard error	t-test
29	Control (0.0)	PBS[2]	2000-C.A.[3]	13:40	.7800 ± .01	
29	0.1% methanol (0.0)	PBS	2000-C.A.	13:40	.6580 ± .03	$p \leq 0.001$
29	0.002 ug/ml	PBS	2000-C.A.	13:40	.5833 ± .04	$p \leq 0.001$; N.S.[4]
29	0.05 ug/ml	PBS	2000-C.A.	13:40	.7211 ± .03	$p \leq 0.05$; N.S.
29	0.1 ug/ml	PBS	2000-C.A.	13:40	.5722 ± .03	$p \leq 0.001$; $p \leq 0.05$
29	0.2 ug/ml	PBS	2000-C.A.	13:40	.6467 ± .03	$p \leq 0.001$; N.S.
29	1.0 ug/ml	PBS	2000-C.A.	13:40	No cells	
40	Control (0.0)	PBS	2000-C.A.	13:40	.4193 ± .01	
40	0.1% methanol (0.0)	PBS	2000-C.A.	13:40	.4013 ± .02	N.S.
40	0.2 ug/ml	PBS	2000-C.A.	13:40	.1693 ± .02	$p \leq 0.05$
40	Control (0.0)	PBS	3000-C.A.	20:29	.3967 ± .01	
40	0.1% methanol (0.0)	PBS	3000-C.A.	20:29	.4242 ± .03	N.S.

[1]The number of surviving cells divided by the number of cells plated.

[2]Phosphate buffered saline.

[3]Cellulose acetate.

[4]Significantly different from the control at the .1% level; no significant difference compared to the 0.1% methanol control.

TABLE 14. Induced mutation frequency of CHO cells exposed to 0.2 ug/ml fluoranthene and a simulated sunlight dose of 2000 j-sec/m^2.

Treatment	Light Dose (j-sec/m^2)	Induced mutation frequency[1]
Control (0.0)	None	9.046×10^{-6}
0.1% methanol (0.0)	None	1.005×10^{-5}
0.2 ug/ml	None	8.849×10^{-6}
Control (0.0)	2000-C.A.[2]	1.392×10^{-5}
0.1% methanol (0.0)	2000-C.A.	1.724×10^{-5}
0.2 ug/ml	2000-C.A.	4.306×10^{-5}

[1]Mutation frequency (induced) is determined by this formula:

$$\frac{\text{number of mutant colonies counted}}{\text{number of cells plated x plating efficiency (PE)}}$$

[2]Cellulose acetate.

untreated cells. The results also indicated that there was no significant difference in induced mutation frequencies between the control and 0.1% methanol control treated cells.

Phenanthrene

The data in Table 15 are concerned with the sister-chromatid exchanges that occurred in phenanthrene-exposed CHO chromosomes, in the absence of light. There was a significant increase in SCEs in chromosomes exposed to 1.0 ug/ml phenanthrene for a period of 26.5 hours (Experiment 16). During a similar period, and under similar conditions, SCEs at the 5.0 and 10.0 ug/ml phenanthrene concentrations were significantly greater than the controls, but there were no differences at the 1.0 ug/ml level. In contrast to the results of Experiment 18, there was no significant increase in SCEs at the 10 ug/ml phenanthrene concentration levels in Experiment 26.

The data in Table 16 indicate that CHO chromosomes exposed to a light dose of 3000 j-sec/m^2 in the presence of 10 ug/ml phenanthrene did not show a significant increase in SCEs when compared to cells not exposed to the PNAH. However, there was a significant difference in the numbers of SCEs induced in those cells exposed to the light filtered with mylar as compared to that filtered with cellulose acetate. Cellulose acetate filtered light doses caused a significant increase in SCEs in both the

TABLE 15. Induced sister-chromatid exchanges (SCEs) in CHO cells exposed only to 1.0, 5.0 or 10.0 ug/ml phenanthrene.

Experiment number	Treatment	Medium	Exposure time (hours)	No. SCEs/chromosome[1] ± standard error	t-test
16	Control (0.0)	McCoy's + 10% K.C.[2]	26.50	.4547 ± .02	
16	1.0 ug/ml	McCoy's + 10% K.C.	26.50	.5609 ± .03	$p \leq 0.01$[4]
18	Control (0.0)	McCoy's + 10% K.C.	26.50	.3619 ± .03	
18	1.0 ug/ml	McCoy's + 10% K.C.	26.50	.4156 ± .03	N.S.[5]
18	5.0 ug/ml	McCoy's + 10% K.C.	26.50	.4564 ± .03	$p \leq 0.05$
18	10.0 ug/ml	McCoy's + 10% K.C.	26.50	.4778 ± .03	$p \leq 0.01$
26	Control (0.0)	PBS[3]	0.37	.5220 ± .02	
26	10.0 ug/ml	PBS	0.37	.4695 ± .03	N.S.

[1]The SCEs were counted in 40 different cells.

[2]McCoy's + 10% Kansas City serum, growth medium.

[3]Phosphate buffered saline.

[4]Significantly different from the control at the 1% level.

[5]No significant difference compared to the control.

TABLE 16. Induced sister-chromatid exchanges (SCEs) in CHO cells exposed to 10.0 ug/ml phenanthrene and a simulated sunlight dose of 3000 j-sec/m^2.

Experiment number	Treatment	Medium	Light Dose (j-sec/m^2)	Exposure time (minutes)	No. SCEs/chromosome[1] ± standard error	t-test
26	Control (0.0)	PBS[2]	3000-mylar	22:13	.5513 ± .02	
26	10.0 ug/ml	PBS	3000-mylar	22:13	.5384 ± .03	N.S.[4]
26	Control (0.0)	PBS	3000-C.A.[3]	22:13	.7395 ± .04	
26	10.0 ug/ml	PBS	3000-C.A.	22:13	.8343 ± .05	N.S.

[1]The SCEs were counted in 40 different cells.

[2]Phosphate buffered saline.

[3]Cellulose acetate.

[4]No significant difference compared to the control.

treated (PNAH) and untreated cells as compared to the mylar filtered exposed cells. A graphic comparison of these results is shown in Figure 6.

Chinese hamster ovary cells exposed to varying phenanthrene concentrations ranging from 3 minutes, 25 seconds to 26.5 hours, in the absence of light, showed few significantly toxic effects. Data from these experiments are shown in Table 17. With the exception of the significant increases in survival of: (1) the 0.1% methanol-treated cells in Experment 18; (2) the 5.0 ug/ml phenanthene-treated cells in Experiment 18; and (3) the 0.01 ug/ml phenanthrene-treated cells in Experiment 35, there were no significant differences in survival between samples compared to controls within given experiments.

The surviving fractions of CHO cells exposed to different light doses in the presence of varying phenanthrene concentrations are categorized in Table 18 and are shown in Figure 10. At a light dose of 2000 j-sec/m^2, the survival of CHO cells exposed to 10.0 ug/ml phenanthrene was significantly decreased when compared to the untreated, light only exposed control cells (Experiment 35). At a similar phenanthrene concentration (10.0 ug/ml), only a light dose of 6000 j-sec/m^2 caused a significant decrease in cell survival (Experiment 36). Light doses of 4500, 2000 and 500 j-sec/m^2, in combination with 10.0 ug/ml phenanthrene, caused no significant decrease in survival. Not only did Experiment 35 reveal a significant decrease in survival at a light dose of 2000 j-sec/m^2 and a

TABLE 17. Surviving fraction of CHO cells exposed only to 0.01-10.0 ug/ml phenanthrene.

Experiment number	Treatment	Medium	Exposure time (hours)	Surviving fraction[1] ± standard error	t-test
16	Control (0.0)	McCoy's + 10% K.C.[2]	24.00	.6400 ± .03	
16	1.0 ug/ml	McCoy's + 10% K.C.	24.00	.6511 ± .02	N.S.[4]
18	Control (0.0)	McCoy's + 10% K.C.	26.50	.5920 ± .02	
18	0.1% methanol (0.0)	McCoy's + 10% K.C.	26.50	.6893 ± .02	$p \leq 0.01$[5]
18	1.0 ug/ml	McCoy's + 10% K.C.	26.50	.6307 ± .02	N.S.
18	5.0 ug/ml	McCoy's + 10% K.C.	26.50	.7253 ± .04	$p \leq 0.05$
18	10.0 ug/ml	McCoy's + 10% K.C.	26.50	.5867 ± .02	N.S.
35	Control (0.0)	PBS[3]	0.25	.5850 ± .02	
35	0.1% methanol (0.0)	PBS	0.25	.5689 ± .02	N.S.
35	0.01 ug/ml	PBS	0.25	.6456 ± .01	$p \leq 0.05$
35	0.1 ug/ml	PBS	0.25	.6022 ± .03	N.S.
35	1.0 ug/ml	PBS	0.25	.6678 ± .03	N.S.
35	5.0 ug/ml	PBS	0.25	.5667 ± .03	N.S.
35	10.0 ug/ml	PBS	0.25	.5356 ± .03	N.S.
36	Control (0.0)	PBS	0.68	.4560 ± .01	
36	10.0 ug/ml	PBS	0.68	.4510 ± .02	N.S.
36	Control (0.0)	PBS	0.51	.5333 ± .01	
36	10.0 ug/ml	PBS	0.51	.5033 ± .02	N.S.
36	Control (0.0)	PBS	0.23	.4893 ± .02	
36	10.0 ug/ml	PBS	0.23	.5040 ± .01	N.S.
36	Control (0.0)	PBS	0.06	.4560 ± .03	
36	10.0 ug/ml	PBS	0.06	.3907 ± .01	N.S.

[1]The number of surviving cells divided by the number of cells plated.

[2]McCoy's + 10% Kansas City serum, growth medium.

[3]Phosphate buffered saline.

[4]No significant difference compared to the control.

[5]Significantly different from the control at the 1% level.

TABLE 18. Surviving fraction of CHO cells exposed to 0.01-10.0 ug/ml phenanthrene and simulated sunlight doses of 500-6000 j-sec/m^2.

Experiment number	Treatment	Medium	Light Dose (j-sec/m^2)	Exposure time (minutes)	Surviving fraction[1] ± standard error	t-test
35	Control (0.0)	PBS[2]	2000-C.A.[3]	13:40	.6562 ± .02	
35	0.1% methanol (0.0)	PBS	2000-C.A.	13:40	.5967 ± .01	$p \leq 0.05$[4]
35	.01 ug/ml	PBS	2000-C.A.	13:40	.6144 ± .01	N.S.[5]
35	0.1 ug/ml	PBS	2000-C.A.	13:40	.6056 ± .02	N.S.
35	1.0 ug/ml	PBS	2000-C.A.	13:40	.6844 ± .04	N.S.
35	5.0 ug/ml	PBS	2000-C.A.	13:40	.4900 ± .05	$p \leq 0.05$
35	10.0 ug/ml	PBS	2000-C.A.	13:40	.5022 ± .02	$p \leq 0.001$
36	Control (0.0)	PBS	6000-C.A.	40:59	.3560 ± .02	
36	10.0 ug/ml	PBS	6000-C.A.	40:59	.1520 ± .01	$p \leq 0.001$
36	Control (0.0)	PBS	4500-C.A.	30:44	.4400 ± .03	
36	10.0 ug/ml	PBS	4500-C.A.	30:44	.3720 ± .02	N.S.
36	Control (0.0)	PBS	2000-C.A.	13:40	.4883 ± .01	
36	10.0 ug/ml	PBS	2000-C.A.	13:40	.4147 ± .03	N.S.
36	Control (0.0)	PBS	500-C.A.	3:25	.4907 ± .03	
36	10.0 ug/ml	PBS	500-C.A.	3:25	.4733 ± .02	N.S.

[1]The number of surviving cells divided by the number of cells plated.

[2]Phosphate buffered saline.

[3]Cellulose acetate.

[4]Significantly different from the control at the 5% level.

[5]No significant difference compared to the control.

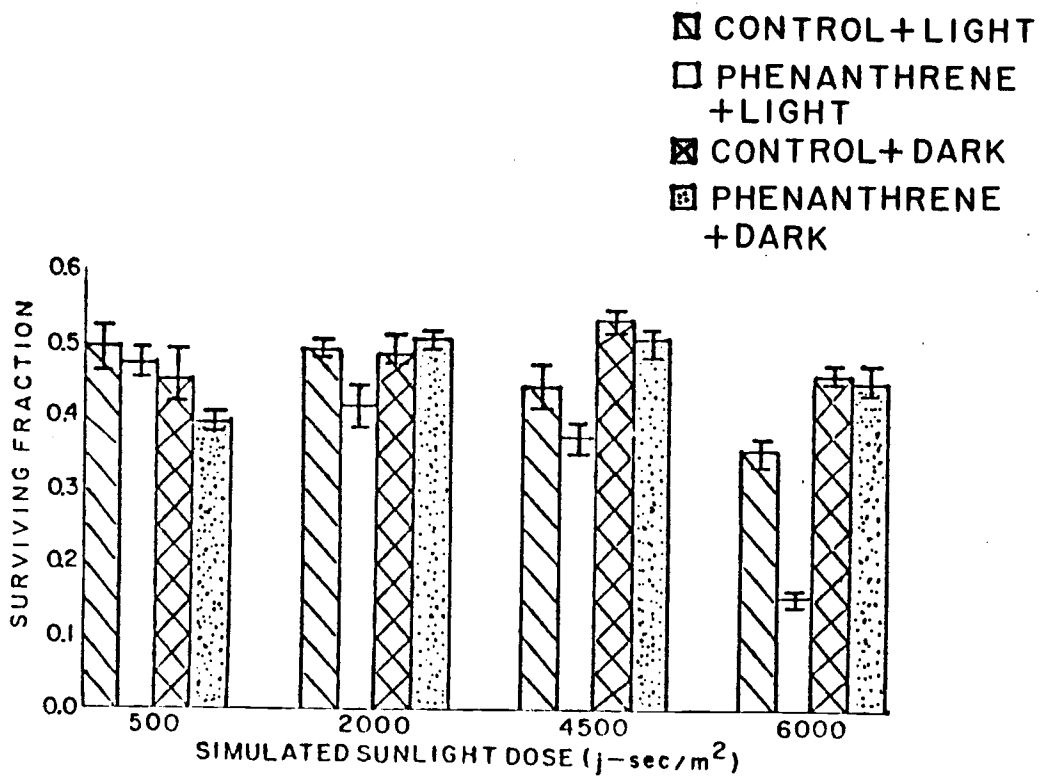


FIGURE 10. Comparison of CHO cell survivals after exposure to simulated sunlight-irradiated phenanthrene (10.0 ug/ml) and cell survivals after exposure to non-irradiated phenanthrene (10.0 ug/ml)

PNAH concentration of 10.0 ug/ml, but also reflected significant survival decreases under a similar light dose at 5.0 ug/ml and in the 0.1% methanol control groups of cells.

DISCUSSION

Development of the System

The development of a reliable system to measure the effects of simulated sunlight-irradiated polynuclear aromatic hydrocarbons (PNAHs) in mammalian cells required the consideration of several problems.

Selection of methanol as the PNAH solvent, monitroing the fluctuating PNAH concentration levels and evaluating the influence of glass vs. plastic tissue-culture flasks on changes in PNAH concentrations, were all important factors in insuring that the defined PNAH concentration was maintained during each experimental exposure. It is noted that continuous measurement of the PNAH concentration throught an experiment was not addressed previously in any PNAH related studies (Huberman and Sachs, 1976; Newbold et al., 1979; Barnhart and Cox, 1980). For example, Barnhart and Cox (1980) dissolved a PNAH in dimethylsulfoxide (DMSO) and stored it for up to 4 weeks with no consideration of measurements to detect any subsequent changes in PNAH concentration levels. My research results indicate clearly that large changes in fluoranthene, and to a lesser extent phenanthrene concentrations occurred over time. Thus, fresh solutions must be prepared prior to each experiment in order to insure accurate concentrations.

Because of the paucity of definitive studies in the literature,

I also found it was necessary to consider the effects of several other experimental materials and substances on CHO cells. The effects of methanol, plastic tissue-culture flasks, phosphate buffered saline (PBS), and 5-bromodeoxyuridine (BrdUrd) on the induction of SCEs and/or survival were all considered prior to any simulated sunlight-irradiated PNAH experiments. For each factor, no significant changes in SCEs or survivals were determined (Tables 1, 2, 3, 7, 8, and 9). My findings agree with others in which no significant effects on CHO cells were associated with phosphate buffered saline (Hsie et al., 1977; Zelle et al., 1980), BrdUrd (Wolff and Perry, 1974) or methanol (Obe and Ristow, 1977). Finally, it should be mentioned that the effect of plastic tissue-culture flasks on the induction of SCEs and survival has not been well-documented in the literature. Therefore, the results of my study can serve as a model for other studies to be conducted using this system. Once these problems had been considered and evaluated, I proceeded to use the system I developed to gather som preliminary data on the effects of simulated sunlight-irradiated PHANs on CHO cells.

Simulated Sunlight-irradiated Polynuclear Aromatic Hydrocarbons (PNAHs)

The process of light-activation of promutagens/procarcinogens as opposed to either enzymatic or metabolic activation was first considered by Lewis (1935). He showed that by combining certain

PNAHs and light, the killing of cultured chick embryo cells was enhanced (Lewis, 1935). More recent studies that have examined the effects of light-activated PNAHs in mammalian cells have been confined to the use of survival and the HGPRT mutation frequency assays (Santamaria et al., 1966; Jones and Leber, 1979; Barnhart and Cox, 1980; Strniste and Brake, 1980; Strniste et al., 1980-1981). No references about the use of the SCE technique as a way to assess simulated sunlight-PNAH effects on CHO cells have been mentioned in the literature.

Mutation frequency and cell survival have been utilized as tools to measure the effects of near-ultraviolet radiation on CHO cells (Todd et al., 1968; Jostes et al., 1977; Rothman and Setlow, 1978; Zelle et al., 1980). The mutagenic and toxic effects of PNAH compounds have also been examined extensively (Huberman and Sachs, 1976; Newbold et al., 1979; Oesch et al., 1981). The use of the sister-chromatid exchange assays, however, have been limited to studies on the effects of near-ultraviolet light (Kato, 1973; Rommelaere et al., 1973; Wolff et al., 1974) or with studies that were concerned only with the effects of PNAH compounds on CHO chromosomes (Bayer and Bauknecht, 1977; Connell, 1979; Roszinsky-Kocher et al., 1979).

Fluoranthene

The sister chromatid exchange results described in Tables 10 and 11 indicate several things. When cells were exposed to

non-irradiated fluoranthene concentrations (Table 10), there were no significant differences between the number of SCEs induced in treated cells as compared to untreated cells in all but one of the experiments; the one exception was the 4.0 ug/ml fluoranthene concentration treatment in Experiment 21. A similar concentration exposure in Experiment 26 did not cause a significant increase in SCEs as compared to the control. However, there was a substantial difference in exposure time between experiments (24 hours for Experiment 21 vs. .37 hours for Experiment 26) which could have accounted for the variability in SCE induction. Another interesting result (Table 10) was that, as the fluoranthene concentrations increased beyond the maximum solubility level (0.2 ug/ml), the number of SCEs per chromosome also increased. At concentrations that exceed the maximum solubility level, the results of the HPLC analysis indicated that fluoranthene was not entirely in solution but was also present in a crystalline form. This fact leads me to believe that the increased number of SCEs at the higher concentrations may be the result of a membrane-type of effect caused by the fluoranthene crystals.

A light dose of 2000 j-sec/m^2 applied to 0.2 ug/ml fluoranthene-treated cells caused no significant increase in the number of SCEs (Experiment 31, Table 11). By contrast, CHO chromosomes subjected to similar conditions in Experiment 41 did show a significant increase in SCEs when compared to an untreated (no PNAH), but light exposed control. This apparent difference may have been

due to experimental differences in plating efficiency, the count in the number of SCEs and/or fluoranthene concentrations. One result that appears in Table 11 is more closely associated with cell survival than sister-chromatid exchanges. Cells exposed to super-saturated fluoranthene concentrations (4.0 ug/ml) only, and to a 3000 j-sec/m^2 light dose did not survive.

It is also of interest to note that the number of SCEs was significantly higher in cellulose acetate filtered light exposures compared to mylar filtered light exposures. Thus, the harmful nature of the near-ultraviolet region (290-320 nm) was reflected in an increased number of sister-chromatid exchanges as well as by the death of CHO cells when fluoranthene was also present at the doses investigated. The results of Elkind and Han (1977) and Rothman and Setlow (1978) both suggest that damage can occur to cells that are exposed to these wavelengths of light.

In another experiment (Experiment 29, Table 13) with a similar result, cells exposed to 2000 j-sec/m^2 cellulose acetate filtered light and 1.0 ug/ml fluoranthene also failed to survive. Light doses of 2000 and 3000 j-sec/m^2 occur in the shoulder region of the light only survival curve (Figure 8) and, thus, could not be solely responsible for the cell death in these two experiments. Therefore, super-saturated concentrations of fluoranthene (>0.2 ug/ml) irradiated with such light doses may have been activated to cause a toxic effect.

Fluoranthene concentrations near maximum solubility levels did

not contribute to significant decreases in survival in non-irradiated conditions (Table 12). Again, these results suggest that some form of activiation is necessary in order to convert a parent PNAH compound to a toxic agent. Though there appeared to be significant differences in survival between untreated and treated cells in Experiment 29 (Table 12), I believe the reason for this was due to an anomalously high control value. When the treatment survival values are compared with the 0.1% methanol control value, there are no significant differences. The high control value was most likely caused by an error in plating. Further, the results of Experiments 12, 16, and 40 all indicated no significant differences in survival between the untreated controls, the 0.1% methanol controls or the 0.2 ug/ml fluoranthene-treated cells.

The results of Experiment 29 shown in Table 13 indicate that CHO cells exposed to simulated sunlight-irradiated fluoranthene showed significant differences in survival when compared to the light only (no PNAH) exposed control cells. However, I believe the control value for this experiment may have been abnormally high and should not be considered as the basis for comparison of the treated values. When the treated cell survival results are compared to the 0.1% methanol control, the only significant decrease in survival occurred at a fluoranthene concentration of 0.1 ug/ml. It is noted that the 0.2 ug/ml value was not significantly different when compared to the 0.1% methanol control. In addition, the results of Experiment 40 (Table 13) show the survival of cells

exposed to a similar fluoranthene concentration (0.2 ug/ml) was significantly decreased when compared to survival of the untreated (no PNAH), but light exposed cells. The results of these two studies indicates the survival of CHO cells exposed to simulated sunlight-irradiated (2000 j-sec/m^2) fluoranthene concentrations at the maximum solubility level (0.2 ug/ml) may be significantly decreased when compared to the survival of light only exposed cells. Also, there may have been some decrease in survival in those cells exposed to a similar light dose in the presence of fluoranthene concentrations that were less than the maximum solubility level (<0.2 ug/ml). However, further studies will be required to confirm or deny these findings.

The interpretation of results described in Table 14 is supported by data included in Tables 10-13. Fluoranthene (0.2 ug/ml) exposed to a light dose of 2000 j-sec/m^2 may have been activated to a mutagenic agent. The fluoranthene-treated cells did show an increase in mutation frequency as compared to the untreated (no PNAH), light exposed cells. Again, additional studies will be required to confirm this preliminary result.

Phenanthrene

The results included in Table 15 are conflicting and difficult to interpret. The maximum solubility level of phenanthrene (1.0 ug/ml) appeared in Experiment 15 to induce SCEs at a significantly

higher level than the control, while in Experiment 18 that was not the case. Also, the resulls of Experiment 18 indicated there was a significant increase in SCE induction at super-saturated levels (5.0 and 10.0 ug/ml), but the results of Experiment 26 at 10 ug/ml did not confirm this. On the basis of other work described in the literature, phenanthrene is not considered to be a mutagenic or carcinogenic agent (Huberman and Sachs, 1976; Bucker et al., 1979, Oesch et al., 1981). The results of Table 16 show there was no significant increase in the induction of SCEs in CHO chromosomes exposed to a light dose of 3000 j-sec/m^2 and 10.0 ug/ml phenanthrene as compared to a similarly light only treated control. Also, the number of SCEs induced by cellulose acetate filtered light was significantly greater than that induced by the mylar filtered light. However, unlike phenanthrene, fluoranthene did not induce any SCEs in Experiment 26, because the cells did not survive.

Tables 17 and 18 summarize the survival results of phenanthrene-exposed cells in the absence or presence of simulated sunlight, respectively. The only significant differences in survival (Table 17) were those where the treated (phenanthrene) cells had a significantly greater survival than untreated control cells. Except for the differences in results between Experiments 18 and 36, it might seem that a super-saturated level of phenanthrene actually enhanced the survival of CHO cells in the absence of light. However, the conclusion I have drawn from this data is that super-saturated levels of phenanthrene did not affect the survival of CHO

cells in the absence of an activation event such as exposure to simulated sunlight. Again, errors in plating cell numbers were probably the cause of the questionable values in Experiment 18.

Chinese hamster ovary cells exposed to super-saturated phenanthrene concentration levels (5.0 or 10.0 ug/ml) and a light dose of 2000 j-sec/m^2 showed a significant decrease in cell survival as compared to the survival of untreated (no phenanthrene) light only exposed cells (Experiment 35, Table 18). However, data from Experiment 36 did not support these results since cells exposed to similar conditions (2000 j-sec/m^2 and 10.0 ug/ml phenanthrene) did not show a significant decrease in survival as compared to the survival of the untreated (no PNAH) cells.

A light dose of 6000 j-sec/m^2 combined with a dose of 10.0 ug/ml phenanthrene was required to cause a significant decrease in cell survival. It is uncertain whether this decrease in survival was the result of the high light dose alone, or due to the combination of simulated sunlight and phenanthrene; 6000 j-sec/m^2 is in the exponential part of the light only survival curve (Figure 8), and hence, could cause a decrease in survival without the presence of a PNAH substance. Nevertheless, in each study listed in Experiment 36, phenanthrene-treated cells consistently showed a lower fraction of survival than did the light only exposed cells to which they were compared. That suggests the decrease in survival reflected in these experiments may have been related to the action of an unknown agent derived from the possible activation of phenanthrene by

simulated sunlight.

The emphasis of this research was to develop an experimental system that could be used to evaluate the effects of PNAHs, simulated sunlight, and simulated sunlight-PNAH interactions on Chinese hamster ovary cells, using sister-chromatid exchanges, survival and mutation frequencies as the endpoints. I believe that the system developed during these studies has significant potential for application in these and other studies using different organic chemicals.

SUMMARY

A system that can simulate the interactions of sunlight (specifically the near-ultraviolet region) and polynuclear aromatic hydrocarbons (PNAHs) in the aquatic environment has been developed as a result of this research. Various components of the system have been tested for their individual effects on Chinese hamster ovary (CHO) cells with respect to inducing sister-chromatid exchanges (SCEs) or mutation frequencies, and decreasing cell survivals. Methanol, plastic tissue-culture dishes, phosphate buffered saline and 5-bromodeoxyuridine (in SCE experiments only) all have been shown not to significantly affect the growth or development of mammalian cells.

A second objective of this research was to measure the effects of simulated sunlight-irradiated PNAHs on CHO cells. Several things were learned in completing this objective, including the following:

- The presence of fluoranthene or phenanthrene at maximum solubility concentrations in medium (24 hours) or phosphate buffered saline (5 hours) did not induce sister-chromatid exchanges or decrease CHO cell survival when compared to control values.
- Simulated sunlight (>290 nm) did contribute to a linear increase in sister-chromatid exchanges with corresponding increases in light dose.

- Simulated sunlight-irradiated fluoranthene at super-saturated concentrations resulted in cell death, while similarly exposed maximum solubility concentration levels of fluoranthene showed only slight decreases in survival, as well as increases in sister-chromatid exchanges and mutation frequencies.

- Simulated sunlight-irradiated phenanthrene at super-saturated concentrations showed a significant decrease in cell survival, but no increases in sister-chromatid exchanges. Phenanthrene present at the maximum solubility level and exposed to simulated sunlight did not contribute to decreases in cell survival or increases in SCEs.

CONCLUSIONS

In this research, the system developed to simulate and measure the effects of sunlight and polynuclear aromatic hydrocarbon interactions in the aqueous environment will serve as a tool for future studies in this area of interest.

The results of this study indicate that solar-irradiated fluoranthene may be mutagenic to mammalian cells. More importantly, at concentrations that exceed the maximum solubility level, the results of such an interaction may be cell death.

By contrast, with respect to solar-irradiated phenanthrene, the results suggest that there may be only a slight decrease in survival at concentrations that exceed the maximum solubility levels. Further, the results of this study give no indication that there is a mutagenic effect induced in CHO cells after exposure to solar-irradiated phenanthrene.

In conclusion, it would be advisable to repeat several of the solar-irradiated PNAH experiments to further confirm or deny the results reported here. In addition, further research could be directed towards testing a variety of other PNAHs and their capacity for being activated to mutagenic or carcinogenic compounds. Both the system developed here and the established assays would make this type of project a feasible one.

REFERENCES CITED

Ames, B. N., W. E. Durston, E. Yamasaki and F. D. Lee. 1973. Carcinogens are mutagens: A simple test system combining liver homogenates for activation and bacteria for detection. Proceedings of the National Academy of Science U.S.A. 70, 2281-2285.

Barnhart, B. J. and S. H. Cox. 1980. Mutation of Chinese hamster ovary cells by near-ultraviolet activation of promutagens. Mutation Research 72, 135-142.

Bauknecht, T., W. Vogel, U. Bayer, and D. Wild. 1977. Comparative in vivo mutagenicity testing by sister-chromatid exchanges and micronucleus induction in mouse bone marrow. Human Genetics 35, 299-307.

Bayer, U. and T. Bauknecht. 1977. The dose-dependence of sister-chromatid exchanges induced by three hydrocarbons, in the in vivo bone marrow test with Chinese hamsters. Experentia 33, 25.

Berger, D. S. 1976. The sunburning ultraviolet meter: Design and performance. Photochemistry and Photobiology 24, 587-593.

Boyland, E. and P. Sims. 1962. Metabolism of Polycyclic Compounds: The metabolism of phenanthrene in rabbits and rats: dihydrodihydroxy compounds and related glucosiduronic acids. Biochemical Journal 84, 571-586.

Boyland, E. and F. Sims. 1964. Metabolism of Polycyclic Compounds: The metabolism of benz(a)anthracene. Biochemical Journal 91, 493-506.

Boyland, E. and P. Sims. 1965a. Metabolism of Polycyclic Compounds: The metabolism of 9, 10-epoxy-9, 10-dihydrophenanthrene in rats. Biochemical Journal 95, 788-792.

Boyland, E. and P. Sims. 1965b. The metabolism of benz(a)anthracene and dibenz(a)a anthracene and their 5, 6-epoxy-5, 6-dihyro derivatives by rat-liver homogenates. Biochemical Journal 97, 7-16.

Bradley, M. O., I. C. Hsu and C. C. Harris. 1979. Relationships between sister-chromatid exchange and mutagenicity, toxicity and DNA damage. Nature (London) 282, 318-320.

Bücker, M., H. R. Glatt, K. L. Platt, D. Avnir, Y. Ittah, J. Blum and F. Oesch. 1979. Mutagenicity of phenanthrene and phenanthrene K-region derivatives. Mutation Research 66, 337-348.

Carrano, A. V., L. H. Thompson, P. A. Lindl and J. L. Minker. 1978. Sister-chromatid exchange as an indicator of mutagenesis. Nature (London) 271, 551-553.

Chasin, L. A. 1973. The effect of ploidy on chemical mutagenesis in cultured Chinese hamster cells. Journal of Cellular Physiology 82, 299-308.

Connell, J. R. 1979. The relationship between sister-chromatid exchange, chromosome aberration and gene mutation induction by several reactive polycyclic hydrocarbon metabolites in cultured mammalian cells. International Journal of Cancer 24, 485-489.

Elkind, M. M., A. Han and C. M. Chang-Liu. 1977. "Sunlight"-induced mammalian cell killing: A comparative study of ultraviolet and near-ultraviolet inactivation. Photochemistry and Photobiology 27, 709-715.

Elkind, M. M., and A. Han. 1977. DNA single-strand lesions due to "sunlight" and ultraviolet light: A comparison of their induction in Chinese hamster and human cells, and their fate in Chinese hamster cells. Photochemistry and Photobiology 27, 717-724.

Gibson, T. L., V. B. Smart and L. L. Smith. 1978. Non-enzymatic activation of polycyclic aromatic hydrocarbons as mutagens. Mutation Research 49, 153-161.

Grover, P. L. and P. Sims. 1970. Interactions of the K-region epoxides of phenanthrene and dibenz(a,h)anthracene with nucleic acids and histone. Biochemical Pharmacology 19, 2251.

Hsie, A. E., P. A. Brimer, T. J. Mitchell and D. G. Gosslee. 1975. The dose-response relationship for ultraviolet light induced mutations at the hypoxanthine-guanine phosphoribosyl transferase locus in Chinese hamster ovary cells. Somatic Cell Genetics 1, 383-389.

Hsie, A. W., A. P. Li and R. Machanoff. 1977. A fluence response study of lethality and mutagenicity of white, black, and blue fluorescent light, sunlamp, and sunlight irradiation in Chinese hamster ovary cells. Mutation Research 45, 333-342.

Huberman, E. and L. Sachs. 1976. Mutability of different genetic loci in mammaliam cells by metabolically activated carcinogenic polycyclic hydrocarbons. Proceedings of the National Academy of Science U.S.A. 73, 188-192.

Ikushima, T. and S. Wolff. 1974. Sister-chromatid exchanges induced by light flashes to 5-bromodeoxyuridine and 5-iododeoxyuridine substituted Chinese hamster chromosomes. Experimental Cell Research 87, 15-19.

Jagger, J. 1967. Introduction to Research in Ultraviolet Photobiology. Englewood Cliffs, New Jersey: Prentice-Hall. 162pp.

Jones, P. W. and P. Leber. 1979. Polynuclear Aromatic Hydrocarbons Third International Symposium on Chemistry and Biology Carcinogenesis and Mutagenesis. Ann Arbor, Michigan: Ann Arbor Science Publishers, Inc., pp. 171-189.

Jostes, R. F., W. C. Dewey and L. E. Hopwood. 1977. Mutagenesis by fluorescent light in mammalian cell cultures. Mutation Research 42, 139-144.

Kao, F. T. and T. T. Puck. 1969. Genetics of somatic mammalian cells. IX. Quantitation of mutagenesis by physical and chemical agents. Journal of Cellular Physiology 74, 245-250.

Kato, H. 1973. Induction of sister-chromatid exchanges by ultraviolet light and its inhibition by caffeine. Experimental Cell Research 82, 383-390.

Keith, L. H. and W. A. Telliard. 1979. Priority pollutants. I-A perspective view. Environmental Science and Technology 13, 416-423.

Koller, L. R. 1965. Ultraviolet Radiation. New York: John Wiley and Sons. 312pp.

Kraybill, H. F., C. J. Dawe, J. C. Harshbarger and R. G. Tardiff (editors). 1977. Aquatic pollutants and biologic effects with emphasis on neoplasia. Annals of New York Academy of Science 298, 1-604.

Latt, S. A. 1973. Microfluorometric detection of deoxyribonucleic acid replication in human metaphase chromosomes. Proceedings of the National Academy of Science U.S.A. 70, 3395-3399.

Lewis, M. R. 1935. The photosensitivity of chick embryo cells growing in media containing certain carcinogenic substances. American Journal of Cancer 25, 305-309.

May, W. E., S. P. Wasik and D. H. Freeman. 1978. Determination of the solubility behavior of some polycyclic aromatic hydrocarbons in water. Analytical Chemistry 50, 997.

May, W. E. 1980. The solubility behavior of polycyclic aromatic hydrocarbons in aqueous systems. In: Advances in Chemistry, Series No. 185; Petroleum in the Marine Environment. (L. Petrakis and F. T. Weiss, eds.), pp. 143-192.

Miller, E. C. and J. A. Miller. 1971. Mutagenicity of Chemical Carcinogens: Correlations, Problems, and Interpretations. In: Chemical Mutagens: Principles and Methods of Their Detection. (A. Hollaender, ed.). New York-London: Plenum Press, Volume 1, pp. 83-113.

Mix, M. C. and R. L. Schaffer. (in press). Concentrations of unsubstituted polynuclear aromatic hydrocarbons in bay mussels (Mytilus edulis) from Oregon, U.S.A. Marine Environmental Research.

Miyata, N., K. Shudo, Y. Kitahara, G. F. Huang and T. Okamoto. 1976. Mutagenicity of K-region epoxides of polycyclic aromatic compounds: Structure-activity relationship. Mutation Research 37, 187-192.

National Cancer Institute, SRI International, Mitre Corporation and Department of Energy. 1981. Evaluation and classification of the potential carcinogenicity and mutagenicity of chemical biorefractories identified in drinking water. First Report. U.S. Government Printing Office (1981-341-132/3582), Washington, D.C.

Neff, J. M. 1979. Polycyclic aromatic hydrocarbons in the aquatic environment. England: Applied Science Publishers, Ltd. 262pp.

Newbold, R. F., and P. Brookes. 1976. Exceptional mutagenicity of a benzo(a)pyrene diol epoxide in cultured mammalian cells. Nature (London) 261, 52-54.

Newbold, R. F., C. B. Wigley, M. H. Thompson and P. Brookes. 1977. Cell-mediated mutagenesis in cultured Chinese hamster cells by carcinogenic hydrocarbons. Nature and extent of the associated hydrocarbon-DNA reaction. Mutation Research 43, 101-116.

Newbold, R. F., P. Brookes and R. G. Harvey. 1979. A quantitative comparison of the mutagenicity of carcinogenic polycyclic hydrocarbon derivatives in cultured mammalian cells. International Journal of Cancer 24, 203-209.

Obe, G. and H. Ristow. 1977. Acetaldehyde, but not ethanol, induces sister-chromatid exchanges in Chinese hamster cells in vitro. Mutation Research 56, 211-213.

Oesch, F., M. Bücker and H. R. Glatt. 1981. Activation of phenanthrene to mutagenic metabolites and evidence for at least two different activation pathways. Mutation Research 81, 1-10.

O'Neill, J. P., P. A. Brimer, R. Machanoff, G. P. Hirsch and A. W. Hsie. 1977. A quantitative assay of mutation induction at the hypoxanthine-guanine phosphoribosyl transferase locus in Chinese hamster ovary cells (CHO/HGPRT system): Development and Definition of the System. Mutation Research 45, 91-101.

Parrish, J. A., R. R. Anderson, F. Urvach and D. Pitts. 1978. Sources of UV-A. In: UV-A, Biological Effects of Ultraviolet Radiation with Emphasis on Human Responses to Longwave UV. New York: Plenum Press, pp. 7-36.

Perry, P. and S. Wolff. 1974. New Giemsa method for the differential staining of sister-chromatids. Nature (London) 251, 156-158.

Perry, P. E. 1980. Chemical mutagens and sister-chromatid exchange. In: Chemical Mutagens: Principles and Methods for Their Detection. (F. J. de Serres and A. Hollaender, eds.). New York-London: Plenum Press, Volume 6, pp. 1-32.

Przybytek, J. T. (editor). 1980. High Purity Solvent Guide. Burdick and Jackson Laboratories, Inc., pp. 46-47 and pp. 72-73.

Puck, T. T., S. Ciecuira and A. Robinson. 1958. Genetics of somatic mammalian cells. III. Long-term cultivation of euploid cells from human and animal subjects. Journal of Experimental Medicine 108, 945-948.

Roe, E. M. F. 1966. Polycyclic Aromatics. In: UV Atlas of Organic Compounds. New York: Plenum Press, Volume 3.

Rommelaere, J., M. Susskind and M. Errera. 1973. Chromosome and chromatid exchanges in Chinese hamster cells. Chromosoma 41, 243-257.

Roszinsky-Köcher, G., A. Basler and G. Röhrborn. 1979. Mutagenicity of polycyclic hydrocarbons, V. induction of sister-chromatid exchanges in vivo. Mutation Research 66, 65-67.

Rothman, R. H. and R. B. Setlow. 1978. An action spectrum for cell killing and pyrimidine dimer formation in Chinese hamster V-79 cells. Photochemistry and Photobiology 29, 57-61.

Santamaria, L., G. G. Giordana, M. Altisi and F. Cascione. 1966. Effects oflight on 3,4-benzpyrene carcinogens. Nature (London) 210, 824-825.

Schwartz, J. L., M. J. Banda and S. Wolff. 1982. 12-O-Tetradecanoylphorbol-13-acetate (TPA) induces sister chromatid exchanges and delays in cell progression in Chinese hamster ovary and human cell lines. Mutation Research 92, 393-409.

Setlow, R. B. 1974. The wavelengths in sunlight effective in producing skin cancer: A theoretical analysis. Proceedings of the National Academy of Science U.S.A. 71, 3363-3366.

Sharp, J. D., N. E. Capecchi and M. R. Capecchi. 1973. Altered enzymes in drug resistance variants of mammalians tissue culture cells. Proceedings of the National Academy of Science U.S.A. 70, 3145-3149.

Smith, R. C., and J. E. Tyler. 1976. Transmission of solar radiation into natural waters. Photochemistry and Photobiology Reviews 1, 117.

Stoien, J. O. and R. J. Wang. 1974. Effect of near-ultraviolet and visible light on mammalian cells in culture. II. Formation of toxic photoproducts in tissue culture medium by blacklight. Proceedings of the National Academy of Science U.S.A. 71, 3961-3965.

Strniste, G. F. and R. J. Brake. 1980. Photoactivity of near-ultraviolet light irradiated benzo(a)pyrene. Federal Proceedings 39, 2010.

Strniste, G. F. and R. J. Brake. 1981. Cytotoxicity in human skin fibroblasts induced by photoactivated polycyclic aromatic hydrocarbons. In: Poly-nuclear Aromatic Hydrocarbons: Chemistry and Biological Effects. (W. M. Cook and A. J. Davis, editors). Columbus, Ohio, Battelle Press, pp. 305-311.

Strniste, G. F. E. Martinez, A. M. Marinez and R. J. Brake. 1980. Photo-induced reactions of benzo(a)pyrene with DNA in vitro. Cancer Research 40, 245-252.

Strniste, G. F. and D. J. Chen. 1981. Cytotoxicity and mutagenic properties of shale oil byproducts 1. Activation of retort process waters with near-ultraviolet light. Environmental Mutagenesis 3, 221-231.

Taylor, J. H. 1958. Sister-chromatid exchanges in tritium-labeled chromosomes. Genetics 43, 515-529.

Todd, P., T. P. Coohill and J. A. Mahoney. 1968. Responses of cultured Chinese hamster cells to ultraviolet light of different wavelengths. Radiation Research 35, 390-400.

Van Zeeland, A. A. and J. W. I. M. Simons. 1976. Linear dose response relationship after prolonged expression times in V-79 Chinese hamster cells. Mutation Research 35, 129-138.

Wang, R. J., J. D. Stoien and F. Lands. 1974. Lethal effect of near-ultraviolet irradiation on mammalian cells in culture. Nature (London) 247, 43-45.

Wolff, S. and P. Perry. 1974. Differential Giemsa staining of sister-chromatids and the study of sister-chromatid exchanges without autoradiography. Chromosoma (Berlin) 48, 341-353.

Wolff, S., J. Bodycote and R. B. Painter. 1974. Sister-chromatid exchanges induced in Chinese hamster cells by UV irradiation of different stages of the cell cycle: The necessity for cells to pass through S. Mutation Research 25, 73-81.

Zafiriou, O. C. 1977. Marine organic photochemistry reviewed. Marine Chemistry 5, 497.

Zakharov, A. F. and N. A. Egolina. 1972. Differential spiralization along mammalian mitotic chromosomes. I. BudR-revealed differentiation in Chinese hamster chromosomes. Chromosoma 38, 341-365.

Zelle, B., R. J. Reynolds, M. J. Kottenhagen, A. Schuite and P. H. M. Lohman. 1980. The influence of the wavelength of ultraviolet radiation on survival, mutation induction and DNA repair in irradiated Chinese hamster cells. Mutation Research 72, 491-509.

Zepp, R. G. 1980. Assessing the photochemistry of organic pollutants in aquatic environments. In: Dynamics, Exposure and Hazard Assessment of Toxic Chemicals. (R. Haque, editor). Ann Arbor, Michigan: Ann Arbor Science Publishers, Inc., pp. 69-104.

Zepp, R. G. and P. Scholtzhauer. 1979. In: Proceedings of Third International Symposium on Polynuclear Aromatic Hydrocarbons. (P. W. Jones and P. Lever, editors). Ann Arbor, Michigan: Ann Arbor Science Publishers, Inc., pp. 141-158.

APPENDICES

APPENDIX A

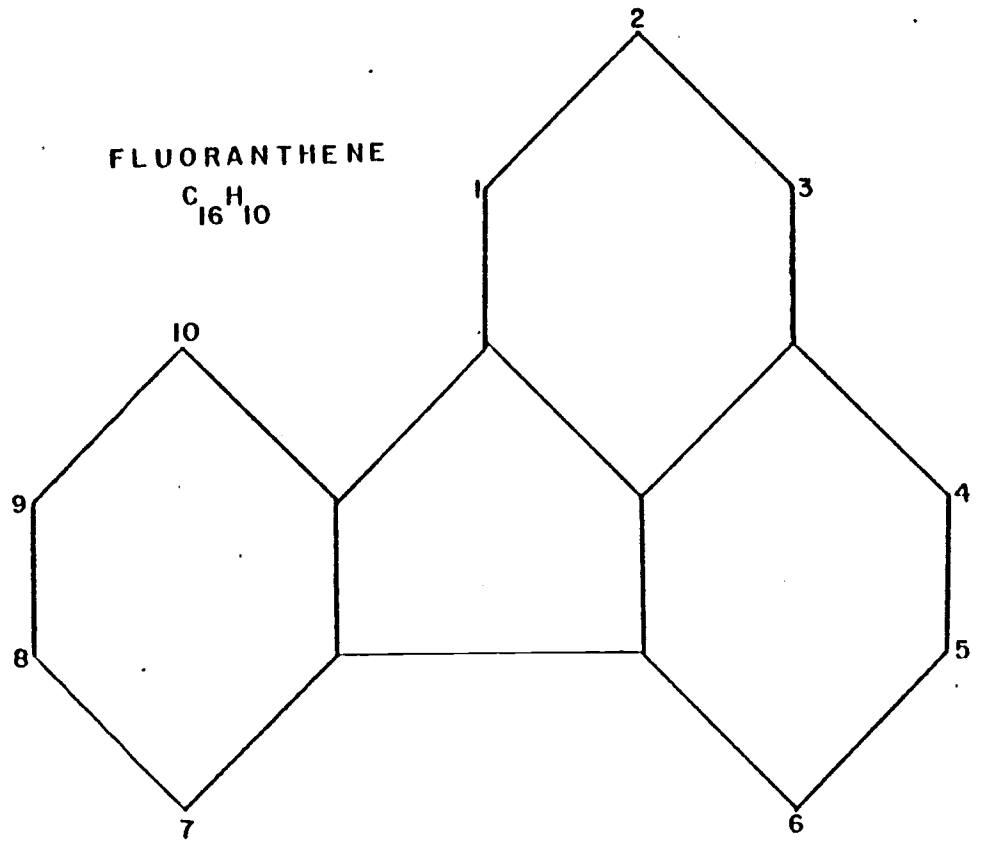


FIGURE 11. Molecular structure of fluoranthene

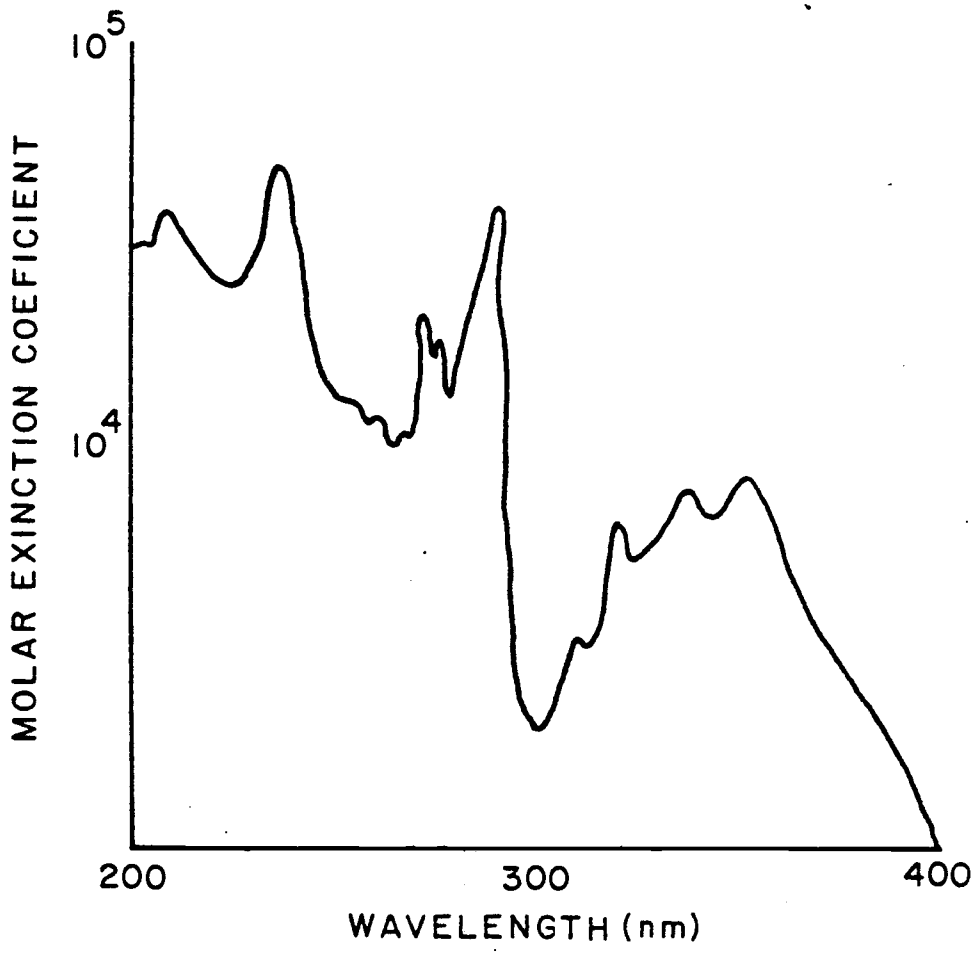
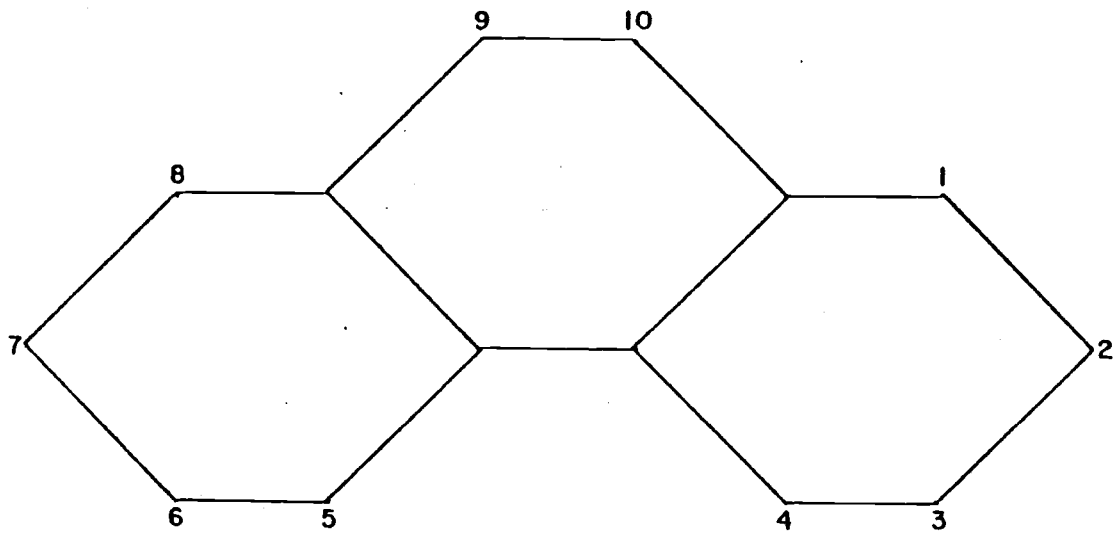


FIGURE 12. Fluoranthene UV absorption spectrum



PHENANTHRENE

$C_{14}H_{10}$

FIGURE 13 Molecular structure of phenanthrene

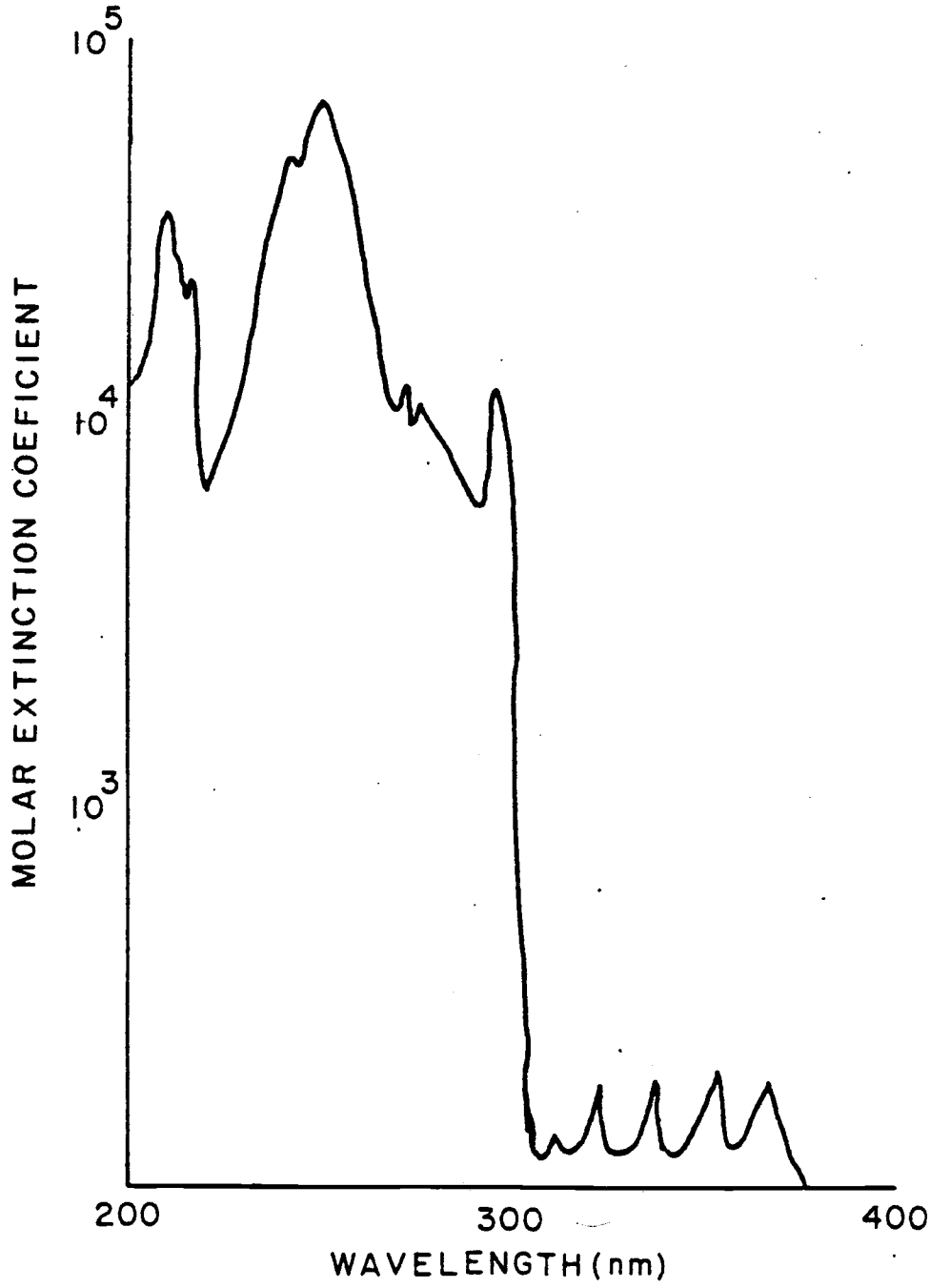


FIGURE 14 Phenanthrene UV absorption spectrum

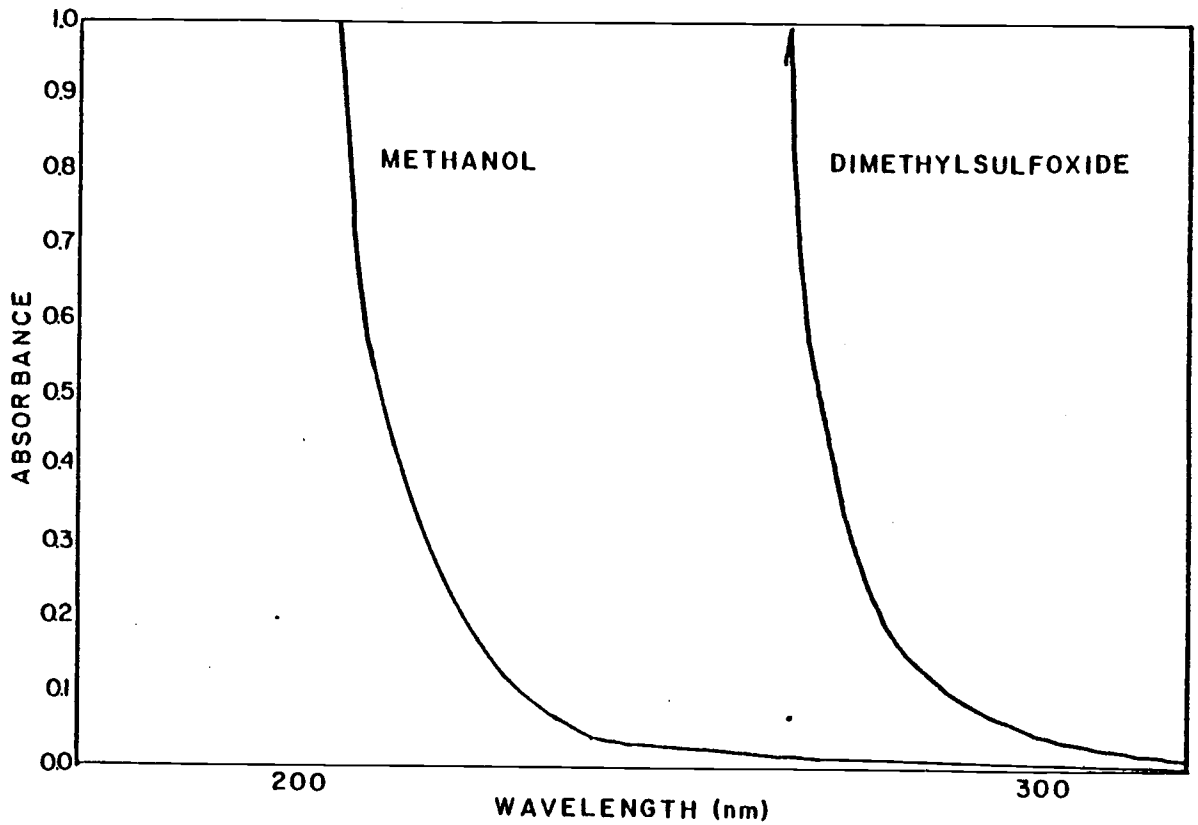


FIGURE 15. Dimethylsulfoxide and methanol absorption spectrum

APPENDIX B

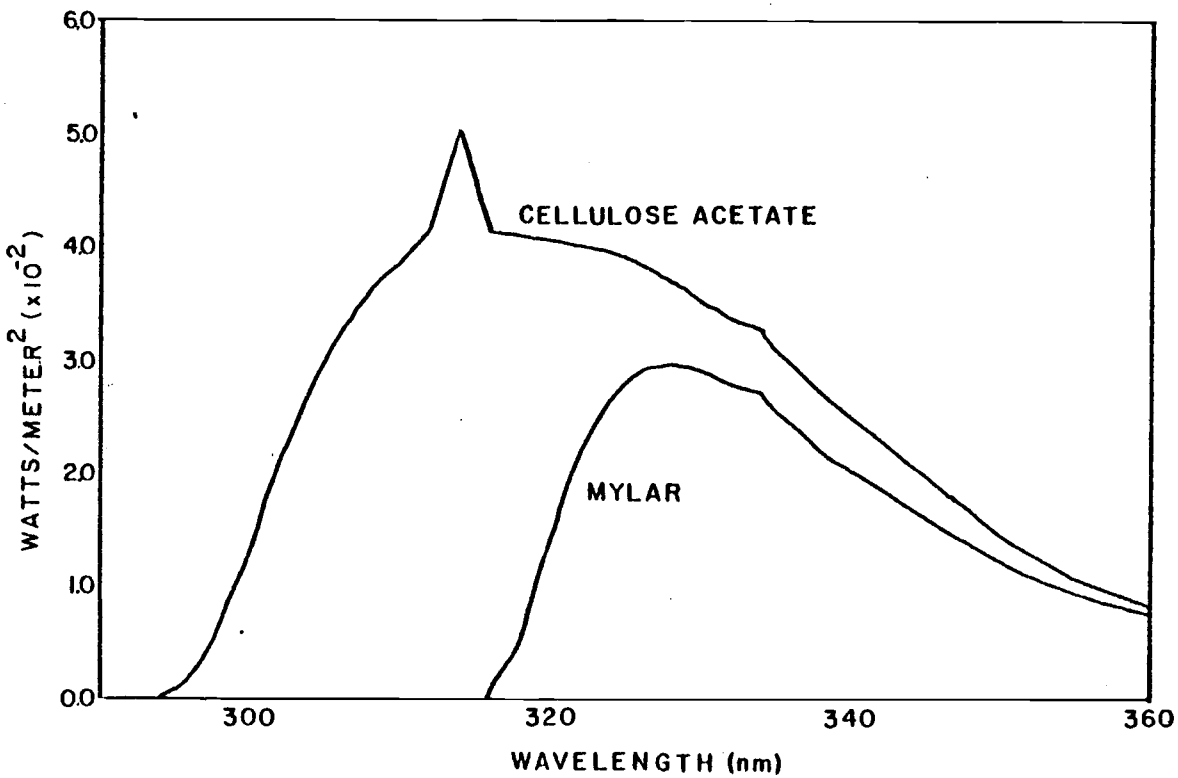


FIGURE 16. Cellulose acetate and mylar filtered sunlamps wavelength spectra

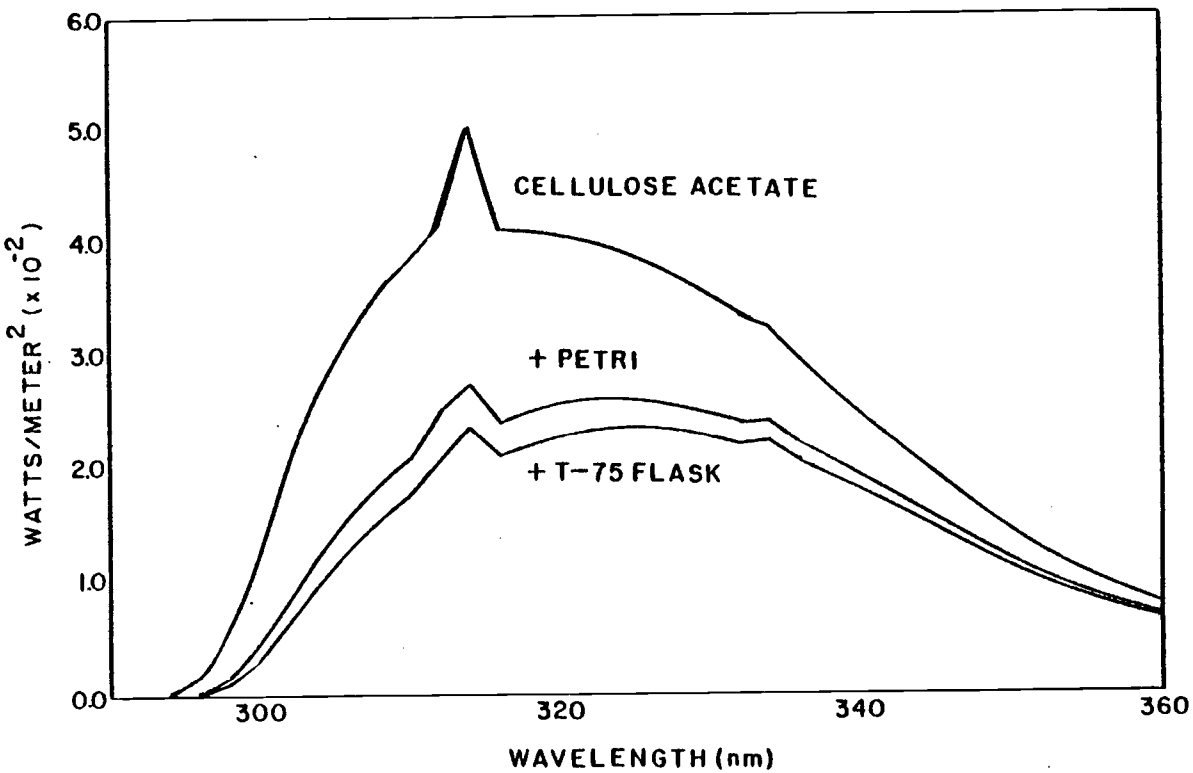


FIGURE 17. Cellulose acetate filtered sunlamps wavelength spectra

TABLE 19. Spectroradiometer dose readings for various exposure conditions.

Tissue-culture container	Medium	Filter	Dose (295-360 nm)	Dose (400-800 nm)	Total dose (j-sec/m^2)
T-75 flask	PBS (10 mls)	Mylar	0.605	1.222	1.83
T-75 flask	PBS (10 mls)	Cellulose acetate	1.002	1.248	2.25
T-75 flask	McCoy's + 10% Kansas City serum (10 mls)	Mylar	0.409	1.020	1.43
T-75 flask	McCoy's + 10% Kansas City serum (10 mls)	Cellulose acetate	0.660	1.080	1.74
60 mm Petri dish	PBS (5 mls)	Mylar	0.658	1.304	1.96
60 mm Petri dish	PBS (5 mls)	Cellulose acetate	1.122	1.314	2.44
60 mm Petri dish	McCoy's + 10% Kansas City serum (5 mls)	Mylar	0.524	1.073	1.60
60 mm Petri dish	McCoy's + 10% Kansas City serum (5 mls)	Cellulose acetate	0.457	1.171	1.63