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MAGOR, Robert Cady, 1935-
FLUORESCENCE ANALYSIS OF BENZO(a)PYRENE AND BENZO(ghi)PERYLENE IN THE SOLID STATE: APPLICATIONS TO QUANTITATIVE ANALYSIS ON CHROMATOGRAPHIC MEDIA AND MIXTURE ANALYSIS.

The University of Michigan, Ph.D., 1967
Chemistry, analytical

University Microfilms, Inc., Ann Arbor, Michigan

FLUORESCENCE ANALYSIS OF BENZO(a)PYRENE AND BENZO(ghi)PERYLENE IN THE SOLID STATE: APPLICATIONS TO QUANTITATIVE ANALYSIS ON CHROMATOGRAPHIC MEDIA AND MIXTURE ANALYSIS

by
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A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy in the
University of Michigan
1967

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ABSTRACT

Binary mixtures of benzo(a)pyrene (BzPy) and benzo(ghi)perylene (BzPer) deposited on Mylar were quantitatively analyzed using both the height of a single fluorescence peak common to both compounds and the amount this peak was decreased by u.v. irradiation of the sample. This analytical method was possible because the mass ratio of the compounds determines (1) the amount by which the peak is decreased by u.v. exposure and (2) the amount by which the fluorescence from the solid mixtures is quenched. A minimum of twenty ng of BzPer were quantitatively determined to within $\pm$ 4.7 ng in the presence of 200 ng of BzPy, and 80 ng of BzPy were determined to within $\pm$ 20 ng in the presence of 100 ng of BzPer.

Attempted analysis on filter paper indicated the presence of two fluorescing species for each compound. The relative abundances of these two fluorescing species in the sample changed in opposite directions, possibly by interconversion, with quantity changes and with exposure to u.v. radiation, producing, for one of the species, peaks, which decreased with increasing quantity and which increased with u.v. exposure. Due to these changes, the use of filter paper was not considered feasible for the analysis of mixtures.

ACKNOWLEDGEMENTS

The completion of this work would not have been possible without guidance, support, and encouragement from a large number of people. I wish to express my appreciation to the following:

The members of my doctoral committee, Professors Paul M. Giever, Chairman, Isadore A. Bernstein, Edward A. Boettner, Herbert H. Cornish, Richard G. Lawton and William Mirsky for their encouragement and guidance, and for making their time and experience available whenever needed.

Mr. J. L. Monkman, who provided reprints and suggestions, and permitted several of his figures to be reproduced in this thesis, and the other authors and editors who granted permission for the reproduction of needed figures.

The Public Health Service and National Institutes of Health for providing financial support, and Professor Paul M. Giever for making this support available.

Mrs. Jean McKown for her contribution of time and talent in preparing this manuscript for reproduction.

Dr. Walter Ruch, Dr. Val Vitols, and my fellow classmates for their interest, encouragement and timely suggestions.

And my wife, Betty, and my parents, whose contributions to this work began many years before the start of the study.

TO MY WIFE

Betty

On the occasion of our
Tenth Wedding Anniversary

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1. INTRODUCTION

1.1 BzPy AND BzPer IN POLLUTED ATMOSPHERES

Few carcinogenic* compounds have received the attention accorded benzo(a)pyrene**. Widespread in the human environment, it is a common contaminant in air, water and foods. Although there is some evidence for its natural occurrence (Blumer, 1961), the presence of benzo(a)-pyrene in the environment must be considered a result of human activity.

Benzo(a)pyrene is one of a group of organic compounds known as the polycyclic aromatic hydrocarbons, which are structured from fused benzene rings. When carbonaceous materials are heated to a sufficient temperature in an inert atmosphere, a spectrum of these compounds ranging from three-ring compounds, such as anthracene and phenanthrene, through five- and six-ring compounds, such as benzo(a)pyrene and benzo-(ghi)perylene***, is generated. These fused ring compounds are formed from simpler hydrocarbons by free radical reactions (Badger, 1962; Badger et al., 1960; Badger and Novotny, 1963; and Tebbens et al., 1956a). A simplification of the growth mechanism, in which the simple

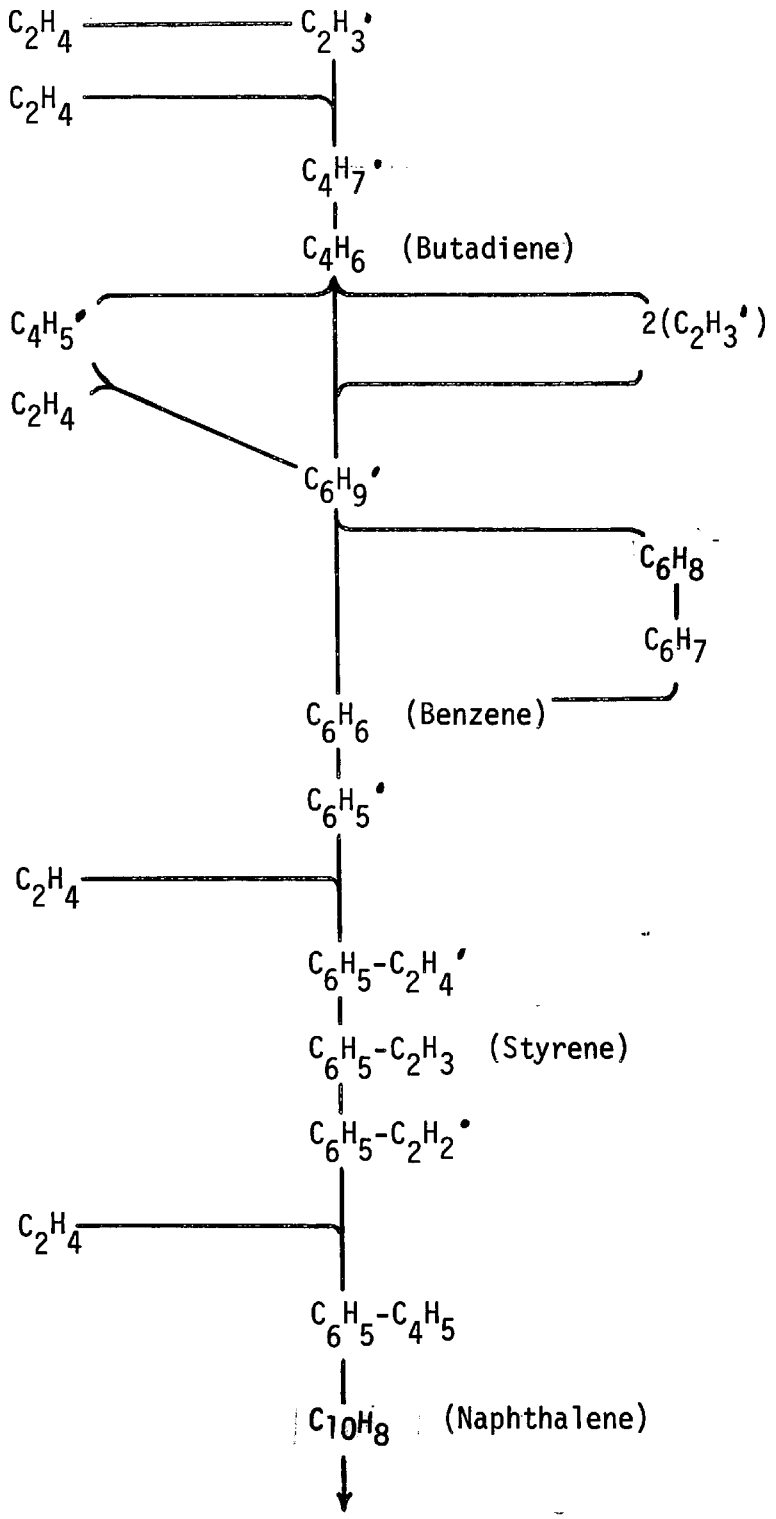
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* As determined by animal testing.

** Also known as 3,4-benzopyrene; Revised Ring Index (RRI) No. 6399 (Patterson et al., 1960).

*** Also known as 1,12-benzoperylene; RRI No. 7036 (Patterson et al., 1960).

hydrocarbon ethylene is the unit building block, is shown below.



(Adapted from Tebbens et al., 1956a.)

The necessary conditions of sufficient heat and an inert atmosphere are the same conditions that result in the incomplete combustion of fuels. These compounds, formed in portions of the combustion zone devoid of oxygen, are present in emissions from internal combustion engines and from the burning of liquid, solid and gaseous fuels. Shabad and Dikun (1959) found the completeness of combustion to be more important in the formation of benzo(a)pyrene than the type of fuel burned. The formation of these compounds during the combustion process, rather than the simple distillation of them, was proven by Tebbens et al. (1956a and 1956b) who identified these aromatic hydrocarbons in smoke from burning cellulose products and gaseous fuels.

Simple hydrocarbons are subjected to high temperatures in the absence of oxygen during the thermal treatment of carbonaceous materials, such as during the distillation of crude oil or the charcoal-broiling of a steak. High concentrations of benzo(a)pyrene have been found in emissions from coking plants and heavy petroleum-products plants (Rondia, 1965; and Shabad and Dikun, 1959). When formed under these conditions, benzo(a)pyrene and the other polycyclics may be retained in the product, requiring a subsequent process, such as the heating of tar (Sawicki et al., 1962a), the wearing of macadam roads, or the wearing of tires containing lamp-black filler (Falk et al., 1951; and Shabad and Dikun, 1959), to make them airborne pollutants.

The importance of temperature on the formation of benzo(a)pyrene has been shown by the results from animal testing of distillation products and the chemical analysis of these products. For the formation of products carcinogenic to test animals, the carbonaceous material must be subjected to a temperature of not less than 400 to 600^{o}C

(Kennaway, 1925a; and Shabad and Dikun, 1959). Badger et al. (1964) found that tars obtained from the pyrolysis of n-butylbenzene do not contain benzo(a)pyrene when the reaction temperature is less than 600°C or greater than 800°C, with 710°C being the optimum temperature for its formation.

After these complex hydrocarbons are formed in the hot combustion zone and leave it, they condense on or are absorbed by solid particulates. Benzo(a)pyrene and the other polycyclics enter the atmosphere, and are transported and dispersed by it, in association with airborne particulates.

In 1933, Cook and his coworkers ended a search begun over 150 years earlier by Pott (1775) when they identified benzo(a)pyrene as the potent carcinogen* present in coal-tar pitch. It was not until many years after the initial observation on the carcinogenicity of combustion products by Pott (1775), who reported that skin cancer was an occupational disease among chimney sweeps, that industrial workers in the soot, tar, shale-oil, petroleum and lubricating oil industries were also found to experience a high incidence of skin cancer (Kennaway, 1925b; Henry et al., 1931; and Kennaway and Kennaway, 1936 and 1947). In their search for the common agent in the large number of mixtures suspected to be carcinogenic, Cook et al. (1933) were helped by the results from animal testing of, first, natural products from the distillation of coal (Yamagiwa and Ichikawa, 1918) and other natural tars and products (Kennaway, 1924), and later synthetic tars and products (Kennaway, 1930), but it remained for Hieger (1930) to

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* As determined by animal testing.

present the information most needed. When he reported the fluorescence spectra from a series of known carcinogenic tars, which displayed similar spectral bands, he not only confirmed the presence of a common agent in these tars but also provided the means for searching for it. Almost anticlimatically, Cook et al. (1933) isolated benzo(a)pyrene from coat-tar pitch and confirmed the identification by synthesizing the compound.

The discovery of benzo(a)pyrene and the other polycyclic hydrocarbons in air was also an incremental process, which started with the biological testing and chemical analysis of obvious air pollutants. Passey (1922) used an ether extract of soot to produce skin cancer in test animals, and later Campbell (1934 and 1939) exposed animals to airborne road dust and soot, which produced lung and skin cancer in test animals. More evidence for the presence of benzo(a)pyrene in the atmosphere was supplied by Goulden and Tipler (1949), who identified benzo(a)pyrene as a component of domestic soot, and by Hieger (1946), who observed fluorescence bands resembling those of benzo(a)pyrene in benzene exposed to the London atmosphere. In 1952 the first concentration data for benzo(a)pyrene in the ambient atmosphere were reported by Waller (1952), who analyzed particulate samples obtained by aspirating known quantities of air through filter papers. Soon thereafter, concentration data were reported for pyrene-5262*, benzo(a)pyrene, benzo(ghi)perylene, anthracene-3618, and fluoranthene-4799 (Cooper, 1954; and Kotin et al., 1954), and the presence of chrysene-5254 and benzo(e)pyrene-6400 were reported (Kotin et al., 1954).

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* RRI No. (See second footnote, p. 1.)

Concentration data for the more abundant polycyclic aromatic hydrocarbons in the atmosphere are given in Appendix A.

1.2 ANALYSIS OF BzPy AND BzPer

Samples of the suspended particulate portion of the atmosphere, obtained by aspirating air through filter paper or glass-fiber filters, are analyzed for airborne polycyclic aromatic hydrocarbons by extracting the hydrocarbons, usually in a Soxhlet extractor with benzene or chloroform, separating the extract chromatographically, and analyzing the fractions spectroscopically. The ideal separation step would separate the extract into fractions containing one compound each and would perform this separation with no material loss in a reasonable length of time. Unfortunately, the methods available for the separation are less than ideal, necessitating a compromise between completeness of separation of the compounds and their easy spectroscopic analysis, on one hand, and time and sample loss* on the other.

Liquid-liquid and liquid-solid chromatography have been found most suited of the methods available for the separation of polycyclic aromatic hydrocarbons in small samples. Generally, a single separation by column chromatography is used, resulting in fractions which contain neither a single compound nor the total amount of the compound. Spectroscopic analysis is performed on these mixtures from the chromatographic separation, and a compound's total quantity is determined from summing the quantities found in successive fractions. Fractions of

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* The sample loss results not only from the increased number of manipulations but also from increased exposure to light and air.

practically pure compounds can be obtained with increased sample loss and time by selectively combining, concentrating, and rechromatographing the fractions by paper or thin-layer chromatography.

The first spectroscopic analysis of air samples for polycyclic aromatic hydrocarbons was by fluorescence spectroscopy (Shabad and Dikun, 1959; and Waller, 1952), the method used in the earlier analyses of soot and tars. Soon thereafter, absorption spectroscopy was introduced for the analysis (Cooper, 1954; and Kotin et al., 1954) and immediately became the more popular technique.

Improvements in instrumentation and the availability of commercial, automatic recording spectrophotofluorometers created a renewed interest in using fluorescence analysis for these compounds. In 1958, Van Duuren (1958a and 1958b) reported the fluorescence excitation and emission spectra for several polycyclic hydrocarbons isolated from cigarette-smoke condensate, and later Sawicki et al. (1960c) reported the excitation and emission spectra for most of the polycyclics of interest in air pollution.

Pairs or groups of compounds that are not separated easily by column chromatography may still be analyzed spectroscopically, provided their spectra are sufficiently different. For benzo(a)-pyrene and benzo(ghi)perylene, the analysis for these compounds in the presence of each other is made difficult because, not only are they not completely separated from each other by column chromatography, but also their absorption, excitation and emission spectra are similar, and they are approximately equally abundant in the atmosphere (Appendix A).

The mutual chromatographic and spectroscopic interferences between these compounds were reported in one of the first analyses of the benzene-soluble fraction of particulate air pollution. Cooper (1954) found that benzo(ghi)perylene and benzo(a)pyrene overlap in the fractions eluted from an activated alumina chromatographic column and their ultraviolet absorption spectra (Figures 1 and 2) display mutual interference except for the 403 nm benzo(a)pyrene peak. For the analysis of these compounds in mixtures (Figure 3), base lines, from which the peak heights are measured, are drawn under the 403 nm and the 385 nm peaks. Since the height of the 385 nm benzo(a)pyrene peak is 5.4 times the height of its 403 nm peak, the 403 nm peak height for the mixture is multiplied by 5.4, the product is subtracted from the height of the 385 nm mixture peak, and the difference is attributed to benzo(ghi)-perylene fluorescence. This base-line technique assumes that these peak heights obey Beer's Law and that the background absorption over short distances is linear (Cooper, 1954). As modified by Commins (1958), who improved the technique by using narrower base lines, this technique is the one used for the analysis of chromatographic fractions containing both of these compounds.

One disadvantage in this technique is immediately apparent. The entire analysis is dependent on a small peak whose height can be only approximated for mixtures. Two additional disadvantages are benzo(k)-fluoranthene-6075, which is not easily separated from benzo(a)pyrene by column chromatography, also absorbs 403 nm radiation (Dubois and Monkman, 1965), and, in mixtures having equal concentrations of benzo(a)pyrene and benzo(ghi)perylene, it is possible for the 403 nm peak to be completely absent. The absence of this peak was noted for the low concentrations which may occur in extracts from air samples (Dubois et al., 1960).

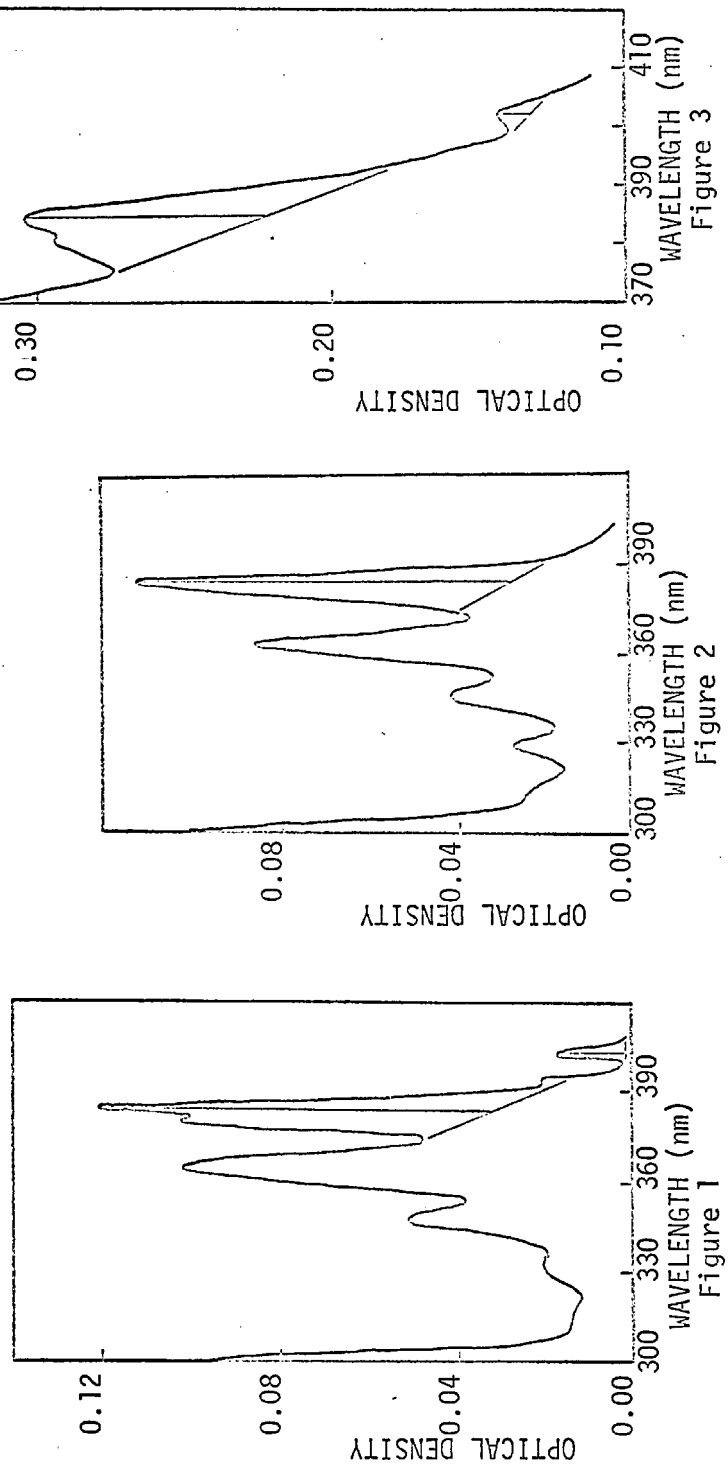


Figure 1. Absorption spectrum of one ug benzo(a)pyrene per ml cyclohexane.
Figure 2. Absorption spectrum of one ug benzo(ghi)perylene per ml cyclohexane.
Figure 3. Graph illustrating the method for determining benzo(a)pyrene and benzo(ghi)-perylene separately in a mixture of the two and in the presence of background absorption.

(Figures 1, 2 and 3 from Cooper, 1954.)

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Fluorescence analysis has offered no improvements. The fluorescence excitation spectra for the two compounds in solution are indistinguishable, and the emission spectrum from mixtures can be used to detect benzo(a)pyrene only when the BzPy/BzPer concentration ratio is greater than approximately 0.1 (Lijinsky et al., 1961). When the concentration ratio is less than or equal to 0.1 only benzo(ghi)perylene can be detected, but for no concentration ratio can both compounds be detected. An additional problem in the quantitative fluorescence analysis of mixtures in solution is the apparent quenching of fluorescence that occurs. Lijinsky et al. (1961) experienced difficulty in relating the fluorescence intensities of mixtures to component concentrations, and Aigina and Mints (1966) found that an analytical method for benzo(a)pyrene, in which benzo(ghi)perylene was used as an internal standard* (Dikun, 1961), was inaccurate because benzo(ghi)perylene quenched the fluorescence from benzo(a)pyrene.

Benzo(a)pyrene and benzo(ghi)perylene can be separated from each other by paper chromatography (Dubois et al., 1960; and Lijinsky and Shubik, 1965). Thus, the problems in the spectroscopic analysis of unseparated mixtures can be avoided by rechromatographing the fractions from column chromatography on paper and then returning the separated compounds to solution for spectroscopic analysis. For routine quantitative analysis of mixtures these added steps would be justified only if a more direct method does not exist, since such a procedure would increase both the time required for the analysis and the amount of sample lost during analysis.

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* The spectral interference noted above for these compounds in mixtures did not prohibit the analysis because a high resolution instrument was used.

1.3 INTENT OF STUDY

The intent of this study was to develop a spectrophotofluorometric method for the quantitative analysis of unseparated mixtures of benzo(a)pyrene and benzo(ghi)perylene. This intent was to be accomplished by exploiting any promising differences in the spectra for these compounds on different solid media or by exploiting any discriminating spectral changes induced by exposure of the compound to ultraviolet radiation. Before the analysis of mixtures could be attempted, extensive background information on the fluorescence from the separate compounds was needed. This background information also has applicability to the analysis of the separated compounds directly on chromatographic media.

2. BACKGROUND

2.1 PREVIOUS LITERATURE

The fluorescence spectra for polycyclic aromatic hydrocarbons on chromatographic media have been used for qualitative analysis of the compounds, but their use for quantitative analysis has been limited to studies on the determination of benzo(a)pyrene. Monkman (Monkman and Porro, 1960; and Monkman et al., 1962) and Thomas et al. (1960) have analyzed benzo(a)pyrene quantitatively on paper chromatograms using the height of its main fluorescence peak, occurring at about 407 nm. This peak height is reported to be linearly related to the quantity of benzo(a)pyrene for quantities from 0 to 10-20 ng (Monkman and Porro, 1960; and Monkman et al., 1962) and from 0 to 500 ng (Thomas et al., 1960). Two disadvantages in the analysis are the sensitivity of benzo(a)pyrene on chromatographic paper to ultraviolet radiation (Thomas et al., 1960), causing its fluorescence to decrease, and self-absorption (Monkman and Porro, 1960; and Thomas et al., 1960), which results in a decreasing rate of peak-height increase with quantity of benzo(a)pyrene.

The quantitative analysis of benzo(ghi)perylene directly on chromatographic media, much less the quantitative analysis of unseparated mixtures, is not reported.

Changes in the spectra from compounds, caused by their crystallization from solution, have been used to aid in the analysis of mixtures in which the components have interfering spectra. The crystallization of benzo(a)pyrene causes its fluorescence spectrum to shift bathochromatically, but the crystallization of benzo(k)fluoranthene-6075 does

not affect its spectrum. Falk and Kotin (1962) were able to analyze for benzo(k)fluoranthene-6075 in the presence of benzo(a)-pyrene by causing the mixture to crystallize, thus removing the spectral interference from benzo(a)pyrene. Several polycyclic hydrocarbons display such spectral shifts in concentrated solutions and in crystals as a result of excimer (excited dimer) formation. Förster and Kasper (1955) first reported excimer formation for solutions of pyrene-5262. They observed that pyrene-5262 in dilute benzene solution (2×10^{-4} mole/liter) has fluorescence peaks at 372, 384 and 392 nm, but the height of these peaks decreases with increasing concentrations of pyrene and a new peak appears at 478 nm. At a concentration of 2×10^{-2} mole/liter the three high energy peaks have almost disappeared, and the 478 nm peak is dominant. This spectral change with concentration is reported for solvents other than benzene (Van Duuren, 1963).

Since the appearence of this 478 nm peak is not accompanied by changes in the absorption spectrum, the ground state of the molecule is unaffected by the concentration change, indicating the occurrence of a concentration-dependent reaction by molecules in the excited state. The reaction sequence, given for pyrene-5262, is (adapted from Förster and Kasper, 1955):

1. Formation of the lowest excited singlet state;

$$Py + h\nu_{ex} \longrightarrow Py^*$$

2. Excimer formation;

$$Py^* + Py \longrightarrow (PyPy)^*$$

3. Radiative decay of excimer to dimer;

$$(PyPy)^* \longrightarrow h\nu_{em} + (PyPy)$$

4. Dissociation of dimer;

$$(PyPy) \longrightarrow Py + Py$$

Excimer formation in solution depends upon the encounter between an excited and an unexcited monomer. The life-time of the excited monomer, along with the viscosity of the solvent and concentration of the solution, are factors which influence this encounter. The temperature of the solution can influence both the formation of the excimer, by promoting either the formation or dissociation reaction, and its fluorescence, by promoting or inhibiting its non-radiative energy losses (Förster, 1963; and Van Duuren, 1963).

The type of crystalline lattice formed (Stevens, 1962) and the mutual energy effect which the molecules exert in the lattice (Perkampus and Pohl, 1964) are important for the occurrence of excimer fluorescence from compounds in the crystalline state. The character of the fluorescence spectra, according to Stevens (1962), is related to the type of crystalline lattice formed, which, in turn, depends on the shape of the molecule. Linear molecules, such as phenanthrene-3619 and chrysene-5254, form a lattice type having large intermolecular distances and little molecular overlapping. For compounds forming this lattice type, there is a close resemblance between the fluorescence spectrum from the compounds in solution and in crystals, the fluorescing species being the monomer in each case. Disc-shaped molecules, such as pyrene-5262 and coronene-7392, form one of two lattice types in which the intermolecular distances are small and the molecules

are grouped in parallel, sandwich-like pairs. The emitting species in such crystals is the excimer, and the fluorescence from these crystals is characterized by a loss of structure and a pronounced bathochromatic shift. Crystals of benzo(a)pyrene and benzo(ghi)perylene are catagorized among those displaying this excimer fluorescence (Table 1).

Table 1. Crystal-lattice assignment* of compounds. (From Stevens, 1962.)

Type A (Monomer fluorescence):
Naphthalene-1754
Anthracene-3618
Phenanthrene-3619
Chrysene-5254
Sublimated benzo(a)pyrene-6399**

Type B (Excimer fluorescence):
Pyrene-5262
Monoclinic benzo(a)pyrene-6399**
Perylene-6401
Benzo(ghi)perylene-7036
Dibenzo(def,mno)chrysene-7033
Coronene-7392
Ovalene-7653

* The assignments are based on molecular shape, fluorescence, and x-ray analysis or exciton capture, or both.

** Benzo(a)pyrene is catagorized A,B by molecular shape. The monoclinic assignment is based on fluorescence and x-ray analysis, and the sublimated assignment is based on fluorescence and exciton capture.

A large mutual energy effect in the lattice is reported to be more important than a favorable spatial order, which merely replaces the need for diffusion motion, for the occurrence of excimer fluorescence (Perkampus and Pohl, 1964). Triphenylene-5256, for example, is a disc-shaped molecule which forms a sandwich-like, crystalline structure when a sublimed deposit of it is annealed at room temperature. According to Stevens (1962), such crystals should exhibit excimer fluorescence,

but, in the case of triphenylene, the typical dimer arrangement in the solid state does not, alone, produce sufficient condition for the appearance of excimer fluorescence (Perkampus and Pohl, 1964). On the other hand, crystals of anthracene-3618, in accord with Stevens (1962) (Table 1), exhibit fluorescence which shows the fine structure characteristic of monomer fluorescence at room temperature, but upon cooling the crystals, excimer fluorescence appears. The appearance of this excimer fluorescence with cooling is believed to result from a repression of excimer dissociation, which occurs at room temperature. A weak, mutual energy effect in the absence of a favorable spatial order causes crystals of this compound to exhibit excimer fluorescence (Perkampus and Pohl, 1964).

The fluorescence spectrum from mixed crystals is influenced by the thickness of the deposit. Thin perylene-pyrene mixed crystals exhibit emission peaks at 478 and 497.5 nm, but, as the thickness of the sample is increased, the 478 nm peak disappears because of reabsorption by other perylene molecules (Kawaoka and Kearns, 1966). The perturbated spectrum was originally interpreted as the fluorescence from a mixed, perylene-pyrene excimer (Hochstrasser, 1962). Unlike the mixtures used in this present study, the ratio of the quantities ranged from 10^{-3} to 5×10^{-6} moles/mole.

The reported fluorescence spectra for small quantities of benzo(a)pyrene on chromatographic media are similar to those reported for benzo(a)pyrene in solution. The spectrum for benzo(a)pyrene on paper is shifted bathochromatically only slightly from its spectrum in solution, the main peaks occurring at 405.5 and 428 nm on paper (Dubois et al., 1960) and at 403 and 427 nm in pentane (Sawicki et al., 1960c). For larger quantities on chromatographic media, benzo(a)pyrene

may possibly yield a mixed spectrum, consisting of monomer and excimer fluorescence, as was observed for pyrene in solutions of high concentrations (Förster and Kasper, 1955). The spectrum reported by Sawicki et al. (1964) for benzo(a)pyrene on a cellulose thin-layer chromatogram appears to be such a mixture of fluorescence. The difference between the spectra for benzo(ghi)perylene in solution and on paper is more striking than just a slight bathochromatic shift. The main emission peaks for the compound in pentane occur at 393, 404, 419 and 440 nm (Sawicki et al., 1960c), and for the compound on paper they occur at 399, 407.5, 420, and 430 nm (Dubois et al., 1960). The reason for the difference between the spectra for benzo(ghi)perylene is not given.

During exposure to ultraviolet radiation, the fluorescence from benzo(a)pyrene on paper decreases slightly in fifteen minutes, whereas the fluorescence from anthracene decreases significantly in less than five minutes (Thomas et al., 1960). This study by Thomas et al. (1960) and other studies, have shown that when polycyclic aromatic hydrocarbons are exposed to visible and ultraviolet radiation in the presence of oxygen, a "loss" of compound occurs, the amount of which differs significantly among the exposed compounds. This loss is believed to result from the photo-oxidation of the compounds to weakly or non-fluorescent compounds.

The effect of visible and ultraviolet radiation exposure on benzo(a)pyrene was shown by Tebbens et al. (1966), who passed smoke through a reaction chamber illuminated with fluorescent lights. The difference between the amounts of benzo(a)pyrene in the smoke at the entrance and exit of the chamber, for three samples each, were -64, -37 and -34 per cent when the chamber lights were on, and -11, +6 and

+22 per cent when the chamber lights were off. Similarly, the differences for perylene for two samples each were -42 and -34 per cent when the lights were on and -5 and -1 per cent when the lights were off. Tebbens et al. (1966), in showing this photosensitivity of benzo(a)pyrene, confirmed the work by Falk et al. (1956), who, using the pure compound deposited on filter paper, earlier reported benzo(a)-pyrene to be light sensitive. When the compound was exposed to flowing air and light, 22 per cent was lost in 48 hours, as opposed to no loss when the compound was exposed to flowing air in the dark.

The presence of air is important to the reaction causing the loss of benzo(a)pyrene. When air was passed through glass-fiber filters irradiated by fluorescent lamps, 60 per cent of the benzo(a)pyrene impregnated on the filters was lost, but when nitrogen was passed through the filters under the same conditions, only 20 per cent* of the benzo-(a)pyrene was lost (Tebbens et al., 1966).

Only benzo(a)pyrene of the compounds studied by Falk et al. (1956) exhibited sensitivity to the irradiating light energy used. Four compounds displayed losses that were unaffected by the presence of light,** and three compounds exhibited no losses under either condition (Table 2). Several of the compounds are sensitive to ultraviolet radiation and differ markedly in their sensitivity (Inscoe, 1964). On thin-layer chromatograms, spots of pyrene-5262 and benzo(a)pyrene, for

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* The amount of benzo(a)pyrene lost in nitrogen is greater than can be attributed to volatilization (Commins and Lawther, 1958).

** The losses for dibenzo(def,mno)chrysene-7033 and pyrene-5262 cannot be attributed to spontaneous oxidation in the absence of light (Rondia, 1965).

Table 2. Losses of pure compounds from filter paper in 48 hours. (From Falk et al., 1956.)

Compound	Per Cent Lost During Exposure to	
	Air in Dark	Air in Light
Dibenzo(def,mno)chrysene-7033	49	44
Phenanthrene-3619	61	60
Pyrene-5262	43	42
Fluoranthene-4799	20	24
Benzo(a)pyrene-6399	0	22
Benzo(ghi)perylene-7036	0	0
Coronene-7392	0	0
Chrysene-5254	0	0

example, are turned brown by five minutes of ultraviolet irradiation, but spots of benzo(e)pyrene-6400 and benzo(ghi)perylene require up to fifteen minutes of exposure to cause them to turn (Inscoe, 1964).

Both reversible and irreversible photo-oxidation of the compounds have been suggested as the reaction causing the loss of material. The quenching effect of oxygen on the fluorescence from polycyclic hydrocarbon solutions and its reversibility are well documented (Weil-Malherbe and Weiss, 1942; and others). It is believed (Thomas et al., 1960) that reversible oxidation may also be responsible for the quenching of fluorescence from solid-state compounds. Suggesting irreversible oxidation of the compounds are the isolation of quinones of pyrene, benzo(a)pyrene and dibenzo(def,mno)chrysene from the particulate portion of air pollution (Tipson, 1965) and the identification of 1,6- and 1,8-pyrenedione among the reaction products from the ultraviolet irradiation of pyrene-5262 on silica gel (Inscoe, 1964).

2.2 DISCUSSION OF APPROACH TO STUDY

The starting point for this study was the observation by Dubois et al. (1960) that the spectra for benzo(a)pyrene and benzo(ghi)perylene on paper are sufficiently different from each other to permit their identification. As originally outlined, the study was to be accomplished by studying the fluorescence from pure compounds on paper, looking for differences both between the spectra and in the effect of ultraviolet radiation exposure on the spectra, then exploiting these differences for the analysis of mixtures on paper.

During the study of benzo(a)pyrene on paper, spectral changes with quantity and with exposure of the compound to ultraviolet radiation were observed which were previously unreported and which presented problems in the quantitative analysis of benzo(a)pyrene on filter paper. Since these changes suggested a re-evaluation of previous literature related to the analysis of benzo(a)pyrene on chromatographic media, the basic study on the fluorescence analysis of mixtures was temporarily suspended at this point to allow the further study of these changes. The fluorescence from benzo(ghi)perylene and its changes with quantity and with exposure to ultraviolet radiation were then studied to determine if its spectrum displayed changes similar to those for the benzo(a)pyrene spectrum.

During this interim period, an unsuccessful attempt was made at inducing chemiluminescence in benzo(a)pyrene on filter paper by an ozone-benzo(a)pyrene reaction. Many organic compounds, when deposited on a porous medium, display such chemiluminescence (Bowman and Alexander, 1966). Analysis using chemiluminescence would have been particularly suited to the analysis of compounds on chromatographic media

since background interference from scattered excitation radiation would not be present. The ozone for the reaction was introduced by recycling a portion of the exhaust from the xenon lamp over the sample using a bellows pump and Tygon tubing.

The basic study was then continued using Mylar instead of filter paper as the support medium. The spectra and changes in spectra with exposure of the compounds to ultraviolet radiation were studied, and promising differences between the spectra and their changes for the two compounds were then applied to the analysis of mixtures.

3. EXPERIMENTAL

3.1 EQUIPMENT AND CHEMICALS

An Aminco-Bowman* spectrophotofluorometer, equipped with a solid-sample accessory (Cat. No. C73-62140)*, a high pressure xenon lamp, a 1P28 photomultiplier tube, and the No. 2 slit arrangement (one nm bandwidth; Grubb, 1967), was used for this study. The slide holding the slits in the first slit position was equipped with a blank disc to effect a shutter between the lamp and the sample. The angle of the mirror in the solid-sample accessory was established by rotating the adjustment screw, notched for convenience, 7-1/2 turns from its "full-in" position. This setting established the mirror angle that yielded the greatest fluorescence-output signal.

The sample holder, used to hold the sample on paper or on Mylar in the excitation beam, was black anodized aluminum, 3/8" square by 1-3/4" high, with a milled 1/4" diameter hole, 3/32" deep, 1/16" from the bottom. Since the 1/4" diameter hole was a modification of a 7/16" x 3/16" slot in the anodized piece, a portion of the sides and bottom of the hole was not anodized.

In use, the sample holder was contained in a spacer to position it in the excitation beam. The spacer was a cuvette from which the bottom and the portion of the side which would have covered the sample had been removed.

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* American Instrument Company, Silver Spring, Maryland

A Houston Omnigraphic Corporation* X-Y recorder, model HR-96T, was used to record the spectra on 7" x 10" graph paper. The wavelength scale was from 225 to 725 nm at 50 nm/inch. The fluorescence intensity scale, except for the spectrum-decay studies, was from 0 to 70 A.U.** at 10 A.U./inch. For the spectrum decay studies the Y-axis span was adjusted to include conveniently both the decaying spectrum and, at a higher meter multiplier (MM) setting, the second order scatter peak.

The benzo(a)pyrene was used as supplied by Distillation Products Industries*** and the benzo(ghi)perylene, by K&K Laboratories, Incorporated.**** The cyclohexane (Matheson Coleman and Bell*****) was slowly distilled once in glass. Whatman No. 42 filter paper, 18.5 cm diameter, and Mylar (Spex Industries, Incorporated******) were used as supplied.

3.2 CALIBRATION AND PURITY CHECKS

The spectrophotofluorometer was calibrated with 0.1 ug/ml

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* Bellaire, Texas

** Arbitrary Units, where one A.U. equals a meter reading of one per cent transmission.

*** Division of Eastman Kodak Company, Rochester, New York

**** Plainview, New York

***** Division of Matheson Company, Incorporated, Norwood, Ohio

****** Metuchen, New Jersey

quinine sulfate in 0.1 N sulfuric acid solution after the solid-sample accessory had been installed.*

It became apparent early in the study that a convenient, secondary standard was needed for checking frequently the instrument's performance. The instrument used was plagued by frequent, randomly occurring, stepwise changes in the output signal. These changes represented increases or decreases of greater than 20 per cent at times. An uranium-glass bead, mounted in plexiglass of the same dimensions as the sample holder, was used to check the performance of the instrument each time before the instrument was used and, for quantitative studies, after practically each determination. The uranium-glass bead was selected instead of the commonly used, small, mercury light, which fits into the sample compartment, because the bead permits monitoring the excitation source and optics in addition to the emission optics, photomultiplier tube, and recorder.

During the spectrum-decay studies, when the uranium-glass bead could not be used, the second order scatter peak was recorded after each spectrum to check the constancy of the instrument's performance during the one-hour periods.

The fluorescence purity of benzo(a)pyrene and benzo(ghi)perylene was determined by comparing the spectra of one ug/ml cyclohexane solutions of these compounds with reported spectra. (The obtained spectra are given in Appendix B along with reported spectra and a table comparing reported peaks.) No fluorescent impurities were detected in either compound.

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* The installation of the solid-sample accessory caused an excitation wavelength shift of five to 10 nm.

Neither the filter paper alone nor the filter paper from which the distilled cyclohexane had been evaporated yielded other than a constant background spectrum, one that would be expected from the scatter of excitation radiation. The Mylar displayed several small excitation peaks, including one at 310 nm that influenced the excitation-wavelength selection for mixture analysis.

3.3 METHOD

The samples were deposited on paper by applying one ul of cyclohexane solutions of benzo(a)pyrene and benzo(ghi)perylene, from one to 400 and from one to 200 ng/ul respectively, to 1/4" diameter pieces of filter paper using a micropipette. For the application the paper was recessed slightly in the hole of the sample holder; only the edges of the paper were in contact with a surface. The solution was forced from the pipette at a rate sufficient to cause the solution to spread 1/32" from the edge of the paper. The paper was then completely recessed into the hole and the sample holder transferred immediately to the sample compartment of the spectrophotofluorometer, with the sample shutter closed. The deposition of the compounds on the 1/4" diameter Mylar mounts was effected by slowly spreading one ul of the cyclohexane solutions over an area less than 3/32" in diameter at the center of the mount using a micropipette, thus allowing the cyclohexane to evaporate rather than to run off the mount. The mount was then placed in the hole of the sample holder. The benzo(a)-pyrene-benzo(ghi)perylene mixtures were prepared by first establishing the concentration ratio of the compounds with one ml of solution

for each compound and then sequentially diluting this mixture. Less than 10 ul of each mixture were withdrawn for analysis.

The compounds were deposited over a smaller area on Mylar than on paper because of reproducibility problems with large area samples on Mylar. Variations in the deposition of the samples on Mylar, coupled with the dependency of the fluorescence intensity upon the position of the sample (Table 3) produced large variations in the results. This dependency of the fluorescence intensity on the sample position was determined by depositing 200 ng of chrysene-5254, determined to be stable during ultraviolet irradiation, approximately 1.5 mm from the center of the Mylar mount and then rotating the mount after recording the spectrum at each position.

Table 3. Height of the 402 nm peak for chrysene-5254 on Mylar for different sample positions.

Determination No.	Sample Position with Respect to Center of Mount	Net Peak Height (A.U.)
1	Bottom	24.3
2	Top	11.3
3	Right	10.0
4	Left	17.7
5	Bottom	24.0

The spectrum-scan rate was about 10 nm per second, with 30 seconds being required for scanning the spectrum and the second order scatter peak in the spectrum-decay studies.

The standard settings for the recorded spectra, unless otherwise noted, are sensitivity = 50 and slit arrangement No. 2.

4. RESULTS

4.1 FLUORESCENCE FROM BzPy ON PAPER

4.1.1 BzPy Spectrum

The emission spectrum for small quantities of benzo(a)pyrene on filter paper (Appendix C*, Figures 52 and 53) resembles the emission spectrum for benzo(a)pyrene in solution (Appendix B, Figure 47) but is shifted slightly bathochromatically. This spectrum for small quantities on paper agrees in shape with reported spectra for benzo-(a)pyrene on chromatographic media (Dubois et al., 1960; Sawicki et al., 1964; and Thomas et al., 1960), and, except as noted below, reported peak positions coincide (Table 4).

Table 4. Reported fluorescence emission peaks for benzo(a)pyrene on chromatographic media.

Medium	Peaks (nm)	Reference
Paper chromatogram	405.5, 428, 456	Dubois et al., 1960
Paper chromatogram	398, 407, 432, 458	Monkman and Porro, 1960
Paper chromatogram	405, 430, 455	Thomas et al., 1960
Cellulose chromatographic plate	408, 430, 455 shoulder 470	Sawicki et al., 1964
Filter paper (one ng)	407, 429, 454 shoulder	This study

* The prolonged exposure of benzo(a)pyrene on paper to ultraviolet radiation, as required for the recording of the several spectra shown in Appendix C for each sample, altered the fluorescence from the sample. The spectra should be examined with a knowledge of the induced changes. (See Section 4.1.3.)

Two reported peaks that appear in disagreement are the 398 nm peak (Monkman and Porro, 1960), which was deleted in a concurrent paper by one of the authors (Dubois et al., 1960), and the 470 nm peak (Sawicki et al., 1964). The 470 nm peak was found in the fluorescence from 500 ng of benzo(a)pyrene on a chromatographic plate. A peak at 469 nm is reported in this present study for large quantities of benzo-(a)pyrene on filter paper (Appendix C, Figures 55 and 56).

Excitation shoulders* at 270, 300, 320, 355 and 400 nm and peaks at 370 and 418 nm for emission wavelength 470 nm are reported by Sawicki et al. (1964) for 500 ng of benzo(a)pyrene. The positions of the excitation peaks for emission wavelength 469 nm were found to be quantity dependent in this present study (Figure 7), but peaks that approximately correspond to those reported by Sawicki et al. (1964) were observed (Appendix C).

4.1.2 BzPy Spectrum Changes with Quantity

The emission spectrum for benzo(a)pyrene underwent several changes with increasing quantity. These changes are described for each of the three regions: from 350 to 400 nm; from 400 to 450 nm; and from 450 to 500 nm.

The 350 to 400 nm region. Benzo(a)pyrene, excited at 301 nm, exhibited no fluorescence in the region between the first order scatter

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* The spectra reported by Sawicki et al. (1964) show a greater contribution from scatter than those reported in this study. These shoulders would probably be peaks if the scatter contribution were reduced.

peak and the first fluorescence peak at 407 nm. To the contrary, the spectral line in this region was consistently below the background recorded for the filter paper alone, the distance between the spectral line and the background line increasing with quantity of benzo(a)-pyrene (Figure 4). This decrease of the spectral line was probably a result of increased absorption, thus less scatter, of the excitation radiation when benzo(a)pyrene was present. The second order scatter peak was similarly reduced by the presence of benzo(a)pyrene.

The 400 to 450 nm region. The region from 400 to 450 nm contained the two prominent peaks for small quantities of benzo(a)-pyrene, the 407 nm and the 429 nm peaks (Figure 4). The 407 nm peak height increased rapidly for quantities of benzo(a)pyrene increasing from 0 to 30 ng and then decreased with further increases of quantity, the peak height for 400 ng being less than one-half the height of the 30 ng peak (Figure 5). The 429 nm peak height also initially increased rapidly with quantity, but it reached a plateau at 20 ng and increased only 18 per cent between 20 ng and 100 ng. Between 100 ng and 200 ng the peak shifted from 429 nm to 425 nm and its height was increased 100 per cent by the 100 per cent increase in quantity. The 407 nm peak, due to this shift of the 429(425) nm peak, its subsequent increase in height, and the decrease of the 407 nm peak height, was no more than a small shoulder on this 429(425) nm peak at 400 ng.

The 450 to 500 nm region. The only fluorescence in the 450 to 500 nm region for small quantities of benzo(a)pyrene was a small shoulder at about 454 nm and a tail from the shoulder (Figure 4). A general increase in the fluorescence in this region occurred for

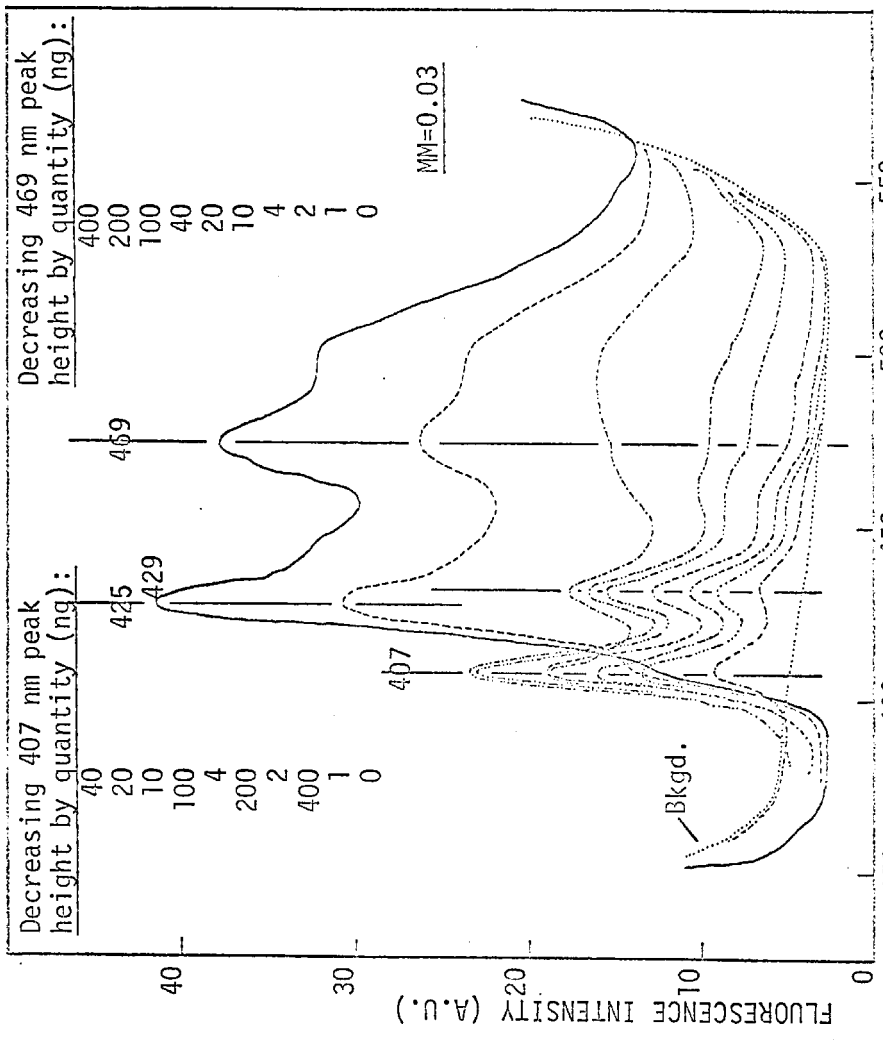
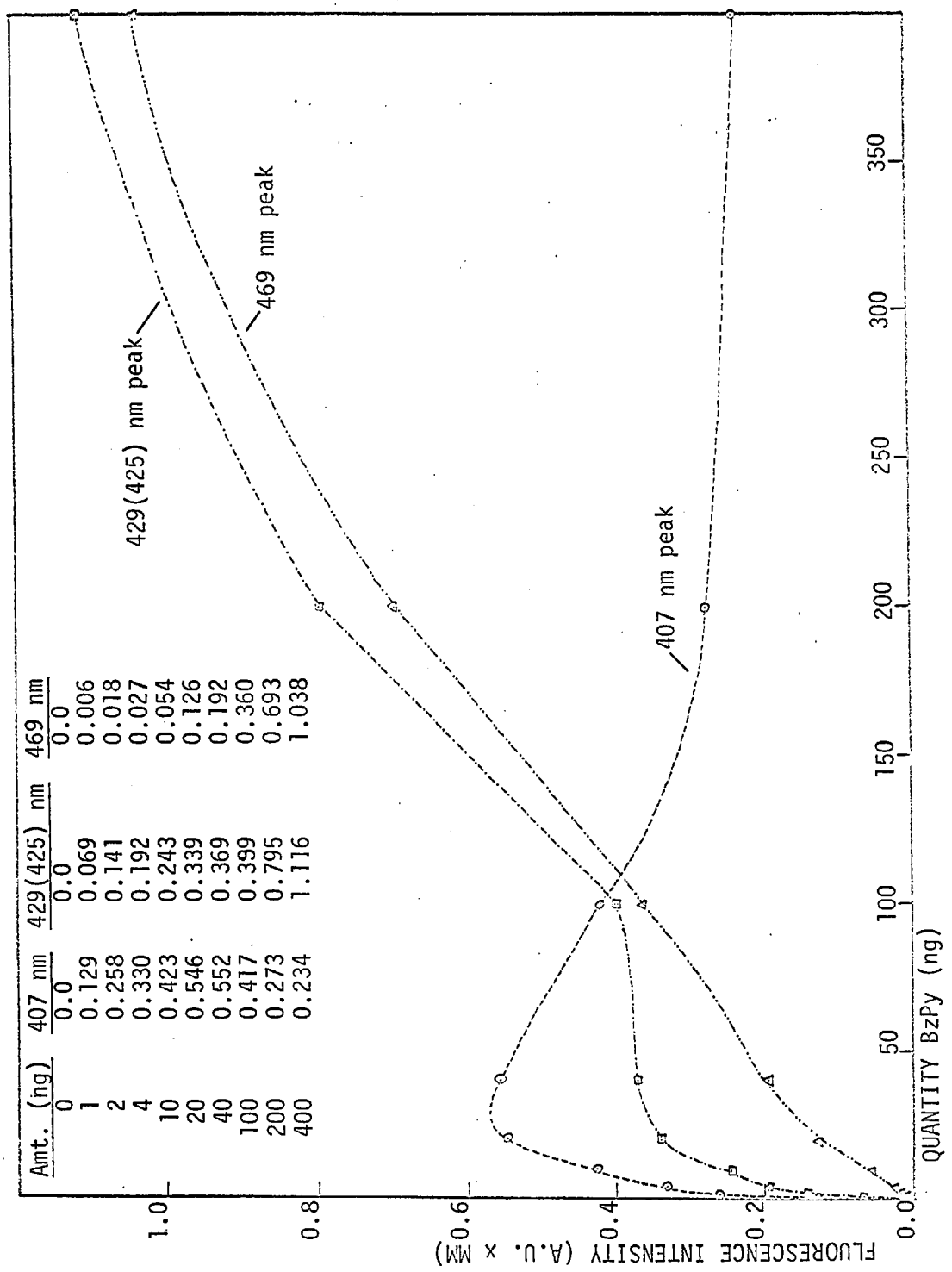


Figure 4. Emission spectra for one to 400 ng of benzo(a)pyrene on filter paper for excitation wavelength 301 nm.



Amt. (ng)	407 nm	429(425) nm	469 nm
0	0.0	0.0	0.0
1	0.129	0.069	0.006
2	0.258	0.141	0.018
4	0.330	0.192	0.027
10	0.423	0.243	0.054
20	0.546	0.339	0.126
40	0.552	0.369	0.192
100	0.417	0.399	0.360
200	0.273	0.795	0.693
400	0.234	1.116	1.038

Figure 5. Peak height versus quantity of benzo(a)pyrene on filter paper for excitation wavelength 301 nm.

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quantities increasing to about 40 ng. This fluorescence had no fine structure or sharp peaks. For quantities increasing from 40 ng to 100 ng, the fluorescence in this region continued to increase while the 407 nm peak height decreased and the 429(425)nm peak height remained approximately constant. It continued to increase with quantity, developing a peak at 469 nm and a broad shoulder at about 500 nm.

The 469 nm peak height for benzo(a)pyrene, measured from recorded spectra (Figure 4), increased approximately linearly with quantity to about 200 ng and then increased at a decreasing rate (Figure 5).

The excitation spectrum for benzo(a)pyrene also changed with quantity. For an emission wavelength of 407 nm, the 301 nm peak (Figure 6) experienced the peak-height reversal between 20 ng and 40 ng noted above for the 407 nm peak. Excitation activity between 385 nm and 400 nm, noted against an intense scatter background for emission wavelength 407 nm, also appeared to exhibit this reversal, but the scatter background prevented good observation of this activity. The changes in the excitation spectrum occurring for emission wavelength 469 nm were different from those occurring for emission wavelength 407 nm. The peak at 301 nm did not exhibit the reversal with increasing quantity that it did for emission wavelength 407 nm, and it shifted position. This 301 nm peak and the peaks at 372 nm and 391 nm, present in the spectrum from 10 ng benzo(a)-pyrene, appeared as a 312 nm peak, a 387 nm shoulder, and a 400 nm peak, respectively, in the spectrum from 400 ng of benzo(a)pyrene, and a new peak appeared at 418 nm (Figure 7).

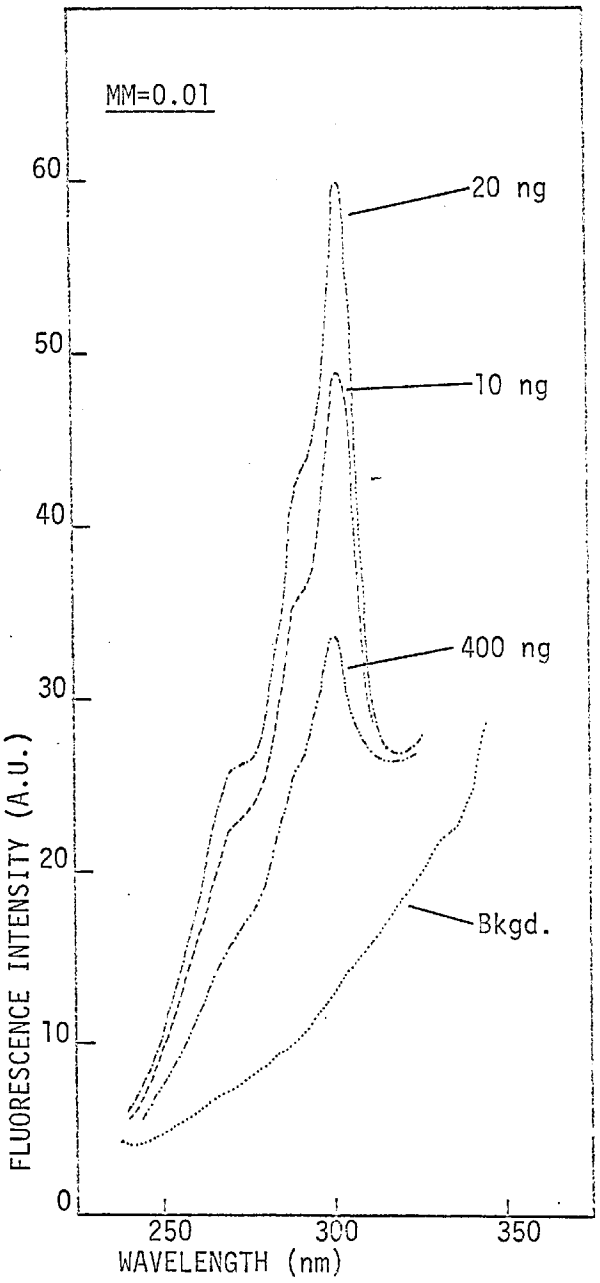


Figure 6. Excitation spectra for 10, 20 and 400 ng of benzo-(a)pyrene on filter paper for emission wavelength 407 nm.

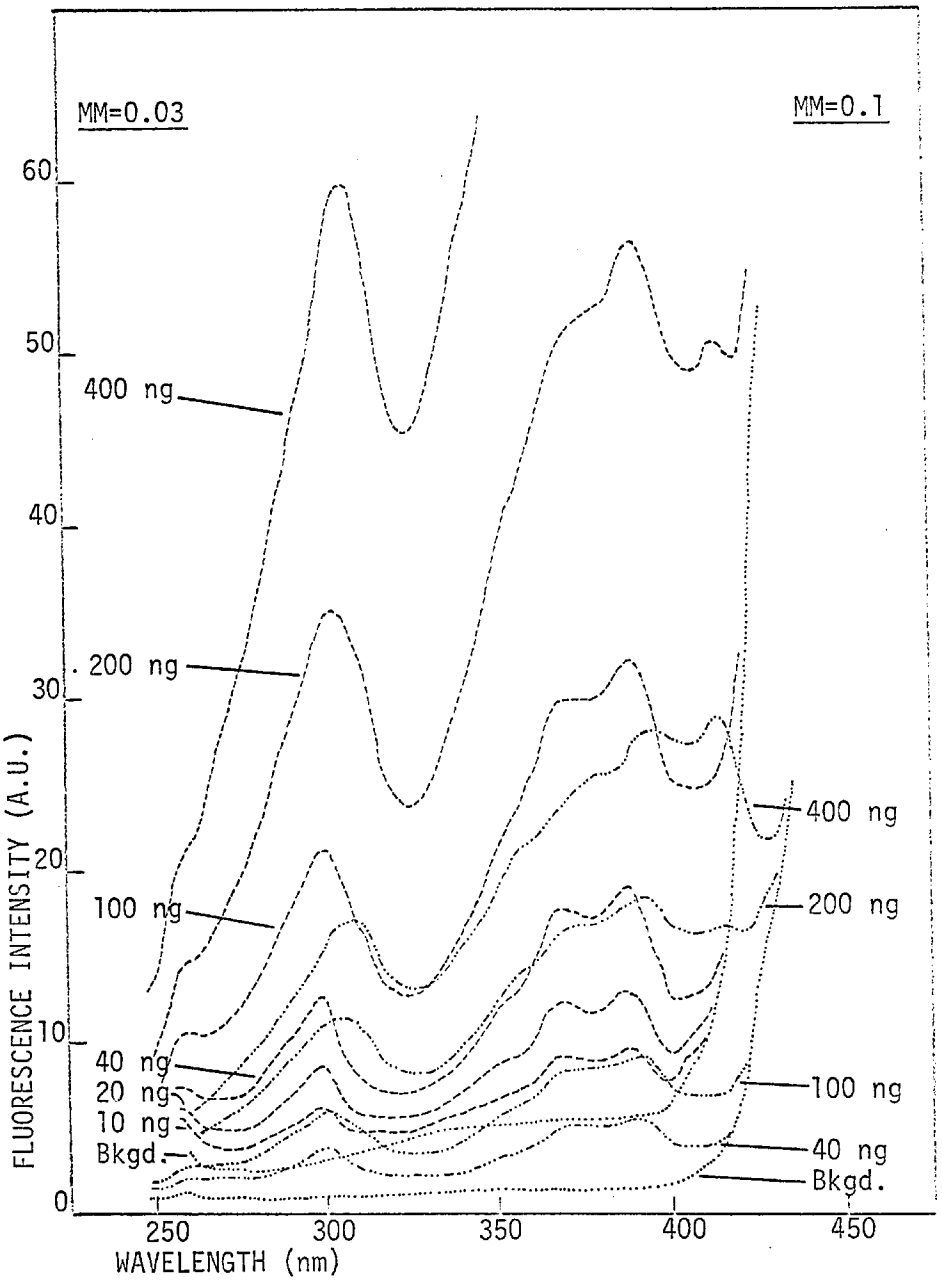


Figure 7. Excitation spectra for 10 to 400 ng of benzo(a)-pyrene on filter paper for emission wavelength 469 nm.

4.1.3 BzPy Spectrum Changes with U.V. Exposure

The changes in the emission spectrum for benzo(a)pyrene on filter paper, resulting from exposure to 301 nm radiation, were studied for two different exposure conditions: continuous exposure, with the photomultiplier shutter being closed between scans; and limited exposure, with the sample shutter being closed between scans. Under the latter condition, ten-30 second scans during the one-hour period resulted in a total exposure of five minutes.

During continuous exposure, the 407 nm peak height initially increased for quantities of 200 ng and less and decreased for 400 ng, and the 429(425) nm peak height initially increased for quantities of 40 ng and less and decreased for quantities from 100 to 400 ng (Appendix D). After five to eight minutes of exposure, both peak heights decreased continuously for the remainder of the one-hour period. The maximum increase in the 407 nm peak height was 28 per cent (for 40 ng after three minutes of exposure), and the maximum increase in the 429(425)nm peak height was 23 per cent (for 40 ng after three and five minutes of exposure).

During limited exposure the 407 nm and the 429(425)nm peak heights initially increased or decreased as mentioned above and for the same quantities, but, except for the 200 ng, 407 nm peak, the peak-height changes continued their initial trend for the full one-hour period. (For 40 ng the peaks reached a maximum height in about 70 minutes.) The exception, the 200 ng, 407 nm peak, reached a maximum height within the first five minutes of exposure. The magnitude of the peak-height changes for the two exposure conditions was extremely different (Table 5). Both peaks displayed larger increases and smaller decreases during

continuous exposure.

Table 5. Maximum and minimum peak heights for benzo(a)-pyrene on paper occurring during one-hour periods of continuous and limited exposure. (In per cent of initial peak height.) (From Appendix D.)

Peak (nm)	Amount (ng)	For 1 Hr. Continuous Exposure		For 1 Hr. Limited Exposure	
		Maximum	Minimum	Maximum	Minimum
407	1	108.1	63.1	144.3	100.0
	10	109.8	71.6	168.0	100.0
	40	128.0	73.6	184.8	100.0
	100	112.0	75.6	116.2	100.0
	200	101.1	59.4	107.0	94.3
	400	100.0	40.3	100.0	72.3
429(425)	1	107.5	69.8	148.5	100.0
	10	111.1	73.6	177.0	100.0
	40	123.0	69.5	179.6	100.0
	100	100.0	51.7	100.0	83.8
	200	100.0	20.5	100.0	37.1
	400	100.0	12.4	100.0	28.9

The 469 nm peak, when present, decreased continuously under both exposure conditions, with the condition of continuous exposure producing the greater decrease (Appendix D, Figure 59).

A second series of changes, in addition to the peak-height changes, occurred during the exposure of 200 and 400 ng quantities of benzo(a)-pyrene to ultraviolet radiation. With decay of the fluorescence, the 425 nm peak shifted to 429 nm, the insignificant shoulder at 407 nm became a peak, and the 469 nm peak decayed rapidly, causing the spectrum to take on the appearance of the spectrum from a small quantity of benzo(a)pyrene (Figures 8 to 11). The spectral changes noted for increasing quantities of benzo(a)pyrene (Section 4.1.2) appeared to be reversed during the decay of the spectrum from a large quantity of benzo(a)pyrene.

Figure 8. Decay of fluorescence from 200 ng benzo(a)pyrene on filter paper during limited exposure for excitation and exposure wavelength 301 nm.

Figure 9. Decay of fluorescence from 200 ng benzo(a)pyrene on filter paper during continuous exposure for excitation and exposure wavelength 301 nm.

*The second degree scatter peak indicated a downward shift of 18 per cent for this spectrum.

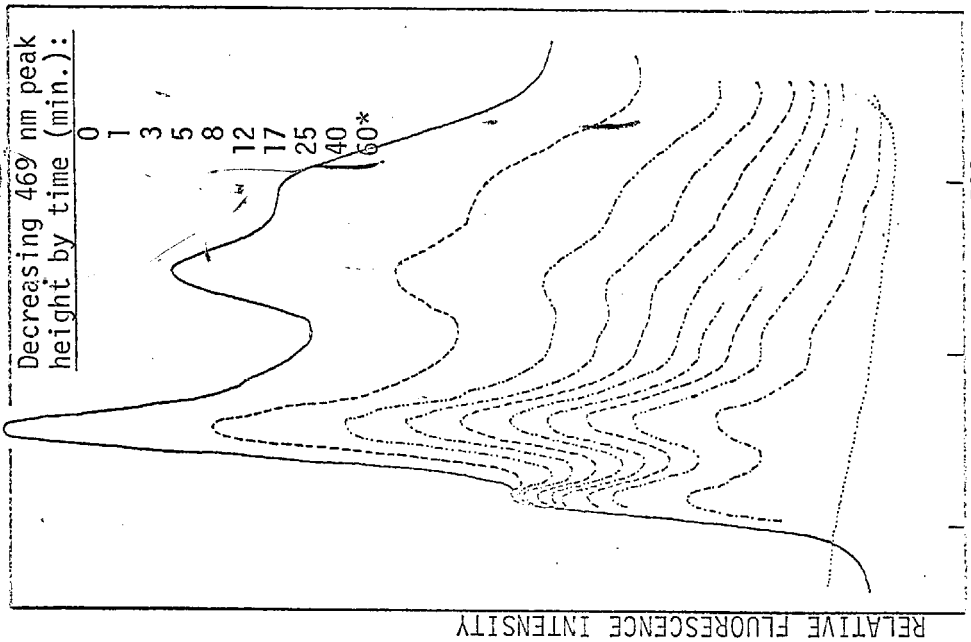


Figure 9

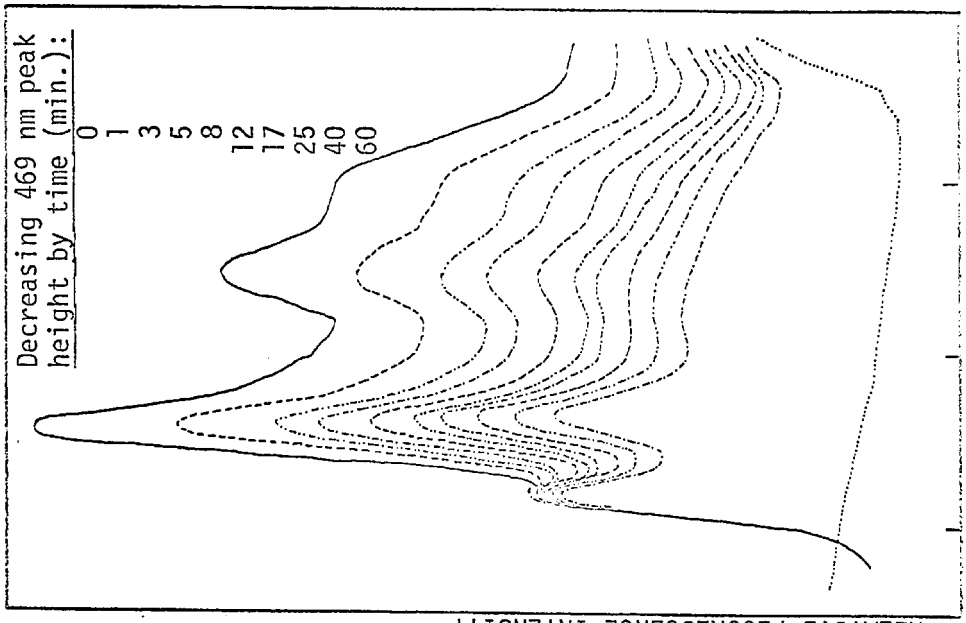


Figure 8

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Figure 10. Decay of fluorescence from 400 ng benzo(a)pyrene on filter paper during limited exposure for excitation and exposure wavelength 301 nm.

Figure 11. Decay of fluorescence from 400 ng benzo(a)pyrene on filter paper during continuous exposure for excitation and exposure wavelength 301 nm.

*The second degree scatter peak indicated an upward shift of 9 per cent for these spectra.

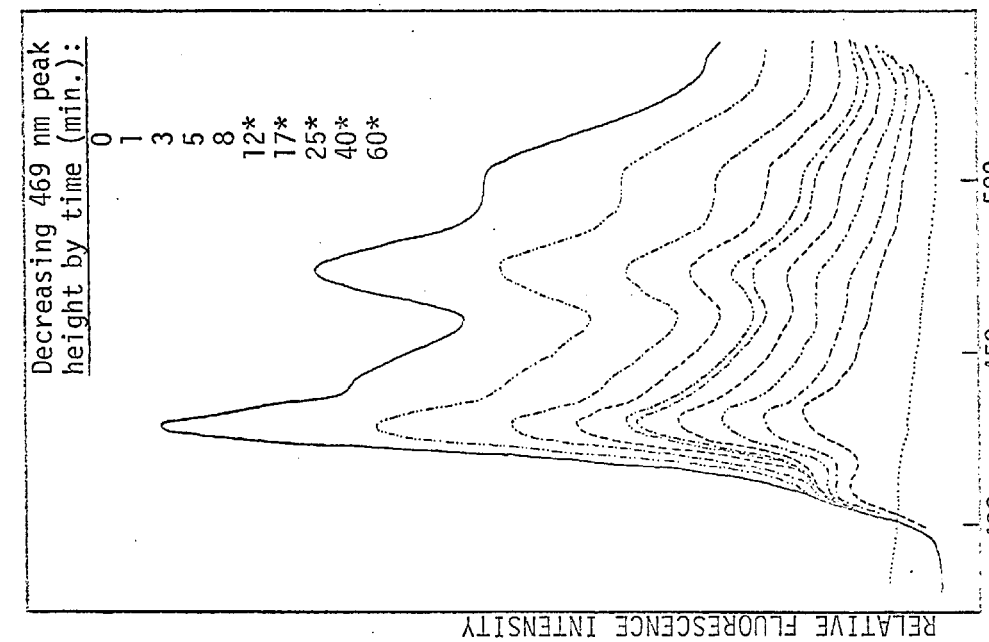


Figure 11

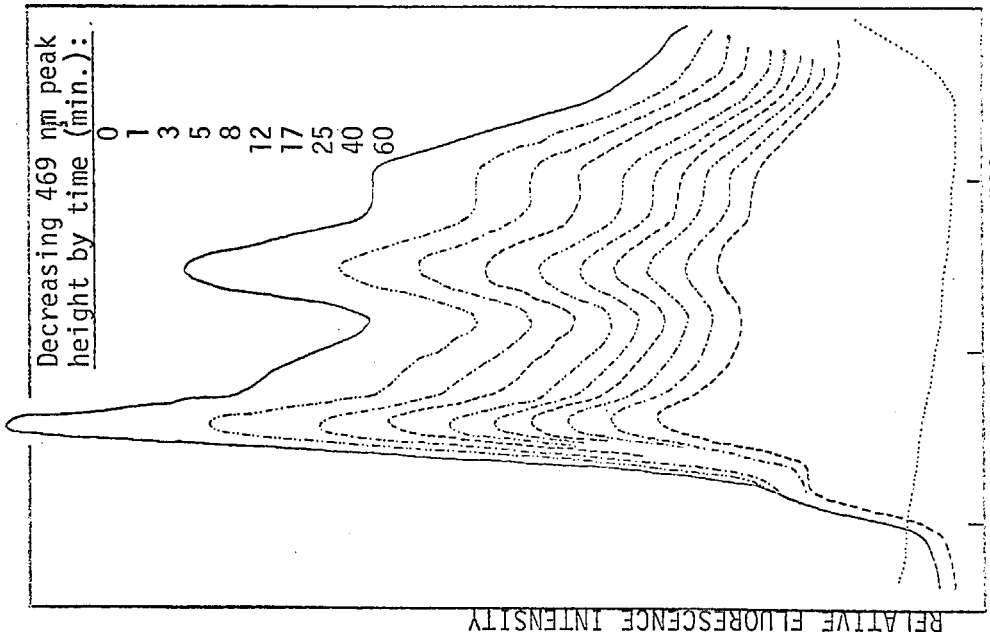


Figure 10

4.2 FLUORESCENCE FROM BzPer ON PAPER

4.2.1 BzPer Spectrum

The emission spectrum for small quantities of benzo(ghi)perylene on paper (Appendix E*, Figure 64) is similar in shape to reported spectra for benzo(ghi)perylene on chromatographic media (Dubois et al., 1960; and Thomas et al., 1960); the reported peak positions also coincide (Table 6). The emission spectrum for benzo(ghi)perylene on paper differs from its spectrum in solution (Appendix B, Figure 50), as described in Section 2.1.

Table 6. Reported fluorescence emission peaks for benzo-(ghi)perylene on chromatographic media.

Medium	Peaks (nm)	Reference
Paper chromatogram	380**, 399, 407.5, 420, 430, 445 shoulder, 460 shoulder	Dubois et al., 1960
Paper chromatogram	398, 407, 420, 430, 442 shoulder 456 shoulder	Thomas et al., 1960
Paper chromatogram	398, 408, 420, 432.5, 445, 460	Monkman and Porro, 1960
Filter paper (one ng)	398 shoulder, 408, 420, 433 shoulder	This study
Filter paper (10 ng)	398 shoulder, 408, 420, 433, 446 shoulder, 460 shoulder	This study

** This peak was deleted in a concurrent paper (Monkman and Porro, 1960) by one of the co-authors.

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* The benzo(ghi)perylene spectrum was affected by exposure of the compound to ultraviolet radiation. (See footnote, p. 27 and Section 4.2.3.)

The main excitation peak found in this study for small quantities of benzo(ghi)perylene is at 305 nm and a shoulder occurs at 297 nm. The only value available for comparison is a reported 297 nm excitation wavelength used in one study (Monkman and Porro, 1960).

4.2.2 BzPer Spectrum Changes with Quantity

The emission spectrum for one ng of benzo(ghi)perylene on paper excited at 305 nm, has peaks at 408 and 420 nm, and shoulders at 398 and 433 nm, but for 200 ng it has only a peak at 469 nm and a shoulder at approximately 500 nm (Figure 12 and Appendix E). The spectral changes taking place with increasing quantity were: the 433 nm shoulder for one ng became a peak for five ng; shoulders at 446 nm and 460 nm for lesser quantities were, for 20 ng, peaks at 446 nm and 469 nm; for 50 ng the 408, 420, 433 and 446 nm peaks were shoulders on the 469 nm peak, and a shoulder at 500 nm was apparent; and for larger quantities the high energy shoulders were less apparent, and only the 469 nm peak and the shoulder at 500 nm were readily distinguishable. The 408 nm peak experienced a height reversal for quantities between 20 ng and 50 ng (Figure 12), whereas the 469 nm peak height increased linearly with quantity (Figure 13).

The changes in the benzo(ghi)perylene spectrum with increasing quantity were similar to those occurring in the benzo(a)pyrene spectrum. The heights of the peaks dominant for small quantities of the compounds increased rapidly and then decreased, becoming obscured by a new peak or peaks which appeared. The changes occurring for the two compounds differed in that only a 469 nm peak appeared in the benzo(ghi)perylene spectrum, whereas a 469 nm and a 425 nm peak developed

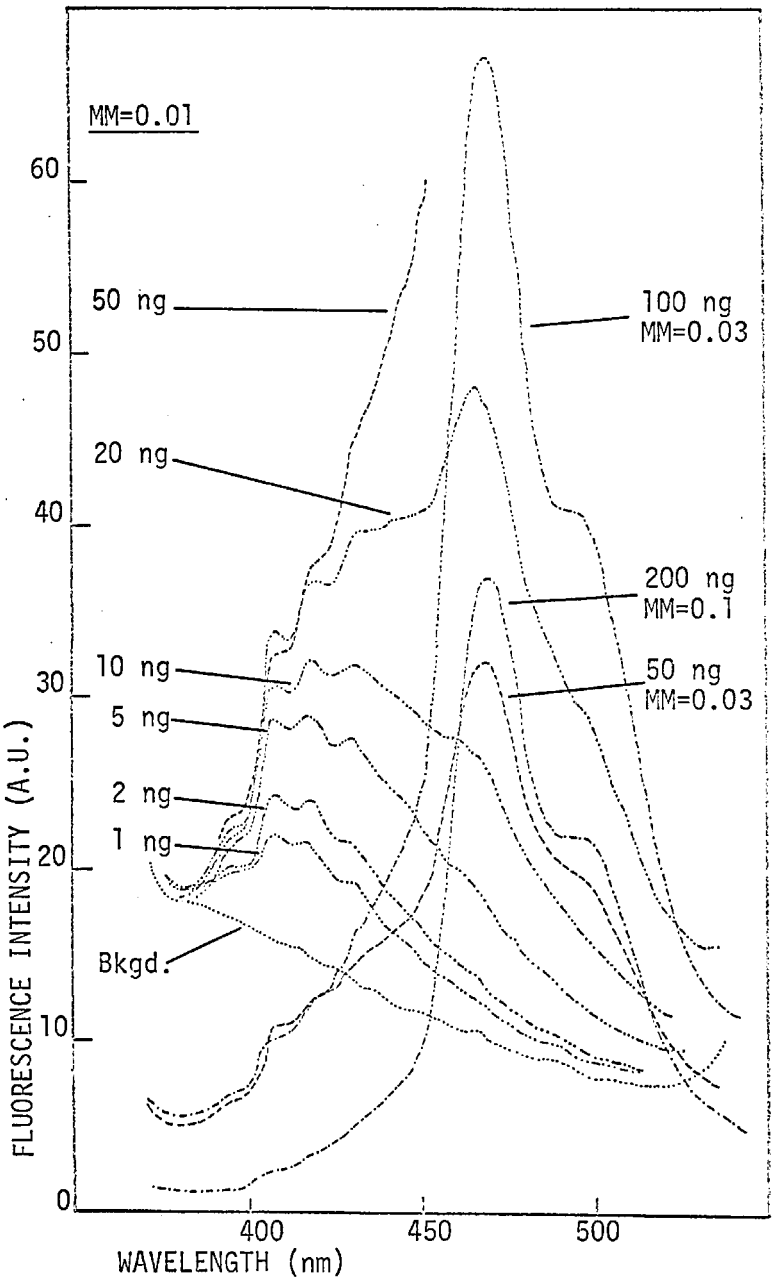


Figure 12. Emission spectra for one to 200 ng of benzo(ghi)perylene on filter paper for excitation wavelength 305 nm.

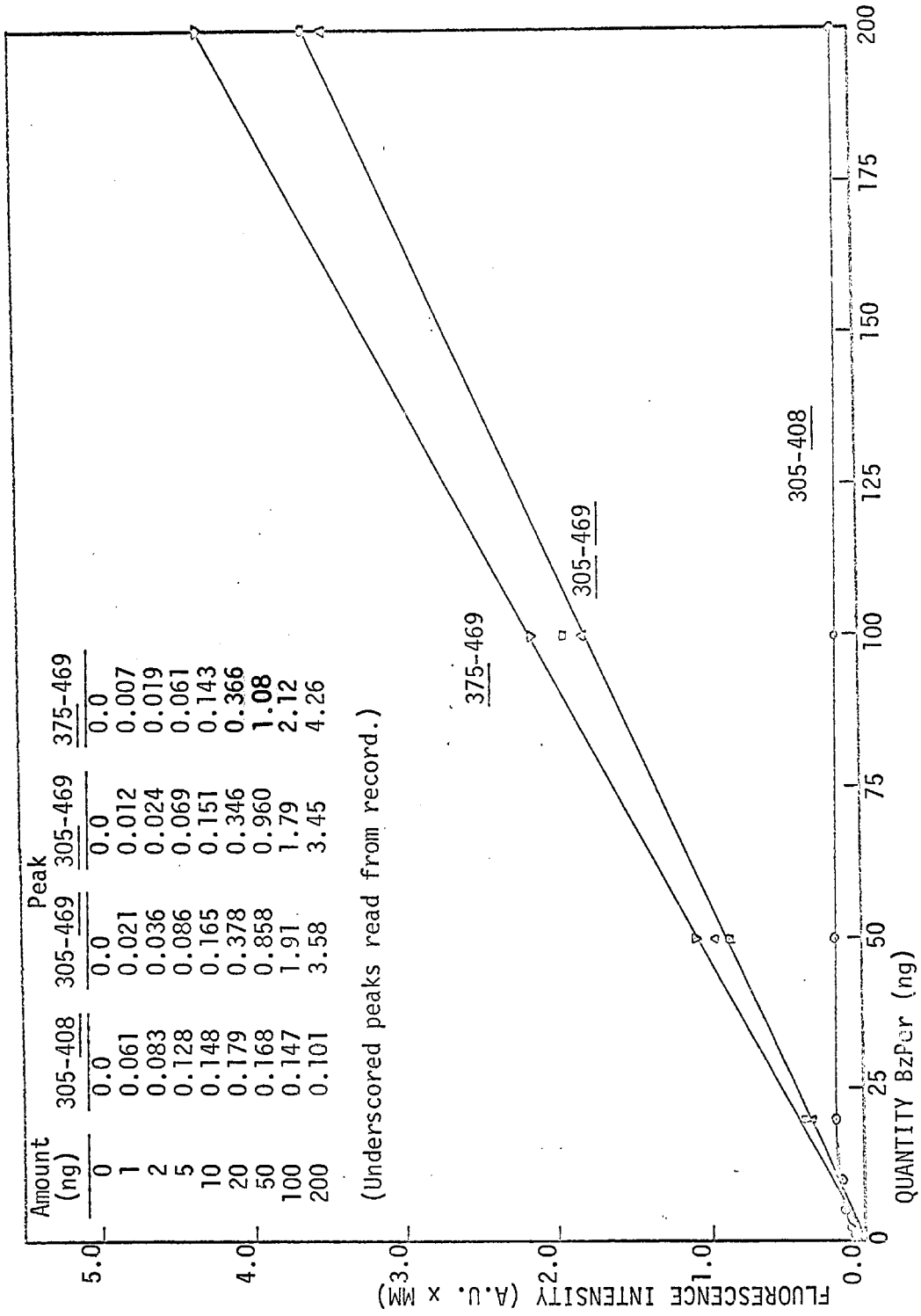


Figure 13. Peak height versus quantity of benzo(ghi)perylene on filter paper.

in the benzo(a)pyrene spectrum.

The excitation spectrum also changed with quantity, the nature of the changes being similar to those for the benzo(a)pyrene spectrum. The 305 nm excitation peak experienced a peak-height reversal for an emission wavelength of 408 nm (Figure 14) and the excitation peaks experienced a wavelength shift for an emission wavelength of 469 nm (Figure 15), the 305, 370 and 390 nm peaks for five ng occurring at 320, 378 and 397 nm for 200 ng.

4.2.3 BzPer Spectrum Changes with U.V. Exposure

The changes in the fluorescence from benzo(ghi)perylene on filter paper, resulting from exposure to 305 nm radiation, were studied for the two exposure conditions given in Section 4.1.3. During continuous exposure, the 408, 420 and 433 nm peak heights initially increased slightly and then decreased for one and 20 ng quantities (Figure 16 and Appendix F). For larger quantities the height changes in these peaks could not be measured because of the small magnitude of the changes (Figure 18). The greatest increase, six per cent, was displayed by the 420 nm peak for 20 ng of benzo(ghi)perylene after five minutes of exposure. After five minutes of exposure or less, the height of these peaks decreased for the remainder of the exposure period. During limited exposure the 408, 420 and 433 nm peaks increased continuously for the one-hour period (Figure 17), resulting in peak-height increases greater than those which occurred during continuous exposure (Table 7), as was the case for benzo(a)pyrene (Table 5). The magnitude of the changes in peak heights occurring during both exposure conditions were considerably less for benzo(ghi)perylene than for benzo(a)pyrene.

Figure 14. Excitation spectra for two to 200 ng of benzo-(ghi)perylene on filter paper for emission wavelength 408 nm.

Figure 15. Excitation spectra for one to 200 ng of benzo-(ghi)perylene on filter paper for emission wavelength 469 nm.

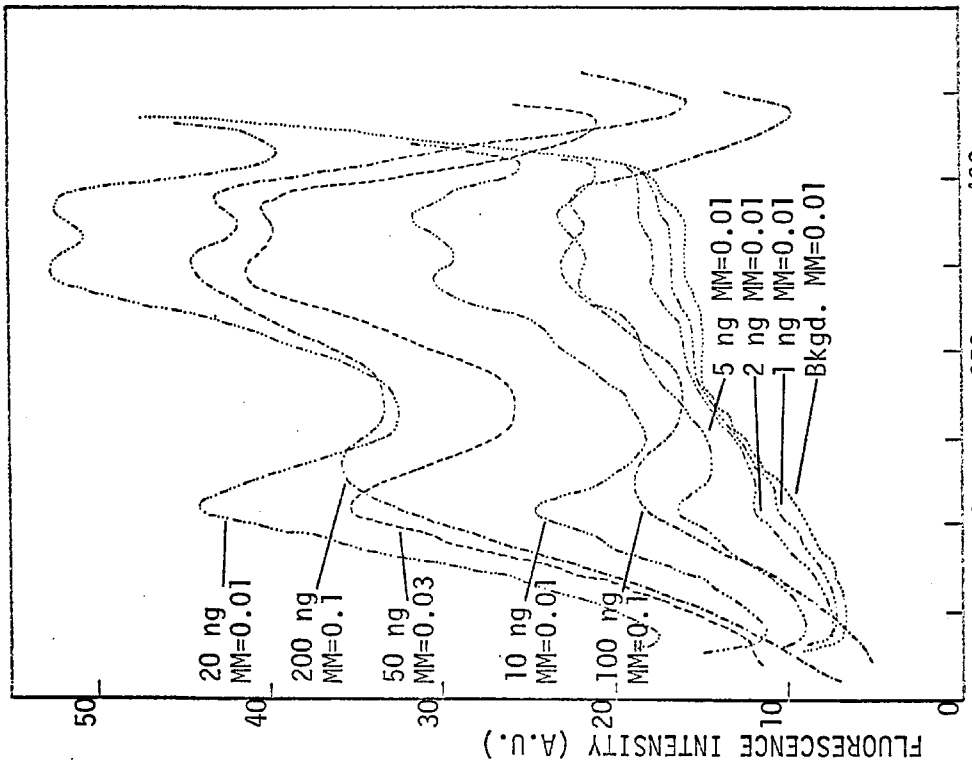


Figure 15

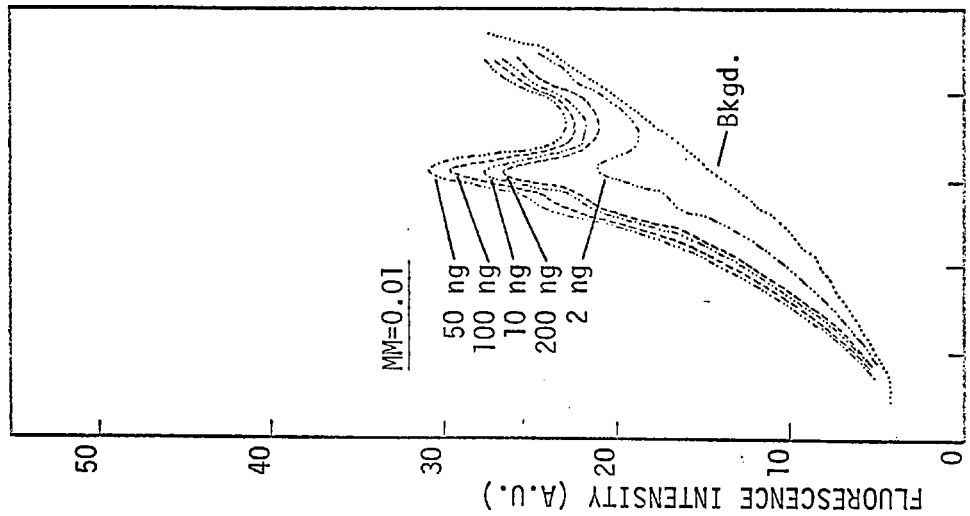


Figure 14

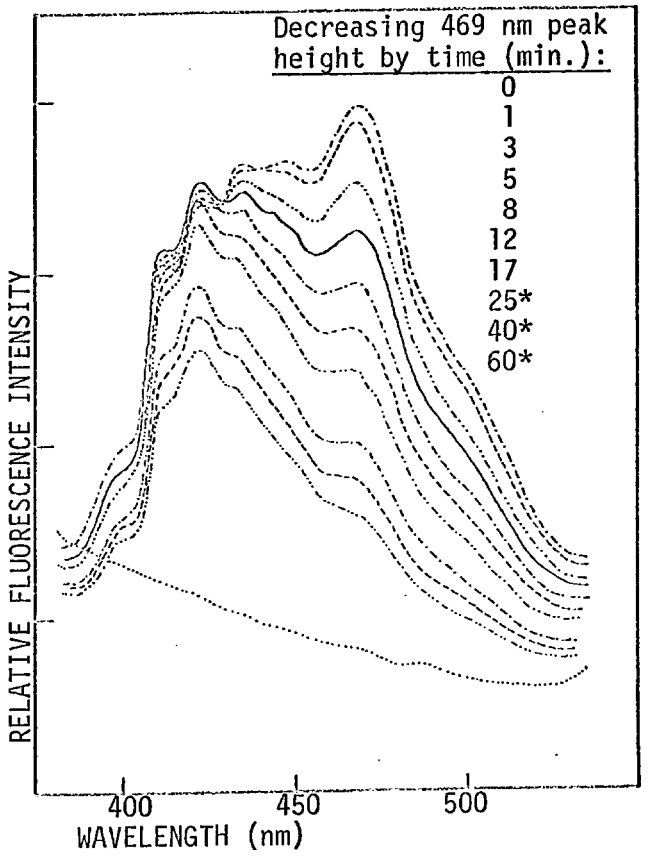


Figure 16

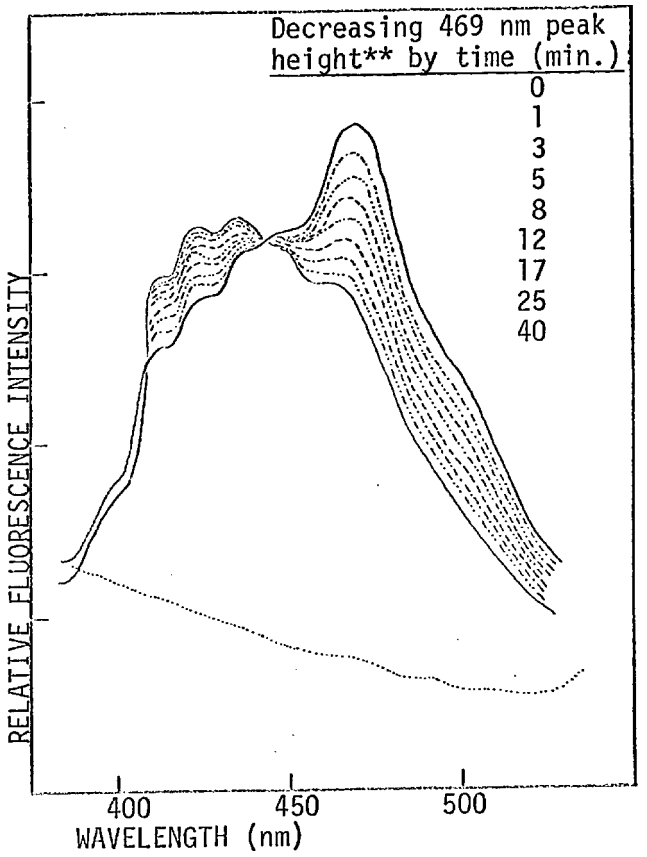


Figure 17

Figure 16. Decay of fluorescence from 20 ng benzo(ghi)perylene on filter paper during continuous exposure for excitation and exposure wavelength 305 nm.

Figure 17. Decay of fluorescence from 20 ng benzo(ghi)perylene on filter paper during limited exposure for excitation and exposure wavelength 305 nm.

* The second degree scatter peak indicated a downward shift of 7 per cent for these spectra.

** The order is reversed for the 408 nm peak height.

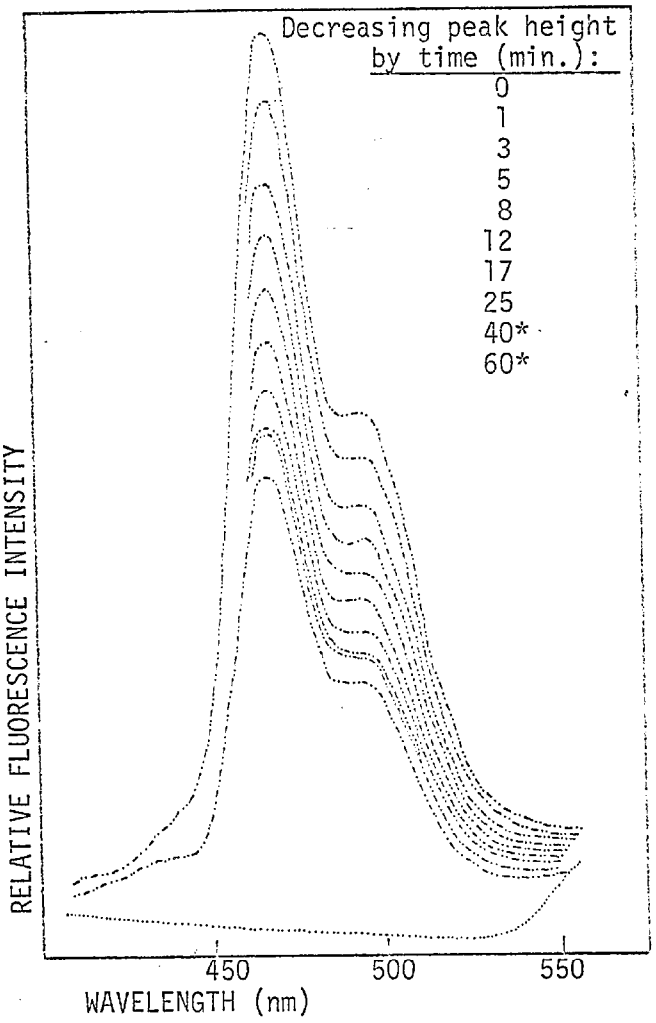


Figure 18. Decay of fluorescence from 200 ng benzo(ghi)perylene on filter paper during continuous exposure for excitation and exposure wavelength 305 nm.

* The second degree scatter peak indicated an upward shift of 15 per cent for the 40 min. and 16 per cent for the 60 min. spectra.

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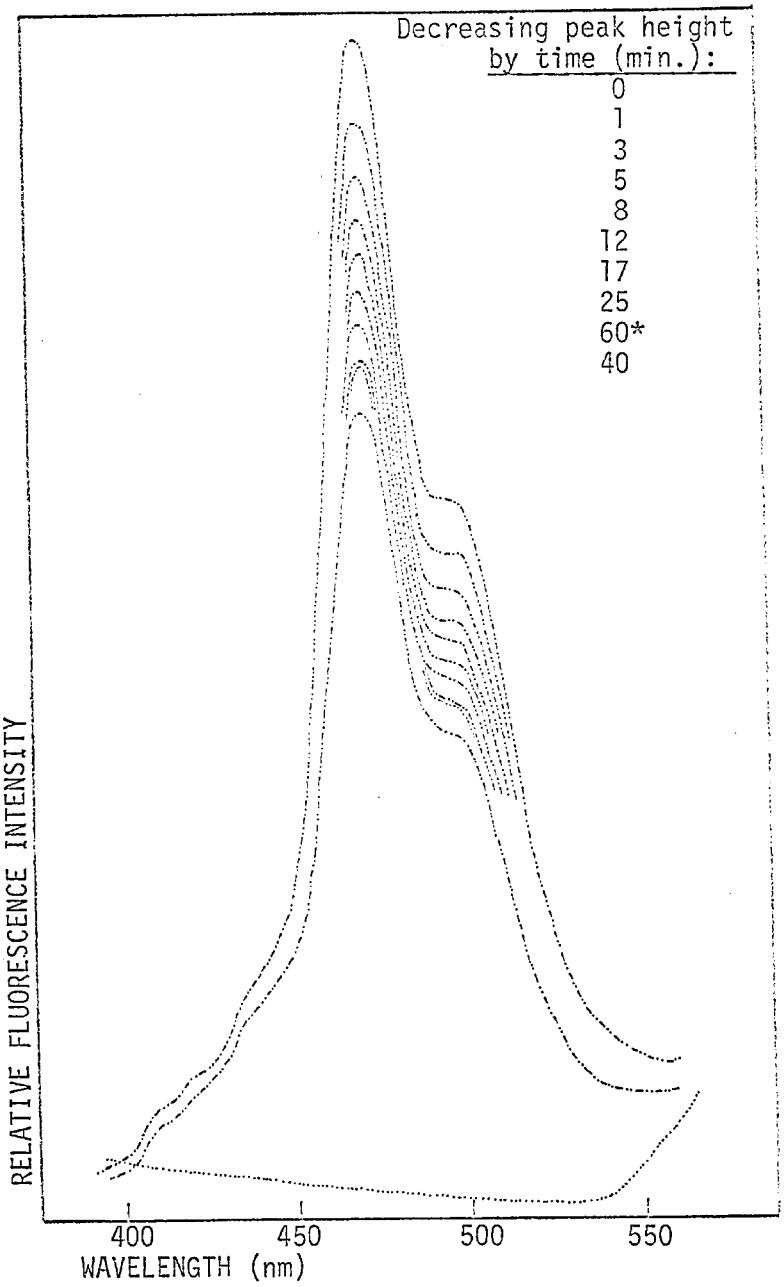


Figure 19. Decay of fluorescence from 200 ng benzo(ghi)perylene on filter paper during limited exposure for excitation and exposure wavelength 305 nm.

* The second degree scatter peak indicated an upward shift of 8 per cent for this spectrum.

Table 7. Maximum and minimum peak heights for benzo-(ghi)perylene on paper occurring during one-hour periods of continuous and limited exposure. (In per cent of initial peak height.) (From Appendix F.)

Peak (nm)	Amt. (ng)	For 1 Hr. Continuous Exposure		For 1 Hr. Limited Exposure	
		Maximum	Minimum	Maximum	Minimum
408	1	101.2	45.2	111.5	100.0
	20	104.4	65.2	130.0	100.0
420	20	105.8	70.6	125.2	100.0
433	20	101.5	54.0	108.8	100.0
469	20	100.0	33.0*	100.0	65.1*
	50	100.0	32.5*	100.0	65.8*
	100	100.0	42.6*	100.0	66.2*
	200	100.0	48.0*	100.0	67.5*

* These results are for the exposure condition given but for 40 minutes duration. The 60-minute minimum for six of the eight values are given in Appendix F.

The height of the 469 nm peak, when it was present, decreased continuously under both exposure conditions, and the condition of continuous exposure produced the greater decrease in peak height (Appendix F, Figure 70; and Figures 18 and 19). The relative change in the 469 nm peak height for benzo(ghi)perylene was less than that for benzo(a)-pyrene for both exposure conditions.

The exposure of benzo(ghi)perylene to ultraviolet radiation did not result in a shifting of its emission peaks, as was noted for benzo(a)pyrene.

4.3 FLUORESCENCE FROM BzPy ON MYLAR

4.3.1 BzPy Spectrum

The emission spectrum for benzo(a)pyrene on Mylar, for excitation

wavelengths of 325 nm* (Figure 20), 301 nm and 312 nm (Appendix G), is characterized by peaks at 425 nm and at 469 nm, and shoulders at 440 nm and at 500 nm. The excitation spectrum for emission wavelengths of 469 nm and 500 nm has major peaks at 312 nm and 418 nm and small peaks and shoulders between these peaks (Appendix G). Benzo(a)pyrene on Mylar was not extremely fluorescent (Section 5.4.5), and its spectra, especially its excitation spectrum, were greatly influenced by the Mylar background, which displayed an excitation peak at 310 nm and several other peaks coincident with the benzo(a)pyrene peaks.

4.3.2 BzPy Spectrum Changes with Quantity

Unlike the fluorescence from benzo(a)pyrene on paper, spectral changes with quantity were slight for quantities of benzo(a)pyrene on Mylar increasing from 0 to 400 ng. In this quantity range the fluorescence increased almost uniformly, with no shifting of peak wavelengths (Figure 20). Changes in the 425 nm and 469 nm peak heights were approximately linear with quantity for quantities from 0 to 200 ng (Figures 33 and 34).

Slight departures from a uniform increase in fluorescence for quantities of benzo(a)pyrene increasing from 0 to 400 ng suggested that significant spectral changes may occur for quantities greater than 400 ng. The observation of these departures, the changing 425/469 peak-height ratio with quantity (1.21 for 40 ng and 0.85 for 400 ng)

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* This excitation wavelength was selected for the analysis of mixtures of benzo(a)pyrene and benzo(ghi)perylene. Therefore, these preliminary studies were made at this wavelength. (See Discussion, Section 5.4.1, for the basis of this selection.)

and the presence of a distinct shoulder at 440 nm for 400 ng of benzo-(a)pyrene, lead to the recording of spectra for larger compound quantities, in which a 439 nm peak was apparent (Figure 21).

4.3.3 BzPy Spectrum Changes with U.V. Exposure

Changes in the fluorescence from benzo(a)pyrene on Mylar resulting from 325 nm irradiation were studied for one-hour periods of continuous and limited exposure and for three-30 minute periods of continuous exposure. The three-30 minute determinations were made to better document the decay of the peaks preparatory to exploiting the stability difference between benzo(a)pyrene and benzo(ghi)perylene during irradiation for mixture analysis.

During continuous and limited exposure, the decay of fluorescence from benzo(a)pyrene was uniform (Figures 22 and 23), with the 425 and the 469 nm peaks decaying at approximately the same rate (Table 8 and Appendix H). For continuous exposure, the decay rate of the fluorescence appeared to be independent of the quantity present for quantities of 100 ng and larger, whereas the decay rate for 40 ng* of benzo(a)pyrene was much greater than for these larger quantities. The condition of limited exposure resulted in less decay of the fluorescence than did the condition of continuous exposure.

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* The decay for quantities less than 40 ng are not presented because low fluorescence intensity and the dependency of the relative peak heights on small background differences make these rates very problematical.

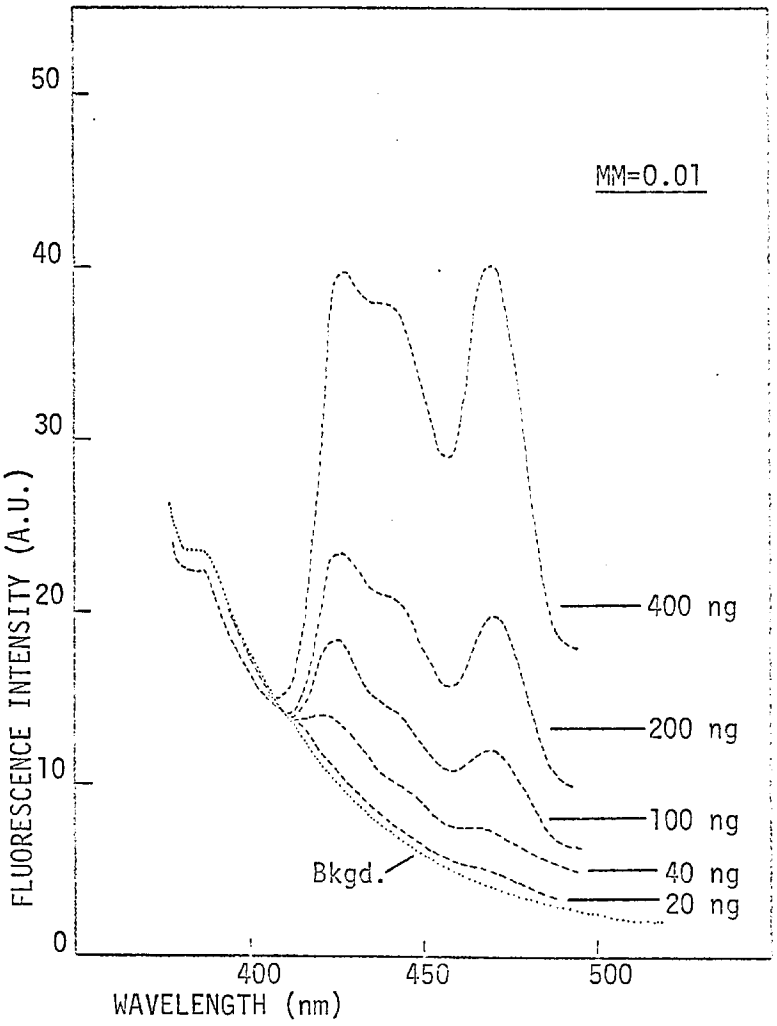


Figure 20. Emission spectra for 20 to 400 ng of benzo(a)-pyrene on **Mylar** for excitation wavelength 325 nm.

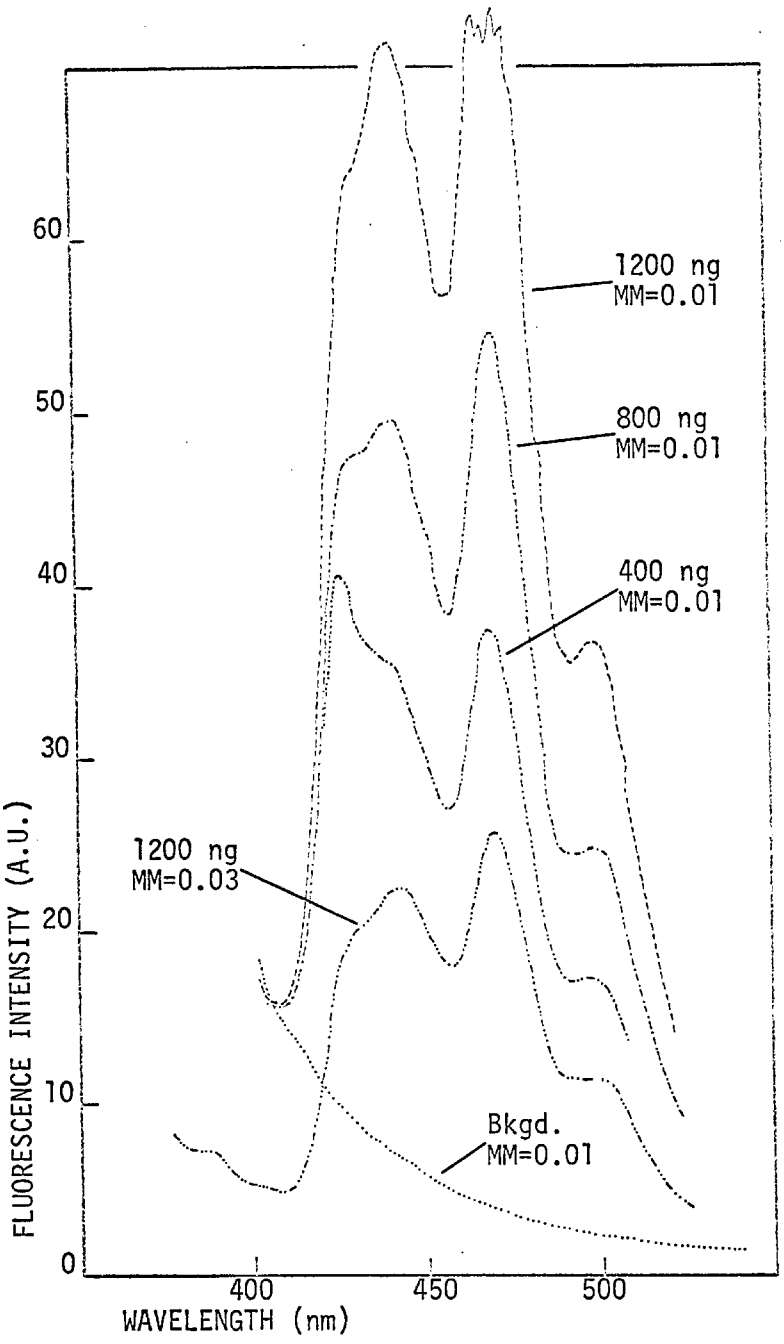


Figure 21. Emission spectra for 400, 800 and 1200 ng of benzo(a)pyrene on **Mylar** for excitation wavelength 325 nm.

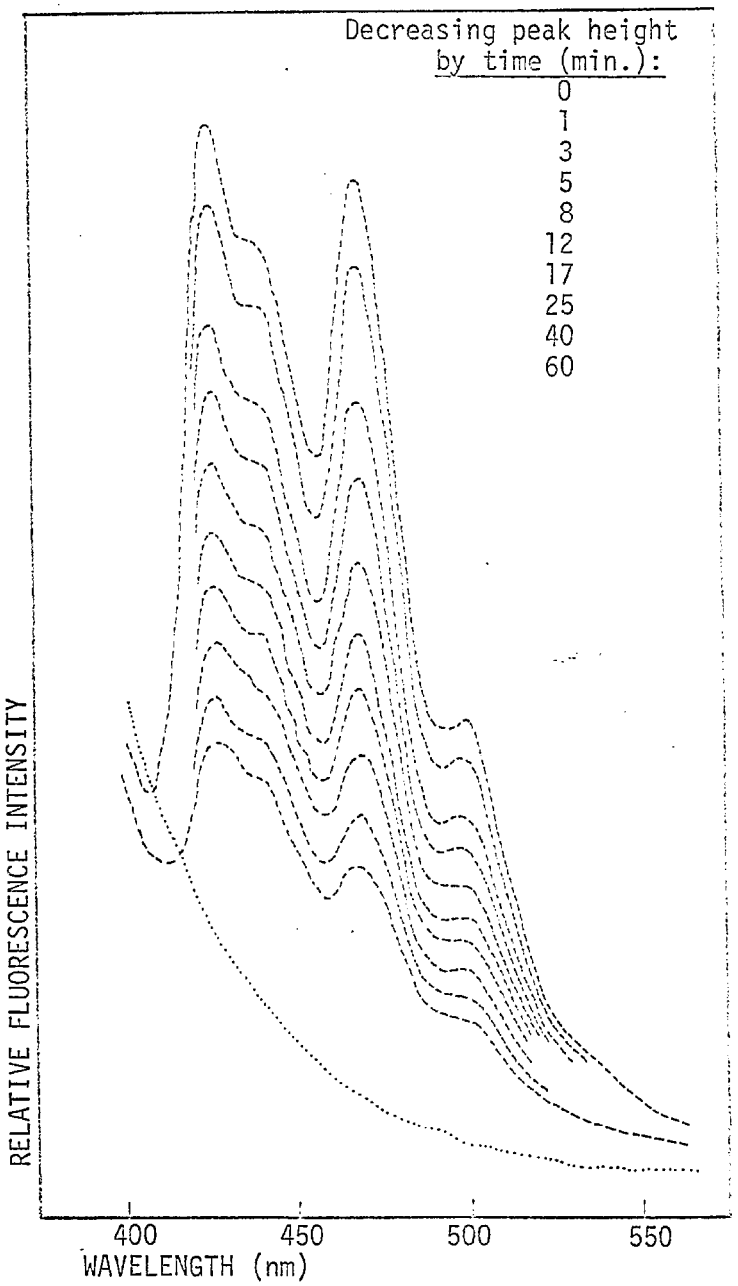


Figure 22. Decay of fluorescence from 400 ng benzo(a)pyrene on **Mylar** during continuous exposure for excitation and exposure wavelength 325 nm.

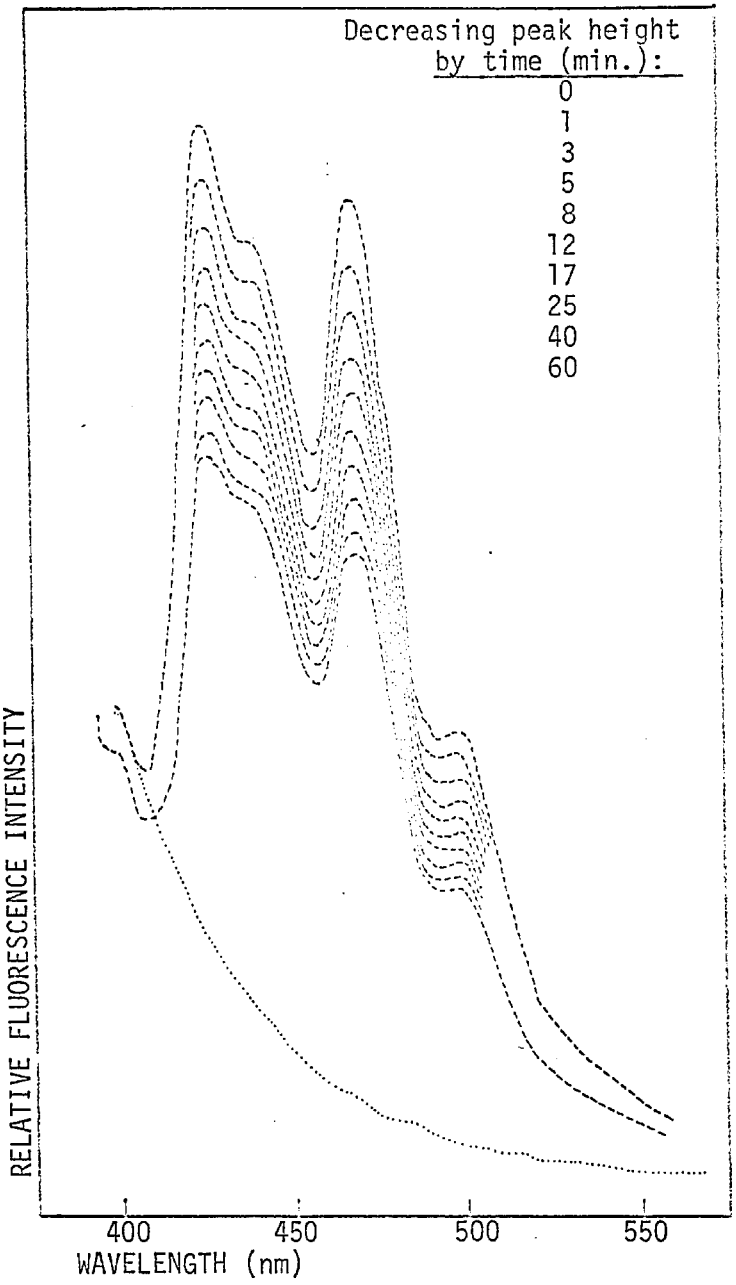


Figure 23. Decay of fluorescence from 400 ng benzo(a)pyrene on **Mylar** during limited exposure for excitation and exposure wavelength 325 nm.

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Table 8. Per cent of peak heights for benzo(a)pyrene on Mylar remaining after exposure to 325 nm radiation. (From Appendix H.)

Peak (nm)	Amt.(ng)	Continuous Exposure 30 min.	Continuous Exposure 40 min.	Limited Exposure 40 min.
425	100	---	26.8	62.0
	400	---	29.8	63.0
469	40	2.0**	---	---
	100	36.9***	31.8	66.4
	200	38.7***	---	---
	400	---	30.8	63.2

** Two determinations

*** Three determinations

Since a quantity effect was present in the spectrum from 1200 ng of benzo(a)pyrene, the decay of this spectrum was studied during a one-half hour period of continuous exposure to determine if a reversal of the quantity effect occurred as it did for benzo(a)pyrene on paper. The reversal did not occur. The 439 nm peak decreased at approximately the same rate as the 425 nm shoulder instead of decreasing more rapidly than the rest of the spectrum (Figure 24).

4.4 FLUORESCENCE FROM BzPer ON MYLAR

4.4.1 BzPer Spectrum

The emission spectrum for benzo(ghi)perylene on Mylar has a peak at 469 nm and a shoulder at 500 nm for excitation wavelengths of 375 nm (Figure 25) and 325 nm (Figures 26 and 27). The excitation spectrum displays a shoulder at 325 nm and peaks at 375 and 392 nm (Figure 25). The fluorescent intensity of benzo(ghi)perylene was greater than that of benzo(a)pyrene for the compounds on Mylar, and the background peaks

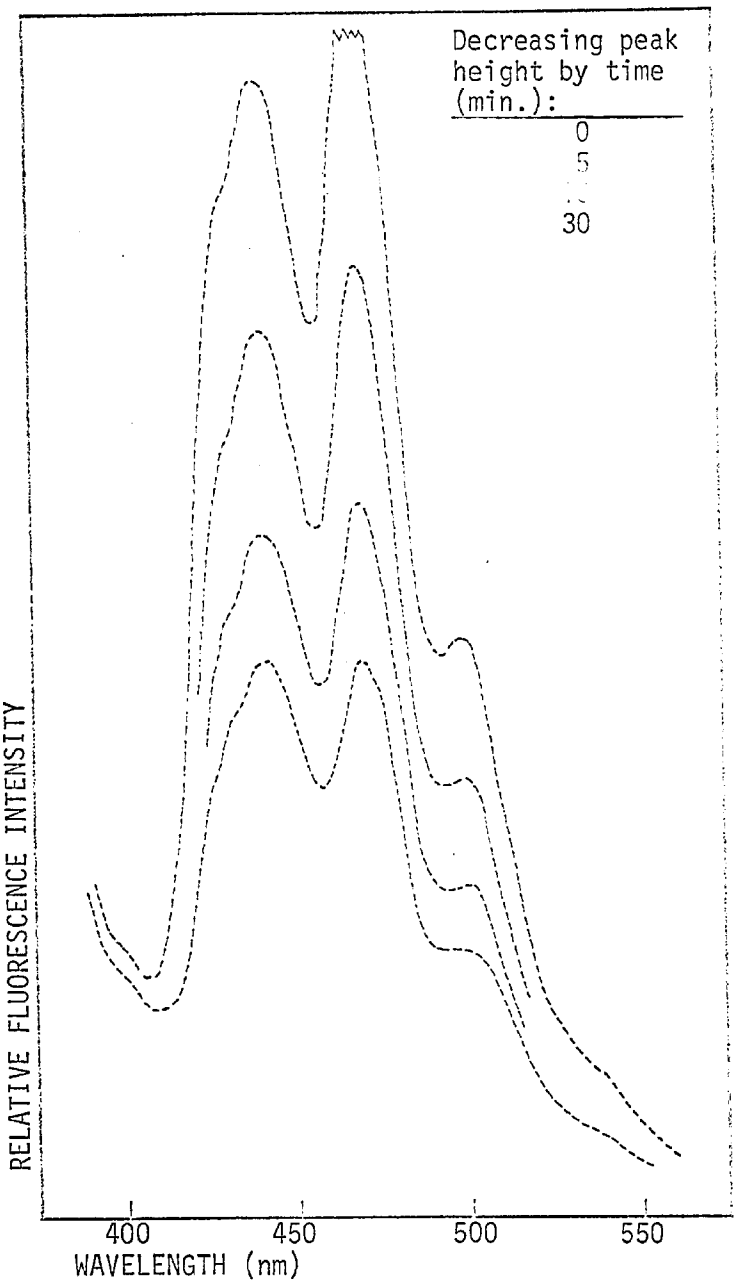


Figure 24. Decay of fluorescence from 1200 ng benzo(a)pyrene during continuous exposure for excitation and exposure wavelength 325 nm.

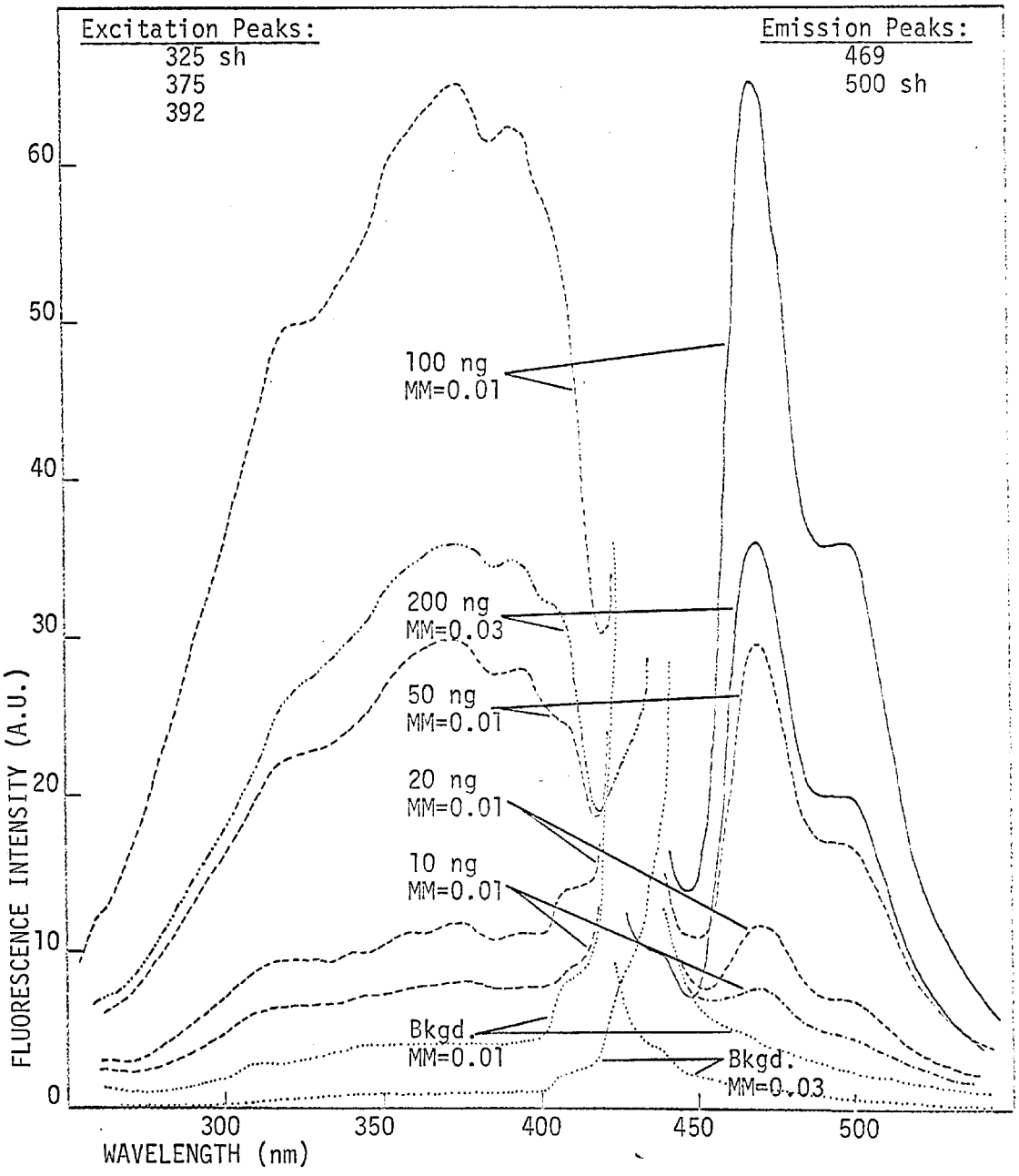


Figure 25. Excitation and emission spectra for 10 to 200 ng of benzo-(ghi)perylene on **Mylar** for excitation wavelength 375 nm and emission wavelength 469 nm.

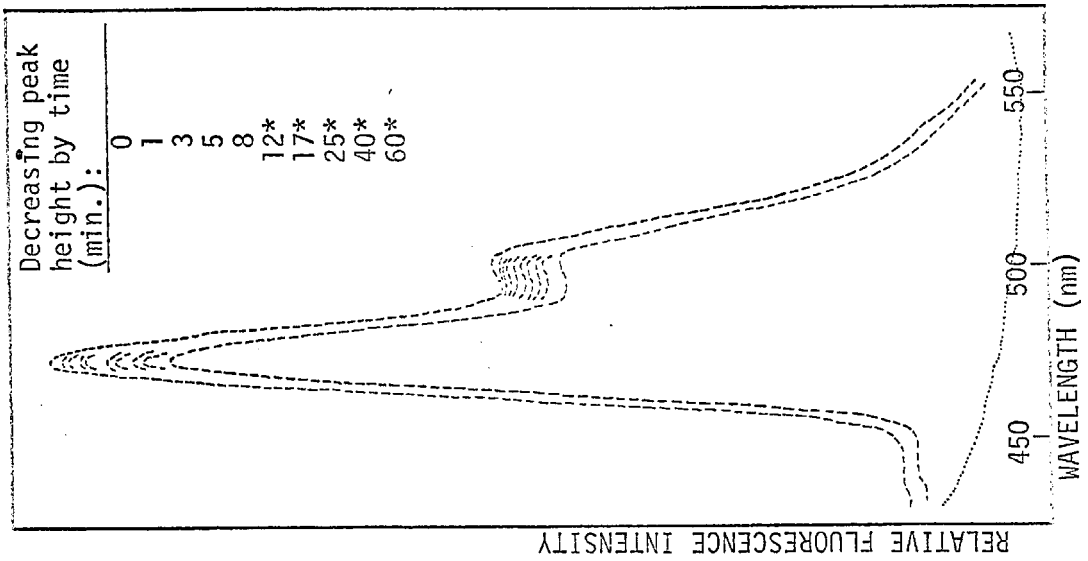


Figure 26. Decay of fluorescence from 200 ng benzo(ghi)perylene on **Mylar** during limited exposure for excitation and exposure wavelength 325 nm.

* The second degree scatter peak indicated downward shifts of 1.6, 2.4, 2.4, 2.4 and 5.4 per cent respectively for these spectra.

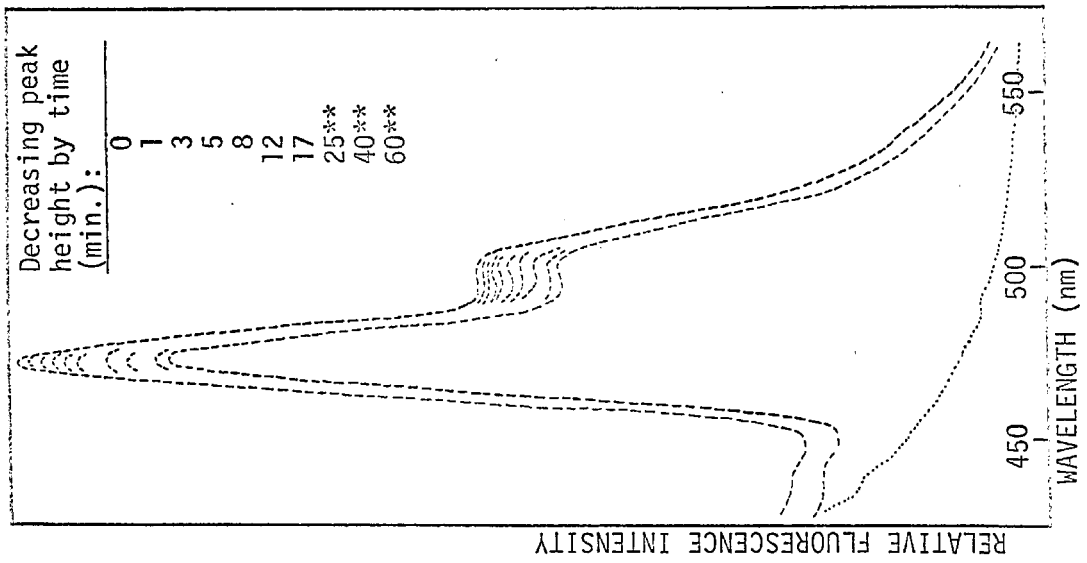


Figure 27. Decay of fluorescence from 100 ng benzo(ghi)perylene on **Mylar** during continuous exposure for excitation and exposure wavelength 325 nm.

** The second degree scatter peak indicated downward shifts of 1.5, 2.7 and 4.6 per cent respectively for these spectra.

from Mylar did not present the interference which occurred in the excitation spectrum for benzo(a)pyrene. The presence of the 325 nm shoulder in the spectrum from benzo(ghi)perylene was a consideration in the selection of the excitation wavelength for mixture analysis (Section 5.4.1).

4.4.2 BzPer Spectrum Changes with Quantity

In the quantity range from 0 to 200 ng the fluorescence from benzo(ghi)perylene increased uniformly (Figure 25) with no shifting of peak wavelengths. The 469 nm peak height changed linearly for quantities increasing from approximately 20 to 100 ng (Figure 34) but departed from a linear relationship for quantities outside this range. For quantities less than 20 ng the 469 nm peak was not well defined, and for quantities greater than 100 ng self-absorption of the fluorescence may have occurred.

4.4.3 BzPer Spectrum Changes with U.V. Exposure

The changes in the fluorescence from benzo(ghi)perylene on Mylar were studied under the conditions given in Section 4.3.3, including the three-30 minute determinations.

The decay of fluorescence from benzo(ghi)perylene was uniform under conditions of continuous and limited exposure to 325 nm radiation (Figures 26 and 27), and the fluorescence from quantities of 100 ng and larger (100 and 200 ng in this case) decreased approximately the same relative amount during equal times of continuous exposure (Appendix I), as was the case for benzo(a)pyrene. The fluorescence from quantities less than 100 ng again decreased more rapidly than it did from larger quantities.

The relative decreases in the 469 nm peak for the two exposure conditions were approximately the same (Table 9).

Table 9. Per cent of 469 nm peak height for benzo(ghi)-perylene on Mylar remaining after exposure to 325 nm radiation. (From Appendix I.)

Amount (ng)	Continuous Exposure		Limited Exposure
	30 min*	40 min.	40 min.
10	67.0	----	----
20	77.0	----	----
50	80.4	----	----
100	91.9	88.4	92.0
200	----	88.5	93.3

* Average of three determinations.

The changes in fluorescence induced by exposure of the compounds on Mylar to ultraviolet radiation were much more pronounced for benzo-(a)pyrene than for benzo(ghi)perylene, as was the case for the compounds on paper.

4.5 FLUORESCENCE FROM MIXTURES ON MYLAR

The fluorescence from benzo(a)pyrene-benzo(ghi)perylene mixtures was studied to determine if quantitative analysis of mixtures was possible. The approaches to the analysis suggested by the preliminary studies were: using the 425 nm peak for the benzo(a)pyrene determination and calculating the amount of benzo(ghi)perylene present by difference using the 469 nm peak; and using the difference in the decay rates of the 469 nm peaks for the two compounds.

4.5.1 Spectrum of Mixtures

Mixtures of benzo(a)pyrene and benzo(ghi)perylene on Mylar yielded

spectra that were less intense than spectra determined from the linear summation of the spectra for the components (Figures 28 to 32). The relative difference between the 469 nm peak heights for the mixture spectrum and the summation spectrum appeared to depend on the ratio of the compounds present (Table 10) rather than on the amount of either compound present or the total amount of the compounds present*, and this difference appeared to increase as the BzPy/BzPer mass ratio approached four from either direction.

Table 10. Difference between the 469 nm peak heights from mixtures* and from the summation spectra of the components**, on Mylar. (Summation peak = 100%.)

Quantity of BzPy (ng)	200	100	50	40	25	20
Mass Ratio (BzPy/BzPer)						
20/1	0	..	..	..	..	..
8/1	29	34	..	..	..	..
4/1	74	78	..	72	..	..
2/1	59	59	..	57	..	..
1/1	..	34	54	..	..	56
0.5/1	..	..	40	..	26	..
0.2/1	..	..	..	..	..	11

* From Appendix J.

** From Figures 33 and 34.

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* The linear correlation coefficients for the amount of quenching to the amount of each compound present and the total amount of compounds present were not significantly different from 0.

Figure 28. Emission spectrum from 200 ng BzPy-100 ng BzPer mixture on Mylar for excitation wavelength 325 nm. (Not corrected to standard uranium-glass bead reading.)

Figure 29. Emission spectrum from 200 ng BzPy-50 ng BzPer mixture on Mylar for excitation wavelength 325 nm. (Not corrected to standard uranium-glass bead reading.)

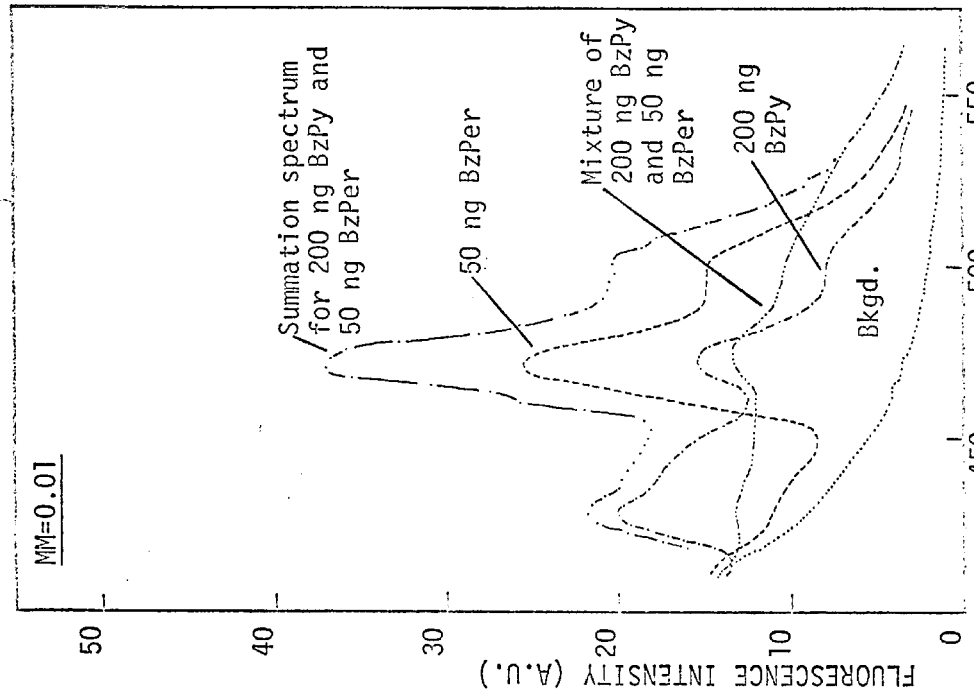


Figure 29

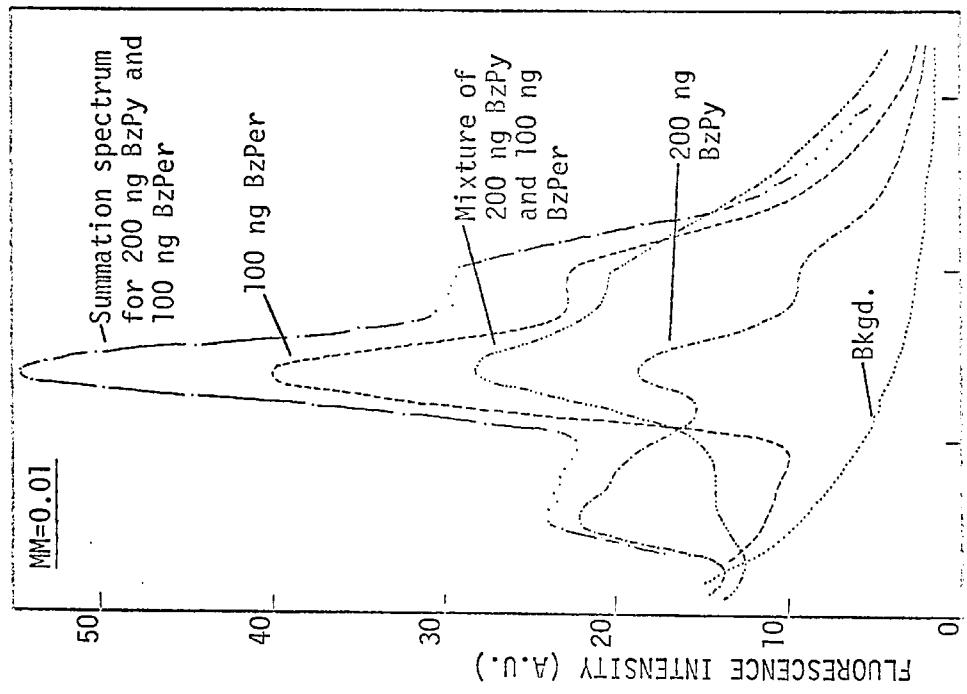


Figure 28

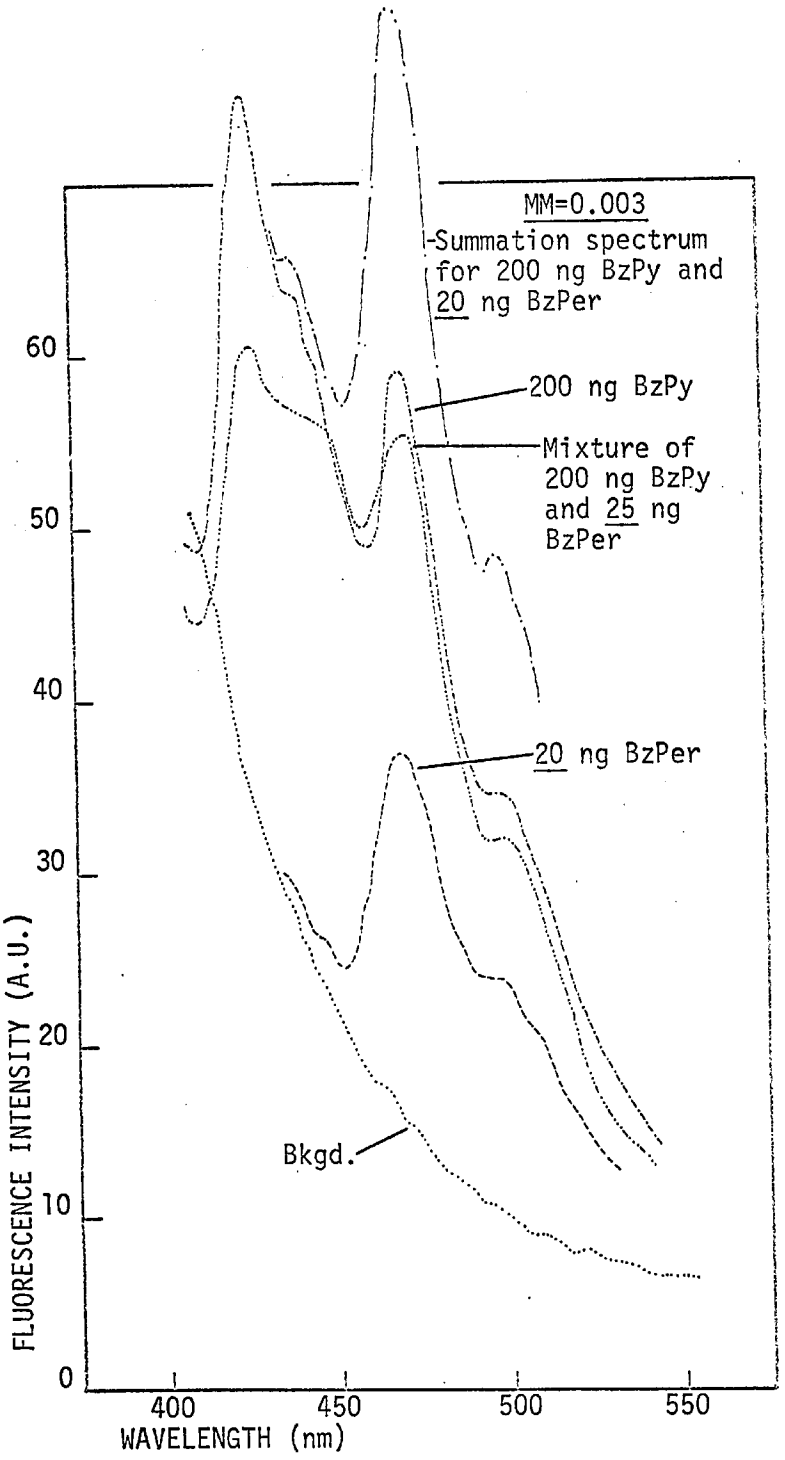


Figure 30. Emission spectrum from 200 ng BzPy-25 ng BzPer mixture on **Mylar** for excitation wavelength 325 nm. (Not corrected to standard uranium-glass bead reading.)

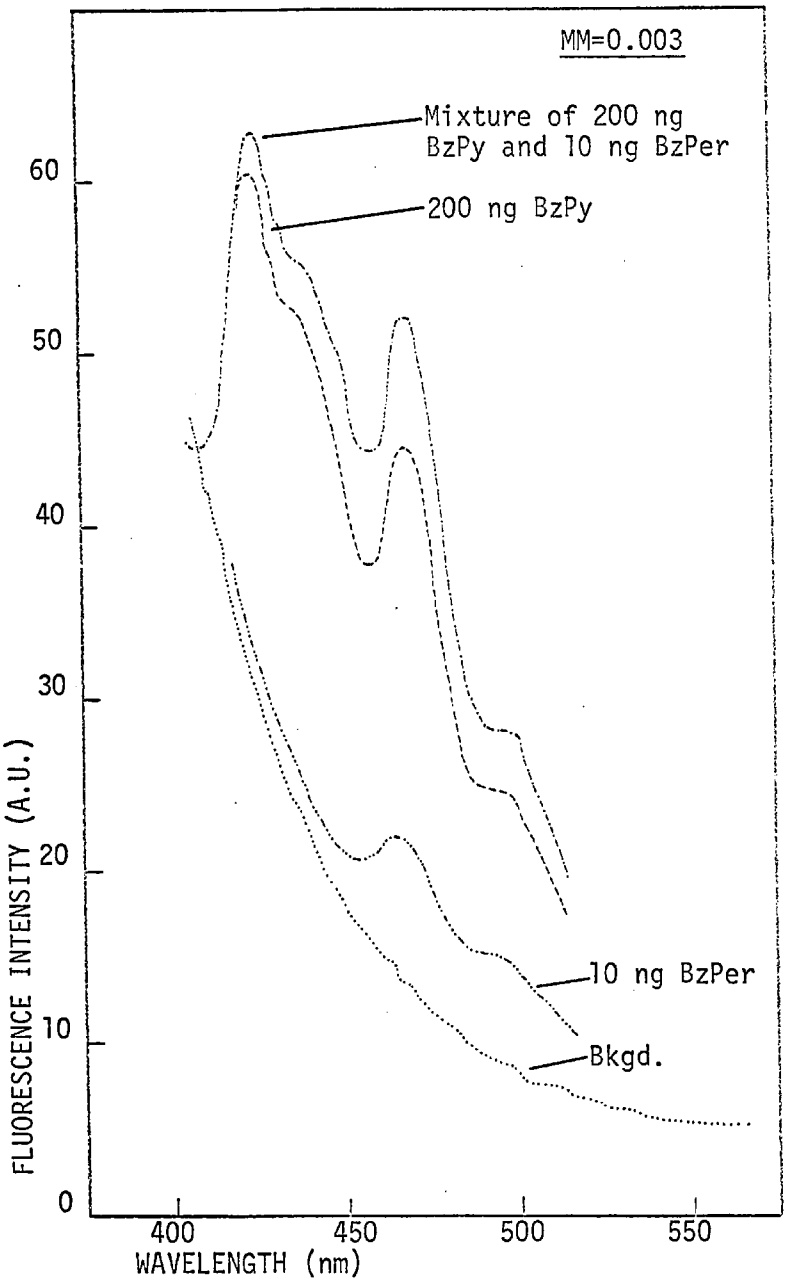


Figure 31. Emission spectrum from 200 ng BzPy-10 ng BzPer mixture on **Mylar** for excitation wavelength 325 nm. (Not corrected to standard uranium-glass bead reading.)

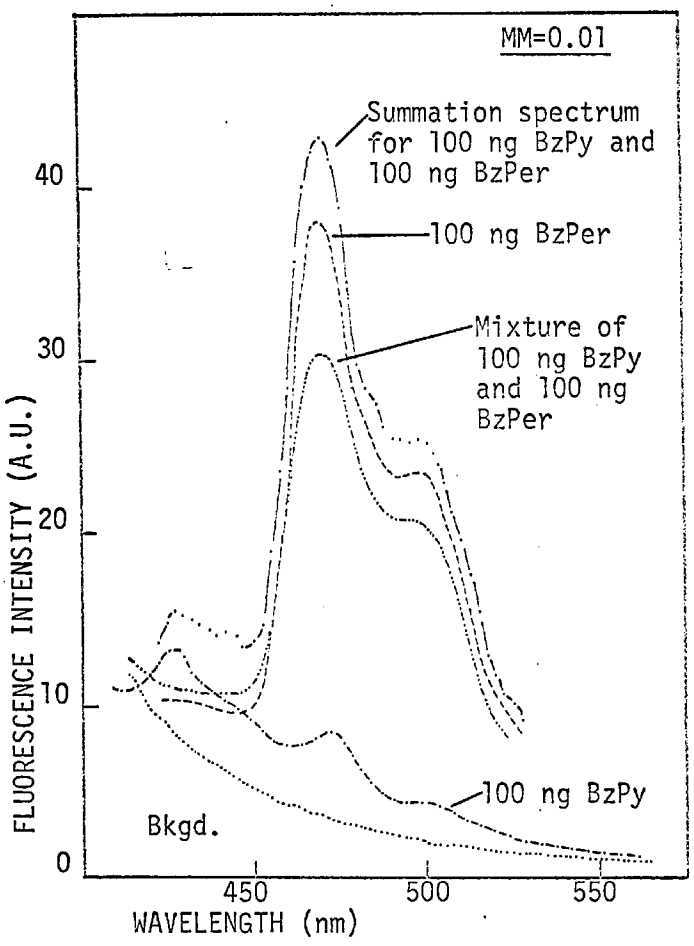
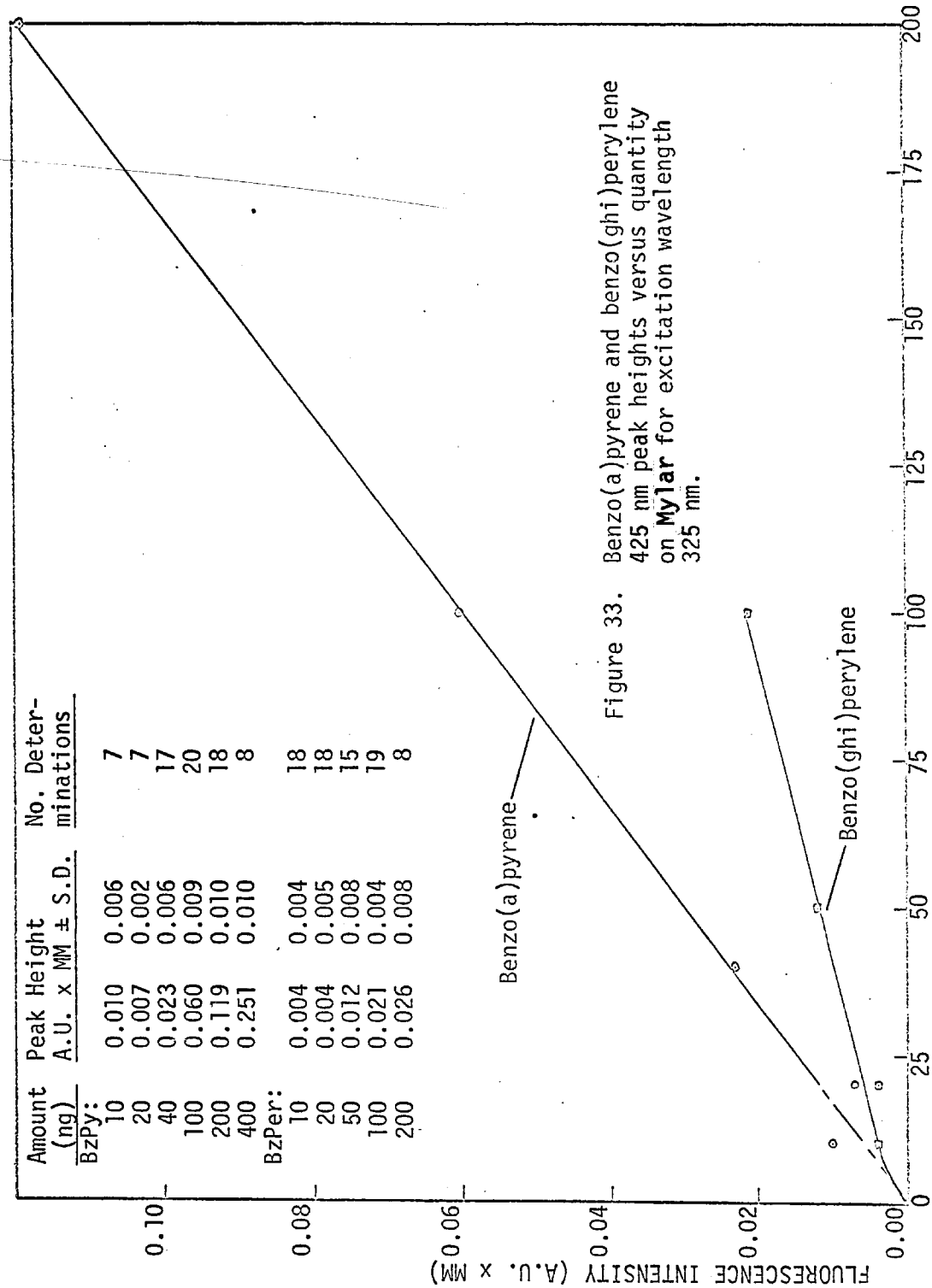


Figure 32. Emission spectrum from 100 ng BzPy-100 ng BzPer mixture on Mylar for excitation wavelength 325 nm. (Not corrected to standard uranium-glass bead reading.)



Amount (ng)	Peak Height A.U. x MM ± S.D.		No. Determinations
BzPy:			
10	0.010	0.006	7
20	0.007	0.002	7
40	0.023	0.006	17
100	0.060	0.009	20
200	0.119	0.010	18
400	0.251	0.010	8
BzPer:			
10	0.004	0.004	18
20	0.004	0.005	18
50	0.012	0.008	15
100	0.021	0.004	19
200	0.026	0.008	8

Figure 33. Benzo(a)pyrene and benzo(ghi)perylene 425 nm peak heights versus quantity on **Mylar** for excitation wavelength 325 nm.

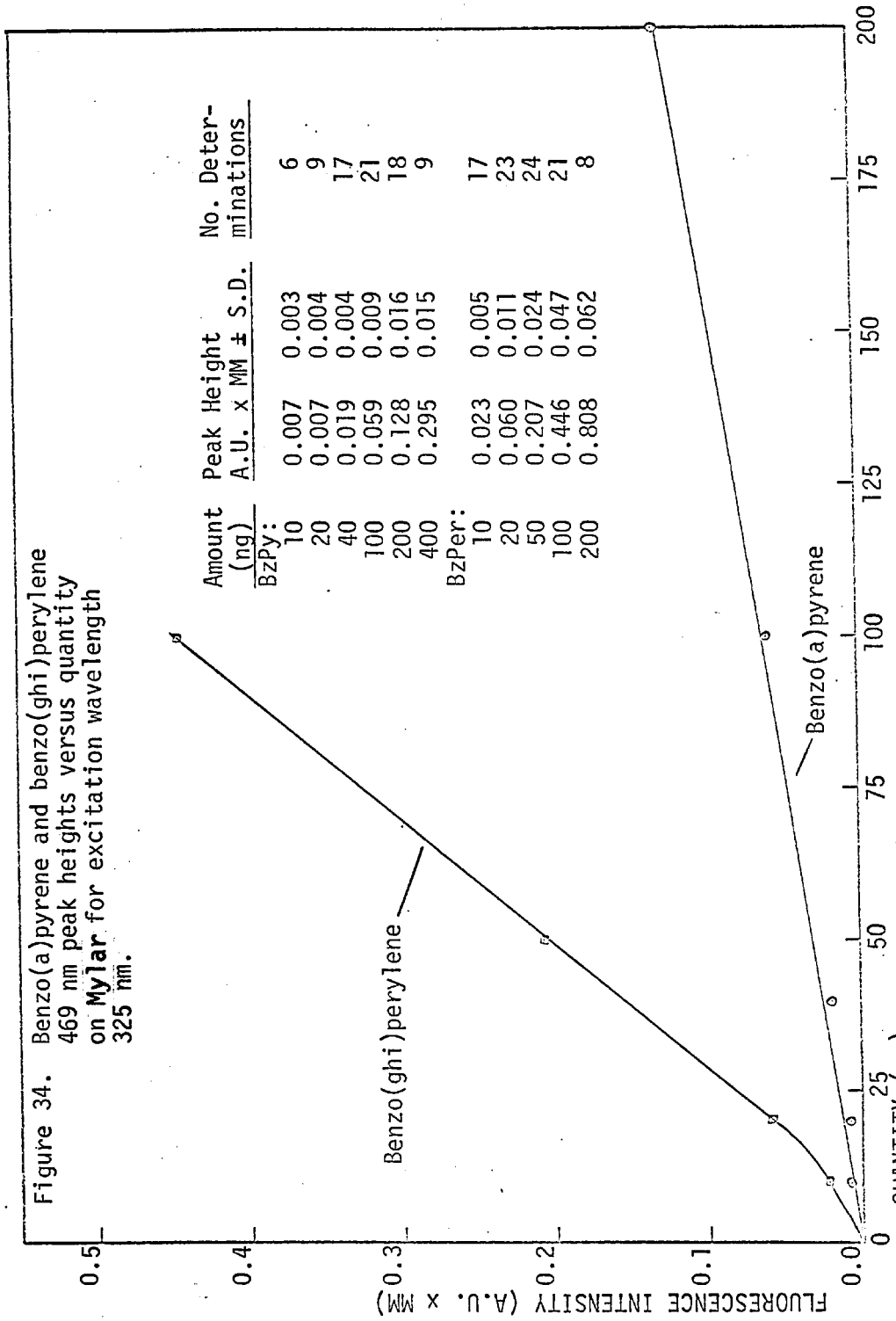


Figure 34. Benzo(a)pyrene and benzo(ghi)perylene 469 nm peak heights versus quantity on Mylar for excitation wavelength 325 nm.

Amount (ng)	Peak Height A.U. x MM ± S.D.		No. Determinations
BzPy:			
10	0.007	0.003	6
20	0.007	0.004	9
40	0.019	0.004	17
100	0.059	0.009	21
200	0.128	0.016	18
400	0.295	0.015	9
BzPer:			
10	0.023	0.005	17
20	0.060	0.011	23
50	0.207	0.024	24
100	0.446	0.047	21
200	0.808	0.062	8

The relative amount by which the fluorescence from each compound was reduced (Table 11) were calculated from the following simultaneous equations:

$$a(x_{425}) + b(y_{425}) = (x_{425} + y_{425}) - M_{425} = R_{425}$$
$$a(x_{469}) + b(y_{469}) = (x_{469} + y_{469}) - M_{469} = R_{469}$$

where:

a = proportion BzPer fluorescence reduced;

b = proportion BzPy fluorescence reduced;

x_{425} and x_{469} = BzPer 425 and 469 nm readings in A.U. x MM (Figures 33 and 34);

y_{425} and y_{469} = BzPy 425 and 469 nm readings in A.U. x MM (Figures 33 and 34); and

M_{425} and M_{469} = Mixture 425 and 469 nm readings in A.U. x MM (Appendix J).

The use of these equations assumes that the relative quenching of the fluorescence from each compound is the same at both emission wavelengths.

The relative reduction in the fluorescence from benzo(ghi)perylene was greater than the relative reduction in the fluorescence from benzo(a)pyrene for the three mixtures having BzPy/BzPer ratios greater than four, and it was less for nine of the 10 mixtures having ratios of four or less. (No estimation of the fluorescence reduction for benzo(a)pyrene was possible for two mixtures.) The relative reduction in fluorescence for both compounds increased as the mass ratio of the compounds approached four.

Table 11. Per cent reduction in the fluorescence from benzo-(a)pyrene and benzo(ghi)perylene in mixtures on Mylar.

Mass Ratio (BzPy/BzPer)	Compound	Amount BzPy (ng) 200	100	50	40	25	20
20/1	BzPy	-7*	..	..	..	..	..
	BzPer	33	..	..	..	..	..
8/1	BzPy	4	25	..	..	..	..
	BzPer	65	51	..	..	..	..
4/1	BzPy	77	93	..	79	..	..
	BzPer	73	67	..	65	..	..
2/1	BzPy	70	84	..	71	..	..
	BzPer	56	52	..	52	..	..
1/1	BzPy	..	82	77	..	..	106*
	BzPer	..	27	51	..	..	48
0.5/1	BzPy	..	..	15	..	**	..
	BzPer	..	..	42	..	21-27	..
0.2/1	BzPy	..	..	..	..	..	**
	BzPer	..	..	..	..	..	9-11

* Values of 0 and 100 for -7 and 106 are within the limits of error.

** The reduction of the 425 nm peak could not be determined for these small quantities of benzo(a)pyrene. The first number for benzo(ghi)perylene was calculated assuming 100% quenching of benzo(a)pyrene's fluorescence and the second number, assuming 0% quenching.

4.5.2 Mixture-Spectrum Changes with U.V. Exposure

The greater sensitivity of benzo(a)pyrene to 325 nm radiation was evident from the changes this radiation produced in the emission spectrum from mixtures. When the maximum quantity of each mixture was exposed continually to ultraviolet radiation, the 425 nm peak decreased more than the 469 nm peak (Table 12), the 425 nm peak being displayed by benzo(a)pyrene only and the 469 nm peak being displayed by both compounds.

The greater sensitivity of benzo(a)pyrene was also shown by the increase in the decay rate for the 469 nm peak from mixtures of increasing BzPy/BzPer ratios (Figure 35).

Table 12. Per cent of 425 nm and 469 nm peak heights remaining after 30 minutes of continuous exposure to 325 nm radiation. (From Appendix K.)

Amount of Compound in Mixture (ng)		Peak Height Remaining (%)	
BzPy	BzPer	425 nm	469 nm
200	100	55	81
200	50	51	74
200	25	35	49
200	10	21	31
100	100	22	84
50	100	78	93
20	100	65	93

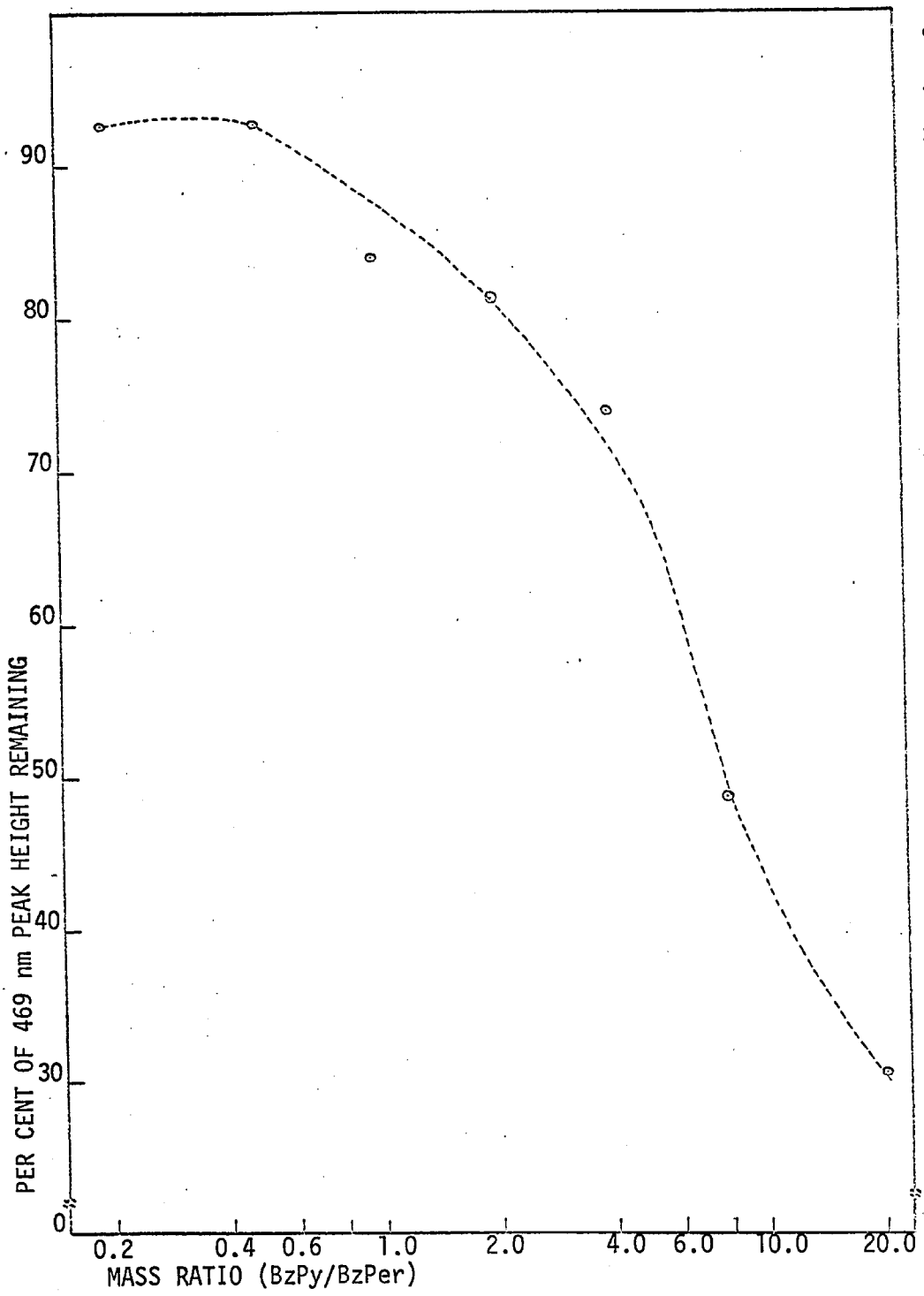


Figure 35. Relative 469 nm peak height for mixtures on **Mylar** exposed to 325 nm radiation for 30 minutes.

5. DISCUSSION

5.1 DISCUSSION OF ERRORS

The reproducibility in the peak-height measurements for the compounds deposited on Mylar must be considered poor. The standard deviations in peak-height measurements, made over a three-week period, for larger quantities of benzo(a)pyrene and benzo(ghi)perylene on Mylar ranged from four to 15 per cent (Table 13); the standard deviations were significantly greater for smaller quantities. The reproducibility in peak-height measurements for the compounds on paper was not determined, but a comparison of the many spectra recorded suggested better reproducibility of results for the compounds on this medium than for the compounds on Mylar. The results for the compounds on paper may approach the five to 10 per cent agreement reported for duplicate determinations of benzo(a)pyrene on paper chromatograms (Monkman and Porro, 1960).

Table 13. Standard deviations in peak-height measurements for benzo(a)pyrene and benzo(ghi)perylene on Mylar.

Compound	Quantity (ng)	Peak (nm)	No. of Determinations	Standard Deviation (%)
BzPy	100	425	20	15
	200	425	18	8
	400	425	8	4
	100	469	21	15
	200	469	18	13
	400	469	9	5
BzPer	50	469	24	12
	100	469	21	11
	200	469	8	8

Random errors in the peak-height measurements are attributed to variations in the position and deposition of the sample, the scatter

contribution to the spectrum, and the performance of the spectrophotofluorometer and recorder. Of these sources for random errors, the dependency of the results on the position of the sample (Table 3) probably exerted the most unequal influence on the variation in results for the two media. This dependency would conceivably be eliminated if all sample depositions were centered, symmetric, and uniform, and these criteria were more closely approached by the deposits on filter paper than by the deposits on Mylar. Variations among samples in the scatter contribution to the spectra made background determinations difficult. This scatter variation, caused by rough edges on the mount, wrinkles or bows in the mount and small changes in the angle of the mount to the excitation radiation, more greatly affected the results for peaks nearer the first order scatter peak.

The problem of randomly occurring, stepwise changes in the output signal from the spectrophotofluorometer has already been mentioned (Section 3.2). These changes appeared to be a constant percentage, as opposed to a constant increment, over the recorded spectrum. The occurrence of these changes and a small amount of drift, which would be acceptable in most convential uses of the instrument, necessitated the use of an uranium-glass bead standard and the second order scatter peak to monitor the performance of the instrument. The use of these controls kept this error from instrumentation to less than an estimated five per cent.

The presence of lint and dust on the Mylar mounts was a source of frustration rather than a source of error. These contaminants would distort the deposition of the sample and resulted in many discarded samples.

In addition to continually monitoring the performance of the instrument, two other precautions were taken to minimize the influence of these errors. The deposits on Mylar were confined to as small an area as possible in the center of the mount in an effort to reduce the dependency of the results on the position of the sample. This precaution was taken because large area samples suffered mainly from not meeting the third criterion, uniformity. The second precaution taken was using recorded spectra, rather than microphotometer readings, for making peak-height determinations. Excessive scatter, maldeposition of samples, and changes in the performance of the instrument were frequently detected from the recorded spectra.

Recording the spectra, rather than using microphotometer readings, resulted in greater exposure of the sample to the excitation radiation and, consequently, greater changes in the peak heights. This error from peak-height changes during exposure was kept as uniform as possible by using a constant scan rate throughout the study. Other systematic errors were a three per cent error introduced by not including the first rinse of the pipette with the sample and, possibly, an error in the peak-height measurements for compounds on paper. This last error is described in the next section.

5.2 BzPy ON PAPER

5.2.1 BzPy Spectrum Changes with Quantity

The changes in the benzo(a)pyrene spectrum with quantity can cause several problems in the quantitative analysis of benzo(a)pyrene directly on chromatographic media.

The height of the 407 nm peak, the peak used in previous studies for the quantitative analysis of benzo(a)pyrene directly on chromatographic media, was difficult to measure in this study due to changes in the scatter background with changes in quantity of the compound present. The standard method of measuring a peak height from the background recorded for a blank was used in this study, but it is believed that the presence of the compound on the paper resulted in increased absorption, thus less scatter, of the excitation radiation and, consequently, a smaller scatter background for the spectrum of the sample than indicated by the background recorded for the blank piece of paper.

The problem of variation in scatter with quantity can be avoided either by reducing the amount of scatter, which is difficult, or by using peaks farther from the scatter peak, such as the 429(425)nm or the 469 nm peaks. A possible solution may also be the use of a reference point within the same spectrum for the peak base. Thomas et al. (1960) used such a reference point, the pit between the 407 nm and the 429 nm peaks (Figure 36), but in this present study this pit was not present in the benzo(a)pyrene spectra for quantities of 200 ng and greater (Figure 4). A more stable reference point for the measurement may be either the minimum preceding the 407 nm peak or the 407 nm reading from a filter paper background line shifted vertically to coincide with the minimum preceding the 407 nm peak. Using either of these reference points for the measurement would change the measured peak heights by amounts which increase with quantity of benzo(a)pyrene (Table 14).

Table 14. Height of 407 nm benzo(a)pyrene peak as measured from three different "peak bases." (In A.U.)

Quantity (ng)	From Filter Paper Bkgd. as Recorded	From Minimum Preceding Peak	From Filter Paper Bkgd. Shifted Vertically to Coincide with Minimum
1	4.3	4.0	4.5
2	8.6	8.3	8.8
4	11.0	10.8	11.3
10	14.1	14.0	14.5
20	18.2	18.5	19.0
40	18.4	18.8	19.3
100	13.9	15.4	15.9
200	9.1	11.0	11.5
400	7.8	10.1	10.6

The relationship between the quantity of benzo(a)pyrene and the 407 nm peak height is approximately linear from 0 to less than 10 ng (Figure 5). This quantity range agrees with the one reported by Monkman (Monkman and Porro, 1960; and Monkman et al., 1962), from 0 to 10-20 ng, more closely than with the one reported by Thomas et al. (1960), from 0 to 500 ng.

The difference between the reported linear ranges was explained previously (Monkman and Porro, 1960) by placing the longer linear range, from 0 to 500 ng, in the region where the 407 nm peak height was believed to approach a maximum asymptotically. This placement of the longer range was justified by a comparison of the slopes. The slope of the shorter range (Figure 37) is 4000 and the slope of the longer linear

Figure 36. Standard curves for the quantitative analysis of benzo(a)pyrene (Insert) based on fluorescent curves. (From Thomas et al, 1960.) (Reproduced with permission from Dr. J. F. Thomas and Pergamon Press, Inc.)

Figure 37. Plot of fluorescence intensity for various quantities of benzo(a)pyrene on paper chromatograms at the 407 nm maximum. (From Monkman and Porro, 1960.) (Reproduced with permission from Mr. J. L. Monkman and the Instrument Society of America.)

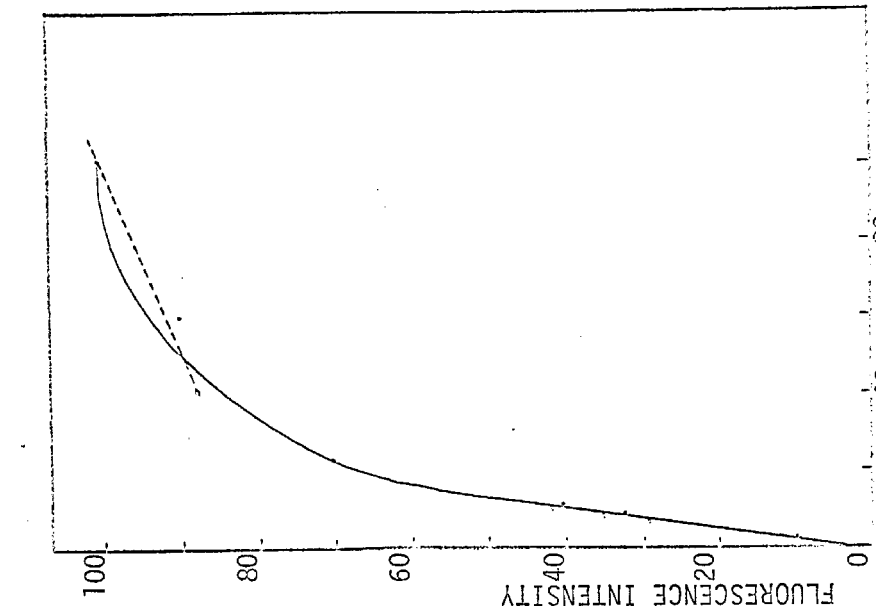


Figure 37

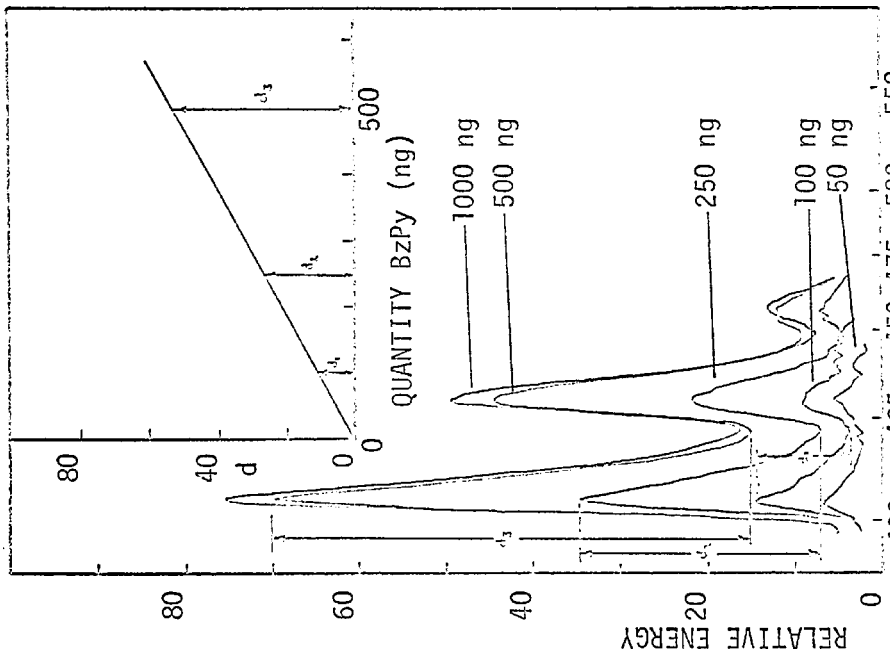


Figure 36

range (Figure 36) is 100. The slope of the line from 50 ng to 100 ng (Figure 37) is 200. A degree of agreement between Figure 37 and a straight line from 0 to 500 ng with a slope of 100 (Figure 36) was concluded.

In this present study the 407 nm peak was seen to experience a peak-height reversal with increasing quantity, rather than to approach a maximum asymptotically. Further, neither of the previous studies reported the spectral changes described in Section 4.1.2 for quantities increasing from approximately 30 ng (Figure 4). Thus, it seems probable that the reported studies were conducted in the same "range," the parameter not considered being the sample area.

Thomas et al. (1960) deposited the reported masses over approximately two cm diameter areas (314 mm^2) and Monkman and Porro (1960) used samples of approximately six mm^2; in this present study the masses were deposited over approximately 3/16" diameter areas (15 mm^2). Replotting Figures 5, 36 and 37, using surface concentration instead of quantity for the abscissa, brought the three sets of data into close agreement (Figure 38). The ratio of the slopes for the two reported linear ranges is 0.77, instead of 40, when the data are expressed in terms of surface concentration instead of mass.

For plotting Figure 38, the peak heights from Figure 36 (Thomas et al., 1960) were determined by measuring the distance between the lines already drawn on the published spectra and between comparable lines drawn on the other two spectra. For the data from Figure 37 (Monkman and Porro, 1960), the peak heights were measured both from the curve fitted to the data points and from the data points themselves.

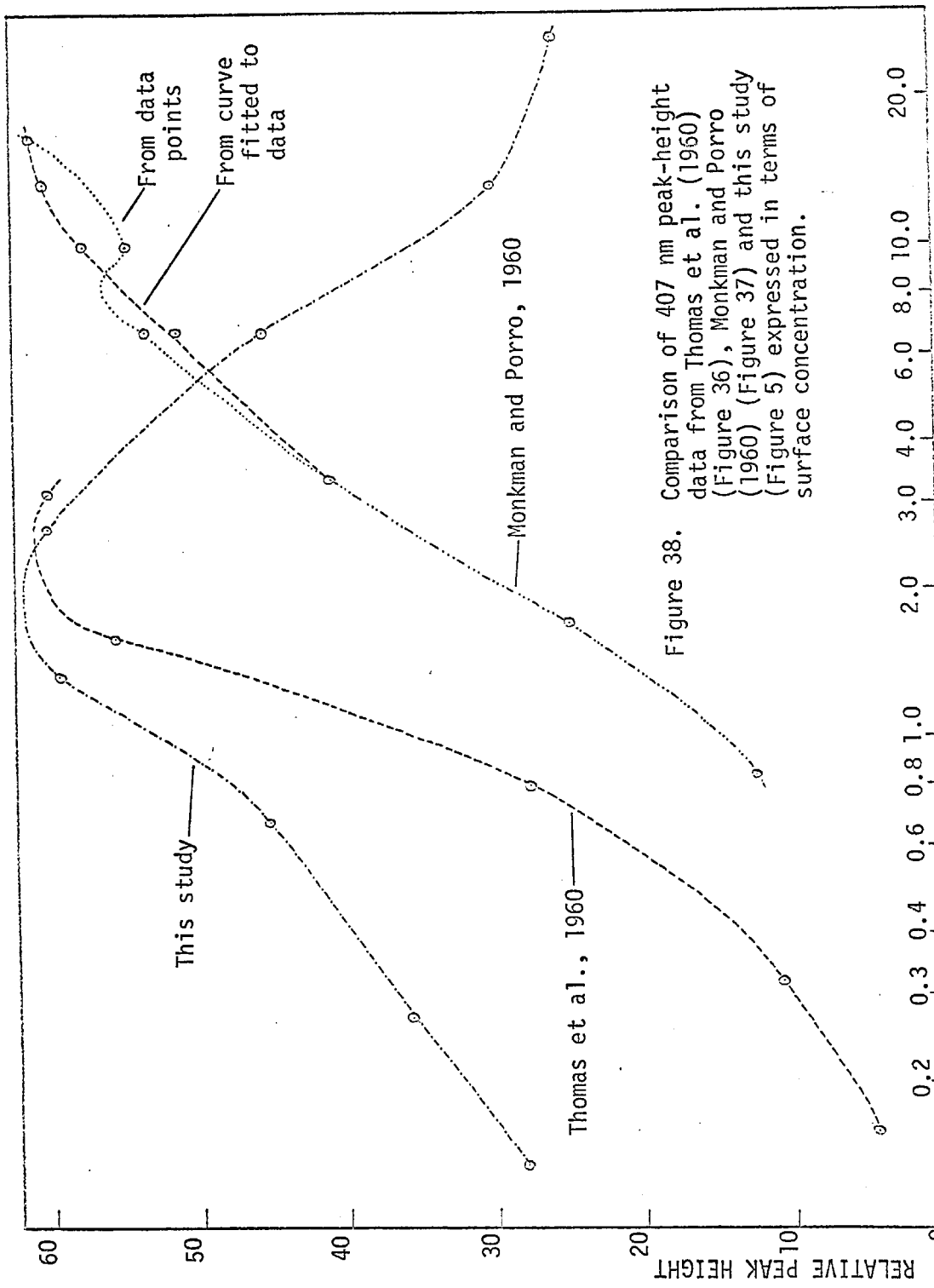


Figure 38. Comparison of 407 nm peak-height data from Thomas et al. (1960) (Figure 36), Monkman and Porro (1960) (Figure 37) and this study (Figure 5) expressed in terms of surface concentration.

The highest or the second highest measurement in each set of data was set arbitrarily equal to 60 for convenience in plotting Figure 38.

In neither of the published papers is it suggested that the 407 nm peak height experiences a reversal with increasing quantities of benzo-(a)pyrene, but the data presented in the papers (Figure 38) do not preclude the possibility of a reversal.

One data point prevents Monkman and Porro's (1960) data from showing the continuing decrease of the peak height with quantity following the reversal of the peak (Figure 38). As shown in this study (Figure 4), for large quantities of benzo(a)pyrene the 407 nm peak height is influenced by the 425 nm peak. For 200 and 400 ng quantities the 407 nm peak is little more than a shoulder on the 425 nm peak. The data point in question, when converted by sample areas, is equivalent to a 250 ng sample in this study. This higher reading (Figure 38) may have resulted from a 425 nm peak contribution to the 407 nm peak-height reading. Since Monkman and Porro (1960) measured the peak heights at a single emission wavelength, rather than scanning the entire emission spectrum, the contribution from the 425 nm peak would have been unnoticed.

The shape of the curves given in Figure 38, with the raw data being interpreted to show maxima and the rise in Monkman and Porro's (1960) data being explained above, are considered in agreement. The position of the maximum is influenced by errors in the determination of the sample area, which was estimated in all three studies, and may also depend on the thickness of the paper. In this study Whatman No. 42 was used; Thomas et al. (1960) used either Whatman No. 44 or acetylated paper; and Monkman and Porro (1960) used acetylated paper.

The fluorescence spectrum for benzo(a)pyrene on paper appears to have two components, each component being contributed by a different fluorescing species. The fluorescence attributed to Species I is dominant for small quantities of benzo(a)pyrene on paper and appears to be characterized by the 407 nm peak described above and a 429 nm peak. The fluorescence attributed to Species II appears to be characterized by peaks at 425 nm and at 469 nm and is dominant for larger quantities of benzo(a)-pyrene. Component I, the fluorescence from Species I, is very similar to the fluorescence from benzo(a)pyrene in solution (Figure 39), the only difference being the slight bathochromatic shift of approximately two nm for the compound on paper. This component of the spectrum is attributed to the presence of benzo(a)pyrene monomers in the sample on filter paper. The origin of Component II, from Species II, is described later.

With increasing quantities of benzo(a)pyrene, Component I increased and then decreased, as shown by the reversal of the 407 nm peak height, and Component II (Figure 39) increased continuously, as shown by the increase in the 469 nm peak (Figure 5). The spectral changes resulting from the decrease in Component I and the increase in Component II were: the 429 nm peak appeared to shift to 425 nm while continuing to increase in height, the decreasing 407 nm peak became a shoulder on the increasing 425 nm peak, and a definite peak developed at 469 nm (Figure 4). The presence of a peak at 470 nm for benzo(a)pyrene on a chromatographic medium is reported (Sawicki et al., 1964), but the 425 nm peak, the resulting 429-425 nm peak shift, and the loss of the 407 nm peak, to the best of my knowledge, have not been reported.

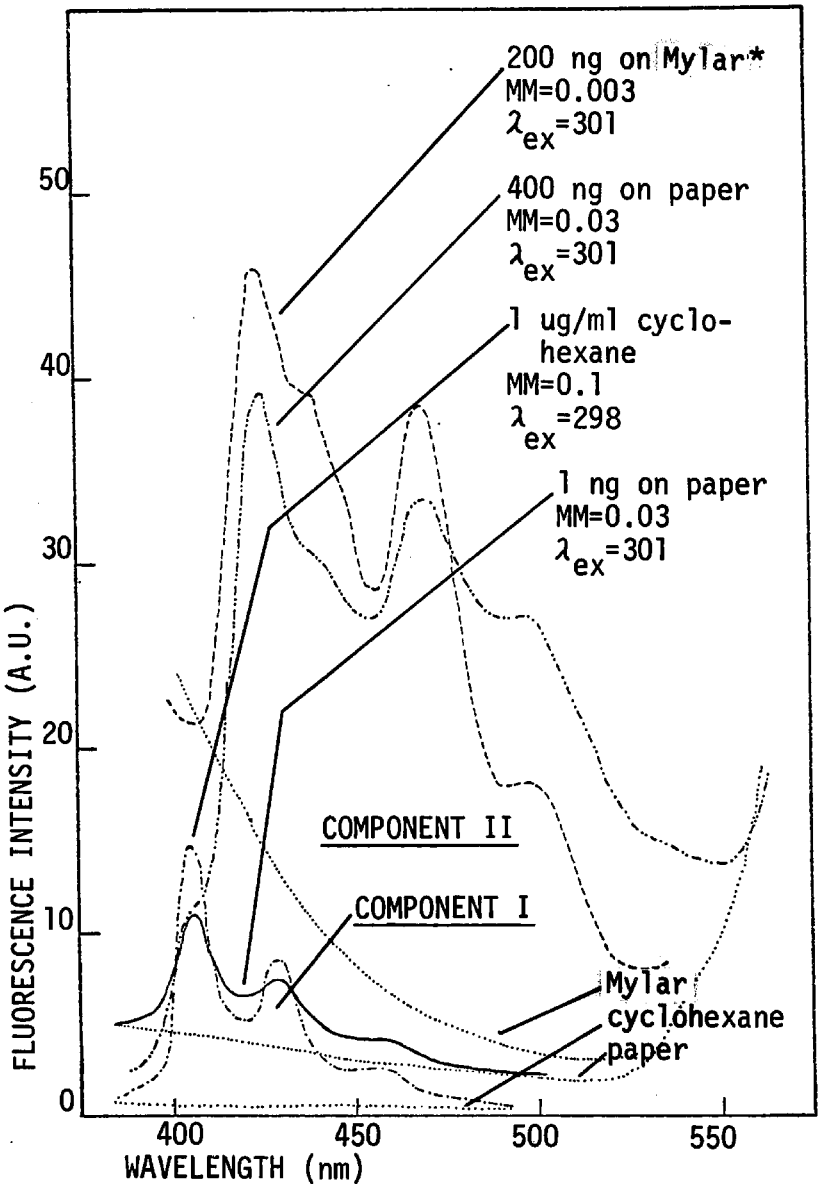


Figure 39. Comparison of benzo(a)pyrene's emission spectra for benzo(a)pyrene on paper, on Mylar, and in cyclohexane solution. (Solid-sample accessory used for all samples.)

* From earlier study using different sample holder.

The decrease of Component I in the spectrum with increasing quantity may have indicated a decrease in the respective fluorescing species and, therefore, a decrease in the number of monomers fluorescing as such with increasing quantity. The explanation given previously (Monkman and Porro, 1960; and Thomas et al., 1960) that self-absorption was responsible for the asymptotic approach of the 407 nm peak height to a maximum implies that the number of monomers fluorescing as such increases with quantity but that a decreasing fraction of the emitted fluorescence leaves the sample.

The fluorescing species contributing Component II is believed to be the benzo(a)pyrene excimer. The increase in Component II with increasing surface concentrations was accompanied by a decrease in Component I. This change is in agreement with the appearance of excimer fluorescence from pyrene solutions of increasing concentrations (Förster and Kasper, 1955). Excimer fluorescence, though, is described as being characterized by a broad, structureless peak shifted bathochromatically (Birks and Cameron, 1959). Component II is not structureless, having sharp peaks at 425 nm and at 469 nm and a broad shoulder at 500 nm (Figure 39), and it is different from reported benzo(a)-pyrene excimer spectra (Birks and Cameron, 1959; and Stevens, 1962), which, in turn, are different from each other.

The spectral changes taking place with increasing average surface concentrations were, at first, believed explainable by the existence of a threshold-surface concentration for the formation and fluorescence of Species II. The existence of such a threshold received tenuous support from a calculation of the theoretical masses which, for increasing quantities, would have surface concentrations less than an assumed

threshold.

The theoretical masses having surface concentrations less than an arbitrary threshold for given mass deposits were determined by starting with the assumption that a compound deposited on filter paper has a normal surface-concentration distribution across a diameter of the deposit. The average surface concentration per annulus (1/4 of a length unit across) and the total mass per annulus for 100 mass units (MU) of a compound deposited as a circle six length units (LU) in diameter would have the distributions shown in Figure 40. The surface concentration for 80 per cent of the mass would be greater than the average surface concentration. (The calculations for this figure are described in Appendix L.) The fraction of different deposited masses having a surface concentration less than an arbitrary surfac-concentration threshold (0.10 MU/LU^2 in this case) was then determined graphically from Figure 41. The intersections of the threshold line with the normal distribution curves, representing surface-concentration distributions for the different deposited masses, occur at the radii where the threshold is exceeded for the different masses. The fractions of the deposited masses outside these radii were read from the cumulative mass distribution curve.

The graphic solutions (Table 15) show, for increasing quantities, first, a linear increase and then a slow decrease in the masses having a surface concentration less than the arbitrary threshold. The 407 nm peak height, with increasing quantity, also displays a linear increase followed by a slow decrease.

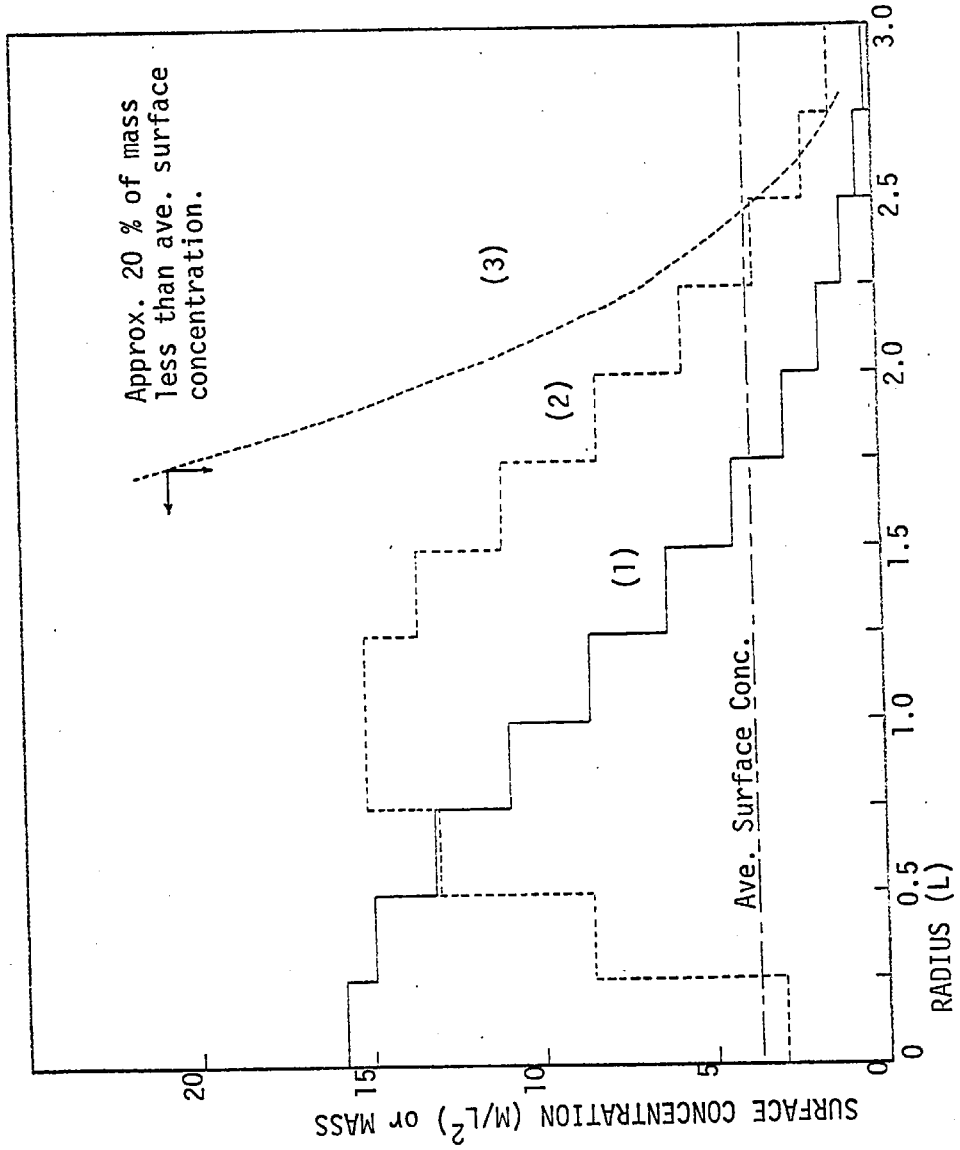


Figure 40. Average surface concentration per annulus (1), total mass per annulus (2), and cumulative mass (3) for 100 mass units having a normal surface-concentration distribution on paper.

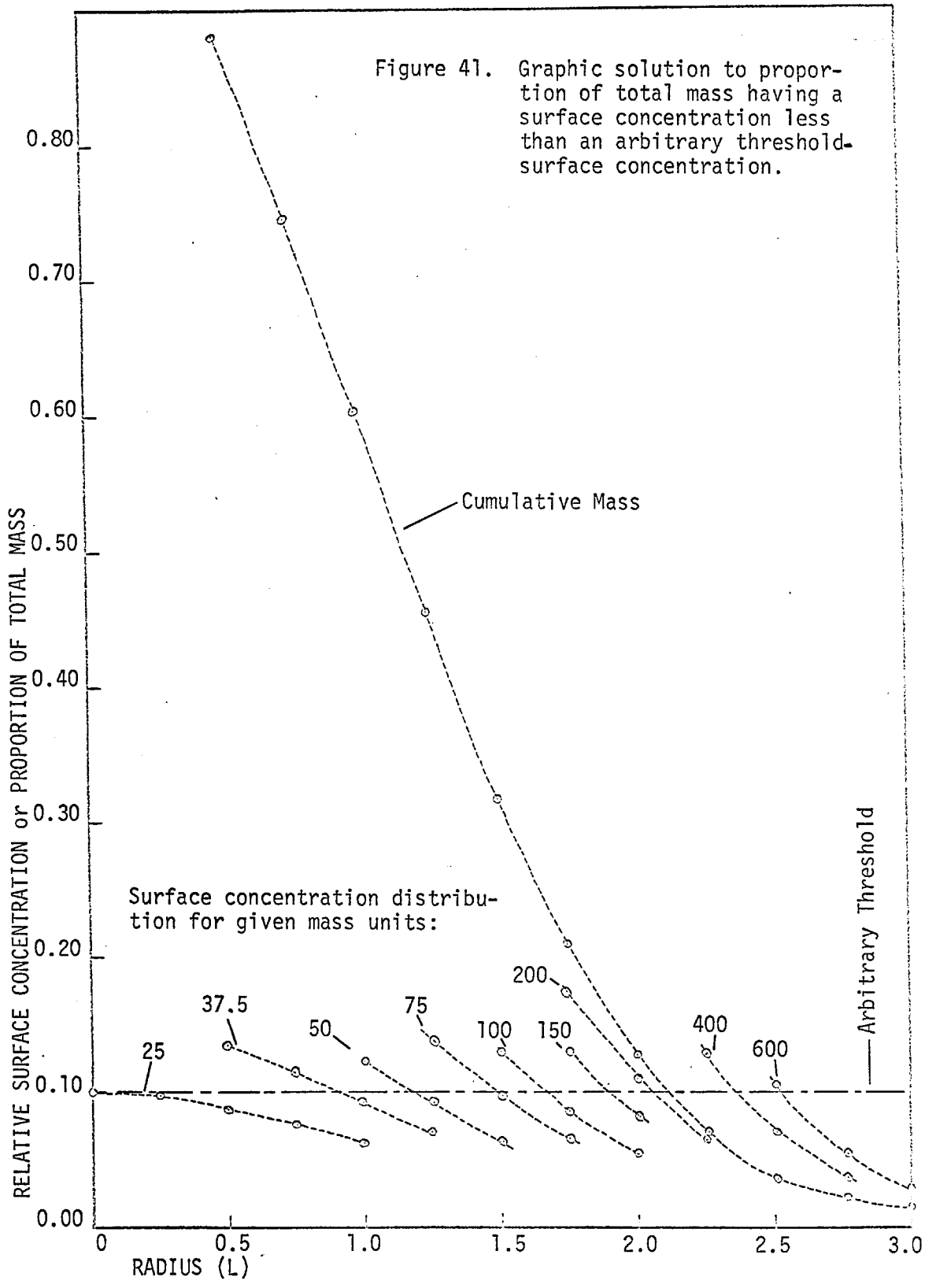


Figure 41. Graphic solution to proportion of total mass having a surface concentration less than an arbitrary threshold-surface concentration.

Table 15. Amount of compound deposited on paper having a theoretical surface concentration less than an arbitrary threshold-surface concentration.

Amount of Compound (Mass Units)	Per Cent Less than Threshold	Mass Less than Threshold
Less than 25	100	Same as first column
25	100	25
37.5	65.5	24.6
50	49.5	24.8
75	32.7	24.5
100	24.0	24.0
150	16.2	24.3
200	11.0	22.0
400	5.0	20.0
600	3.2	19.2

The study designed to show the existence or non-existence of this threshold was conducted by allowing one ml of benzo(a)pyrene solution, 40 ug BzPy/ml cyclohexane, to spread radially over a three-inch diameter circle on filter paper. One-fourth inch diameter samples were then taken from a diameter of the circle and analyzed immediately. Although this macrospot was not a true extrapolation of the samples studied because of asymmetry of deposition (Figure 42, Samples 1 and 12) and solvent evaporation during the deposition, it was sufficient for studying the possible presence of the threshold since the samples taken from the macrospot did not have the peripheral areas of low surface concentration. It was these areas that were believed responsible for the presence of

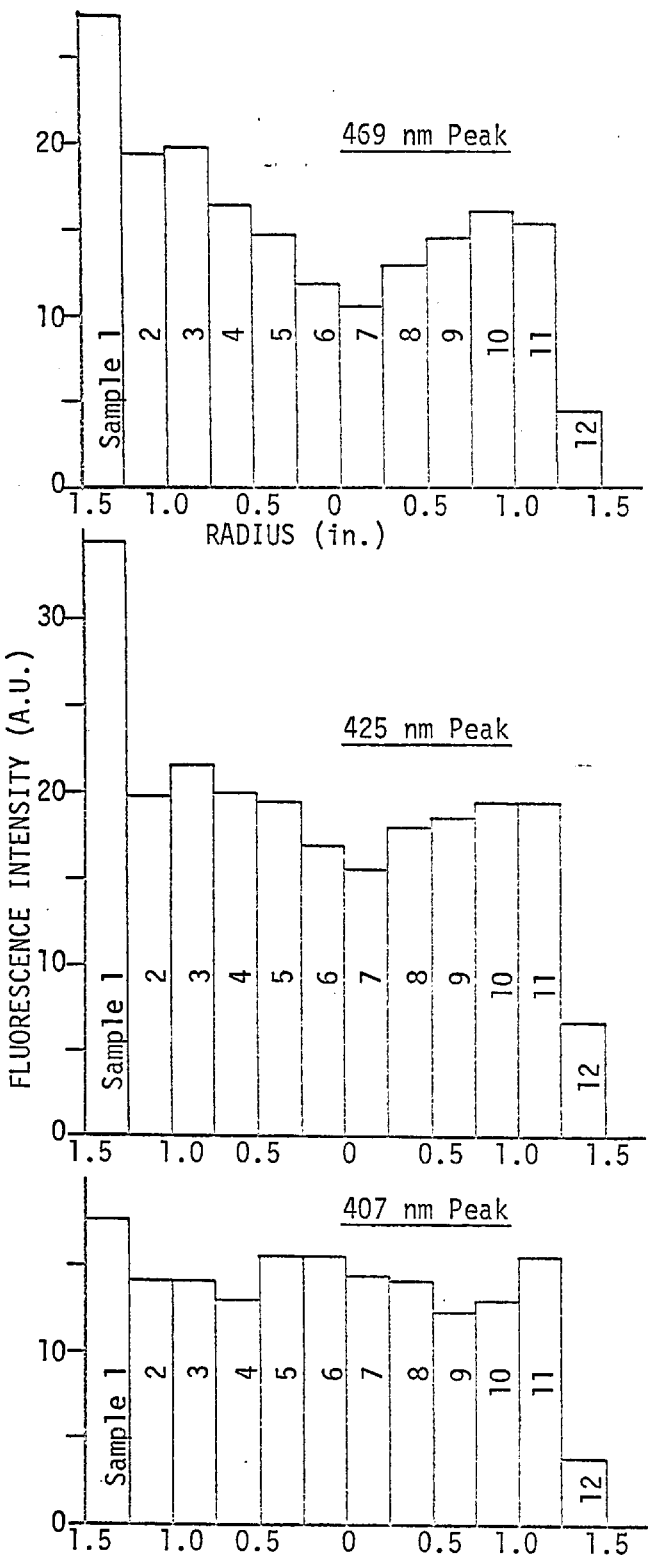


Figure 42. Peak heights of samples taken across a three in. diameter deposit of 40 ug benzo(a)-pyrene on filter paper for excitation wavelength 301 nm. (MM=0.03.)

the 407 nm monomer peak in the fluorescence from samples having large average surface concentrations.

The finding of both monomer and excimer fluorescence in all samples taken from across the diameter of the macrospot (Figure 42) discounted the existence of a threshold-surface concentration. The total disappearance of the 407 nm peak from the fluorescence spectrum for the interior samples would have been accepted as proof of the existence of this threshold.

5.2.2 BzPy Spectrum Changes with U.V. Exposure

The changes in the fluorescence from benzo(a)pyrene on filter paper during exposure of the compound to ultraviolet radiation further indicate the presence of two fluorescing species of benzo(a)pyrene. The following explanation, based on the presence of two fluorescing species, is consistent with the spectral changes observed except for those in the spectra for very small quantities of benzo(a)pyrene.

> During exposure to ultraviolet radiation Species II, yielding Component II, reacts to form a product that fluoresces like Species I. Species I reacts to form a non-fluorescing species, Species 0. The above reactions also procede in darkness following an initial exposure to ultraviolet radiation.

In the following discussion this explanation is used to predict, after the fact, the spectral changes observed for the purpose of illustrating the two-component character of the fluorescence.

The conversion of Species II to Species I during exposure to

ultraviolet radiation should result in the simultaneous decrease of the 425 nm and the 469 nm peaks and increase of the 407 nm and the 429 nm peaks. The concurrent conversion of Species I to Species 0 should result in the decrease of the 407 nm and the 429 nm peaks. The predicted peak-height changes are shown below.

Peak	Species II ⟶ Species I	Species I ⟶ Species 0
469 nm	Decrease due to loss of 469 nm peak.	No effect.
429(425) nm	Decrease due to loss of 425 nm peak. Increase due to gain of 429 nm peak.	Decrease due to loss of 429 nm peak.
407 nm	Increase due to gain of 407 nm peak. Depending upon quantity, decrease due to loss of 425 nm peak.	Decrease due to loss of 407 nm peak.

Increases in the 407 nm and the 429 nm peak heights are thus predicted, as opposed to their decrease only (Thomas et al., 1960), during exposure of benzo(a)pyrene to ultraviolet radiation.

The relative 407 nm and 429(425) nm peak heights should be altered equally by increases and decreases in the amount of Species I resulting from exposure. Therefore, any differences in behavior between these peaks during exposure must be consistent with the loss of Species II. For small quantities of benzo(a)pyrene the relative changes in the two peak heights should be the same, since the 425 nm peak is not significant in the fluorescence from small quantities. For increasing quantities, the 429(425) nm peak should exhibit progressively less increase or greater decrease than the 407 nm peak because of the presence of a significant 425 nm peak, which is decreasing. For very large

quantities, the difference between the relative height changes of the two peaks during exposure should be less than for lesser quantities, since, for these large quantities, changes in the 425 nm peak influence both the 429 nm and the 407 nm peak heights.

For consistency with the above explanation, the differences between the relative 407 nm and the 429(425) nm peak heights (Table 16) should: (1) be positive, indicating less increase or greater decrease of the 429(425) nm peak height; (2) be much smaller for small quantities than for larger quantities, indicating no significant 425 nm peak for small quantities; and (3) decrease for very large quantities, indicating the influence of a large 425 nm peak on both the 407 nm and the 429 nm peaks.

Table 16. Difference between the relative 407 nm and 429(425) nm peak heights for different times of continuous exposure. (Calculated from Appendix D.)

Time (min)	1 ng	10 ng	40 ng	100 ng	200 ng	400 ng
0	0.0	0.0	0.0	0.0	0.0	0.0
1	-0.1	0.7	3.8	15.3	26.1	18.6
3	2.0	-2.4	5.0	23.4	40.2	31.3
5	1.8	-1.3	3.6	26.4	44.4	34.8
8	0.4	-2.2	3.5	27.1	47.4	40.0
12	-3.5	-2.2	3.8	25.9	47.1	38.1
17	-9.8	-3.1	4.5	28.1	47.9	37.6
25	-4.2	-2.7	4.5	26.7	45.3	35.9
40	-4.1	-2.2	2.8	25.0	41.3	32.2
60	-6.7	-2.0	4.1	23.9	38.9	27.9

The departure from the predicted for one and 10 ng quantities is not readily explainable. The increasing difference between the peak heights for quantities increasing from 40 to 200 ng is in accord with the increasing influence of the 425 nm peak on the 429(425) nm peak height, and the decrease in the difference for 400 ng, as compared with the difference for 200 ng, is in accord with the increasing influence of the 425 nm peak on the 407 nm peak for 400 ng. The 469 nm peak decreases for 200 ng and for 400 ng quantities (Figure 59) as predicted, as does the 469 nm reading for 100 ng and for 40 ng quantities.

According to this explanation, any peak height increases are due to a conversion of Species II to Species I. Therefore, Species II would have to be present in quantities of benzo(a)pyrene as small as one ng since the fluorescence from Species I for this quantity increases during exposure to ultraviolet radiation (Table 5). Although the presence of Species II in the sample is not apparent from the emission spectrum for one ng of benzo(a)pyrene, the uniform increase in the 469 nm reading for quantities from one to 10 ng (Figure 4) suggests that Species II may be present for very small quantities. The conversion of Species II to Species I in small quantities of benzo(a)pyrene may result, therefore, in an indiscernible decrease in Component II of the spectrum and a discernible increase in Component I.

The postulated conversions of Species II to Species I and Species I to Species 0 in darkness following an initial exposure of benzo(a)-pyrene to ultraviolet radiation are supported by a comparison of the relative peak-height changes occurring during continuous exposure to

those occurring during limited exposure. During limited exposure the sample received ten 30-second exposures to ultraviolet radiation during a one-hour period, thus allowing the comparison of peak-height changes occurring during a specific exposure time to changes occurring during the same exposure time and a period of darkness. The comparison (Table 17) shows that the relative increases or decreases in peak heights resulting from continuous exposure are exceeded by the relative increases or decreases in peak heights occurring during the same exposure time and a period of darkness, and that the magnitude of the difference between the peak-height changes for the two exposure conditions increases as the period of darkness increases. Two exceptions in this comparison are the 407 nm peak for 200 ng and the 429(425) nm peak for 100 ng, both of which displayed only small relative changes during exposures.

The reactions causing the spectral changes were probably photo-oxidation reactions in the presence of ultraviolet radiation and spontaneous oxidation reactions in the dark following an initial exposure to ultraviolet radiation (Inscoe, 1964). It is interesting that, if oxidation reactions were responsible for the conversion of the species, the oxidation of Species II, believed to be the excimer, yielded Species I, the monomer. This conversion would mean that, for a pair of interacting molecules, oxidation affected only one molecule and not the pair. The second molecule was freed of the interaction by the oxidation of the first and fluoresced as the monomer.

Table 17. Comparison of relative changes in benzo(a)pyrene peaks occurring during continuous and limited exposure periods for equal, actual exposure times. (Calculated from Appendix D.)

Peak (nm)	Amt. (ng)	One Minute Exposure			Three Minute Exposure			Five Minute Exposure		
		Limited*	Cont.	Diff.	Limited**	Cont.	Diff.	Limited***	Cont.	Diff.
407	1	14.1	4.7	9.4	34.0	8.1	25.9	44.3	7.9	36.4
	10	19.6	4.2	15.4	46.5	7.7	38.8	68.0	9.8	58.2
	40	24.5	20.5	4.0	56.9	28.0	28.9	84.8	26.6	58.2
	100	11.2	9.5	1.7	14.0	12.0	2.0	16.2	10.8	5.4
	200	3.5	1.1	2.4	2.9	- 0.6	3.5	- 5.7	- 3.4	- 2.3
	400	-13.6	-10.5	- 3.1	-21.0	-15.8	- 5.2	-27.2	-21.0	- 6.2
429 (425)	1	17.2	4.8	12.4	38.2	6.1	32.1	48.5	6.1	42.4
	10	19.8	3.5	16.3	48.3	10.1	38.2	77.0	11.1	65.9
	40	20.8	16.7	4.1	52.2	23.0	29.2	79.6	23.0	56.6
	100	- 3.3	- 5.8	1.5	-12.4	-11.4	- 1.0	-16.2	-15.6	- 0.6
	200	-29.0	-25.0	- 4.0	-49.2	-40.8	- 8.4	-62.9	-47.8	-15.1
	400	-34.3	-29.1	- 5.2	-57.6	-47.1	-10.5	-71.1	-55.8	-15.3
469	200	-33.1	-31.7	- 1.4	-56.3	-53.2	- 3.1	-70.6	-62.7	- 7.9
	400	-31.5	-30.3	- 1.2	-57.0	-51.0	- 6.0	-70.8	-61.4	- 9.4

* One minute of exposure and two minutes of darkness.

** Three minutes of exposure and 14 minutes of darkness.

*** Five minutes of exposure and 55 minutes of darkness. (These relative peak-height changes were calculated from the spectral scan made during the last 30-seconds of the exposure period.)

5.2.3. The 425-429 nm Peak

The changes in the fluorescence spectrum for benzo(a)pyrene on paper strongly suggest that the 425-429 nm "peak" consists of a Component I peak at 429 nm, which parallels the 407 nm Component I peak in its height changes with quantity and with exposure of the compound to ultraviolet radiation, and a Component II peak at 425 nm, which parallels the Component II 469 nm peak in its height changes. The heights of these peaks are believed to change in opposite directions during quantity changes and ultraviolet exposure. Unfortunately, neither the No. 2 slit arrangement used previously nor the narrower No. 1 slit arrangement resolved this 425-429 nm "peak" into the suggested components (Figures 43 and 44).

The improved resolution of the benzo(a)pyrene spectrum effected by the narrower slits yielded the by-product observation of a 407 nm peak-height reversal during the continuous exposure of 400 ng of benzo(a)pyrene to ultraviolet radiation. This reversal was noted for quantities of 200 ng and less when the No. 2 slit arrangement was used, but the greater contribution from the decaying 425 nm peak to the 407 nm peak, allowed by the No. 2 slit arrangement, probably masked the increase in the 407 nm peak from 400 ng.

5.2.4 Quantitative Analysis of BzPy on Paper

Each of the benzo(a)pyrene emission peaks, at 407 nm, 429(425)nm and 469 nm, has its own advantages and disadvantages for use in the quantitative measurement of benzo(a)pyrene on filter paper.

The 407 nm peak, excited at 301 nm, is the highest peak in the

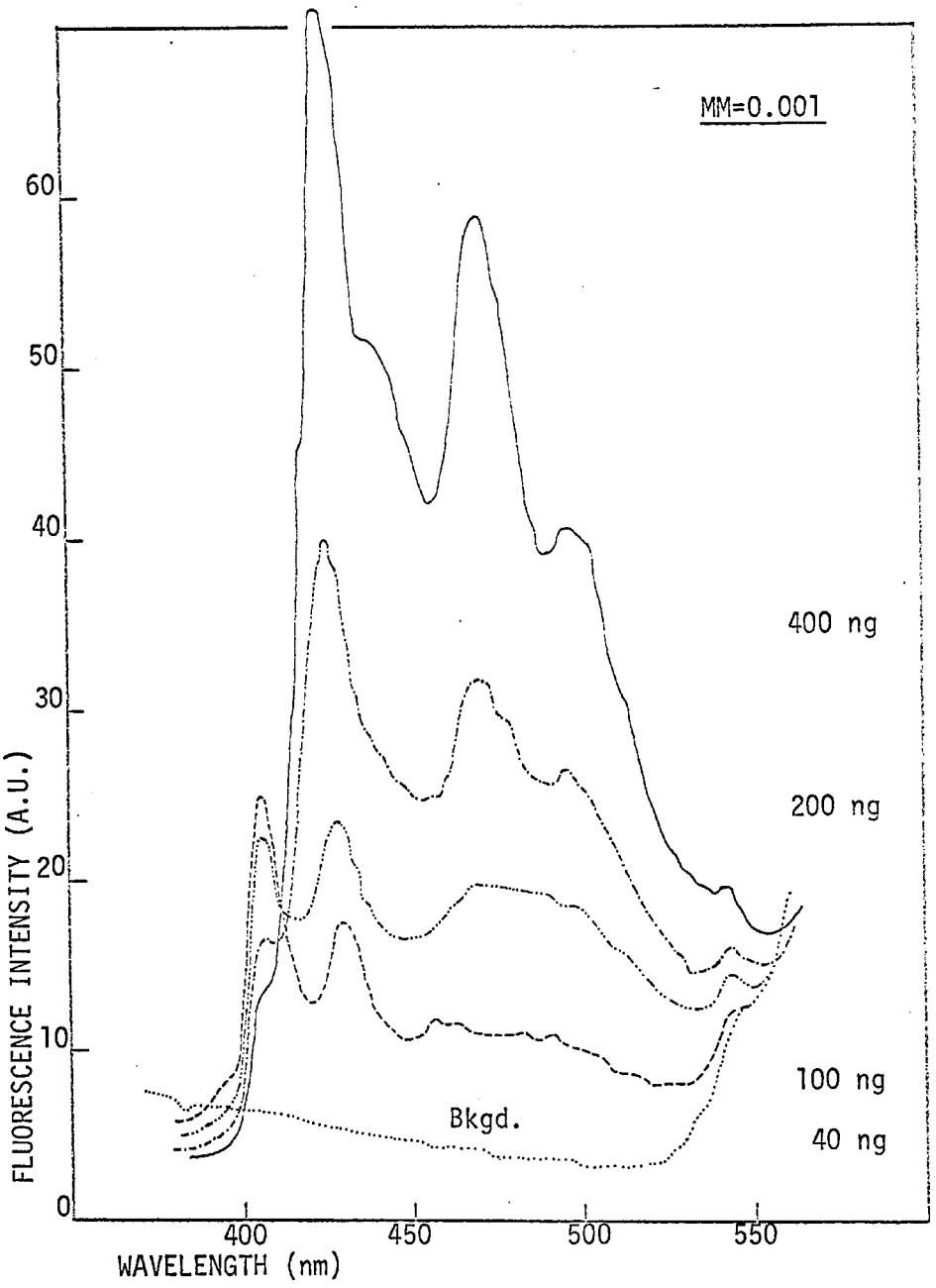


Figure 43. Emission spectra for 40 ng, 100 ng, 200 ng and 400 ng of benzo(a)pyrene on filter paper for excitation wavelength 301 nm and slit arr. No. 1.

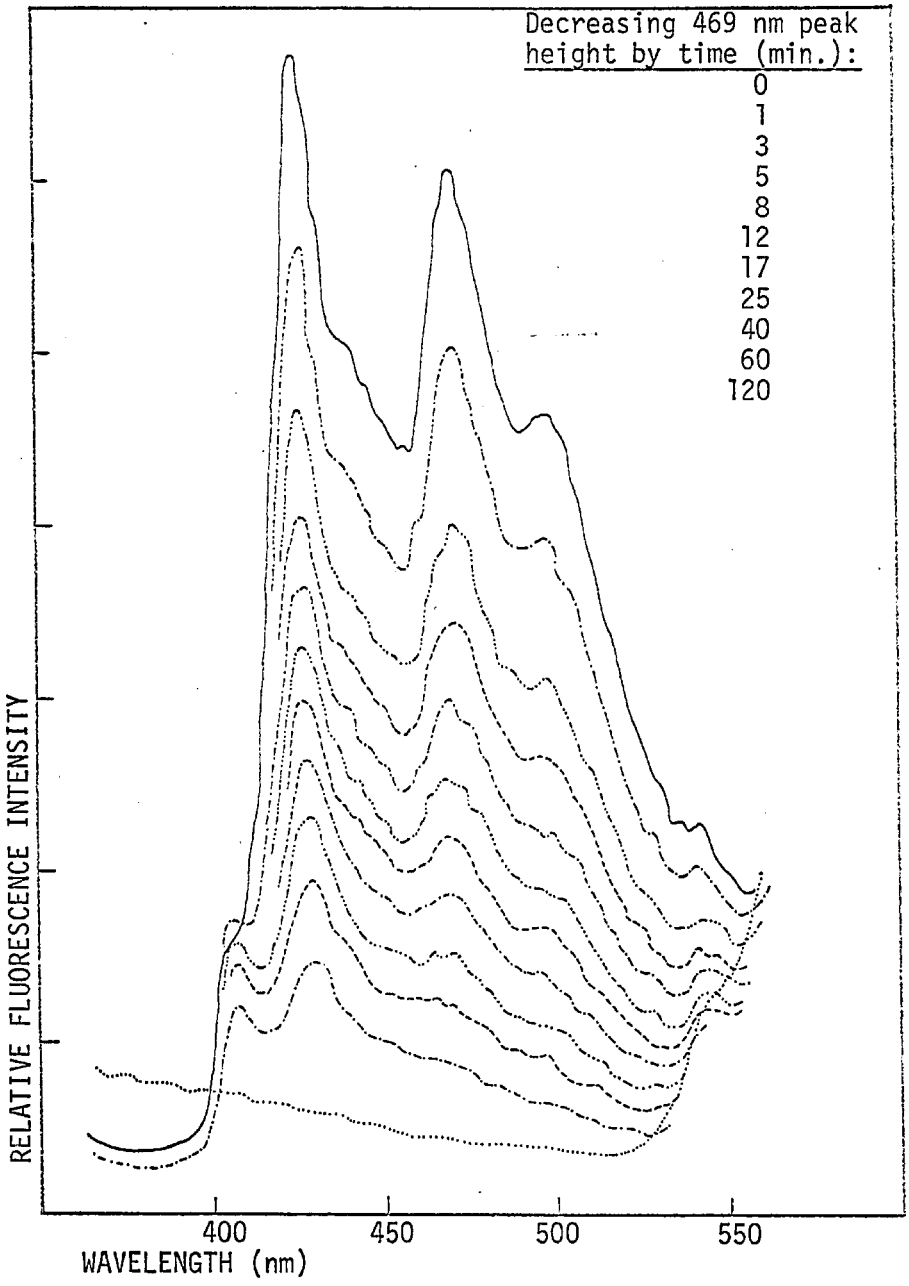


Figure 44. Decay of fluorescence from 400 ng benzo(a)pyrene on filter paper during continuous exposure for excitation and exposure wavelength 301 nm and slit arr. No. 1.

emission spectrum for small quantities of benzo(a)pyrene on paper. Under the conditions of this study, this peak could have been used to detect subnanogram quantities of benzo(a)pyrene. (For comparison, the 407 nm peak height for one ng of benzo(a)pyrene on paper is equal to the 469 nm peak height for 200 ng of benzo(a)pyrene on Mylar.) This is the peak that has been used for quantitative analysis to date (Monkman and Porro, 1960; and Thomas et al., 1960). The disadvantages in using this peak are the short range for which its height is linearly related to quantity (Figure 45), its height reversal with quantity, its loss into the 425 nm peak for large quantities, the problem in measuring its height due to background-scatter variations, and its decrease or increase in height during exposure to ultraviolet radiation.

The 429 nm peak for benzo(a)pyrene is also an intense peak for small quantities (Figure 5). For use in quantitative analysis, the 429 nm peak has the advantage of being farther from the first order scatter peak than the 407 nm peak and is, therefore, less influenced by variations in the scatter background. The disadvantages in using this peak are its short linear range, its shift to 425 nm with increasing quantity, and its decrease or increase in height during exposure to ultraviolet radiation.

The 469 nm peak height for benzo(a)pyrene can be used with either of two different excitation wavelengths for quantitative analysis. Exciting the 469 nm peak at 301 nm is not the best use of this peak for analysis since the 469 nm peak is not sharply defined for quantities less than 200 ng. A 469 nm reading for this excitation wavelength, though, is linearly related to quantity for quantities from approximately 50 ng

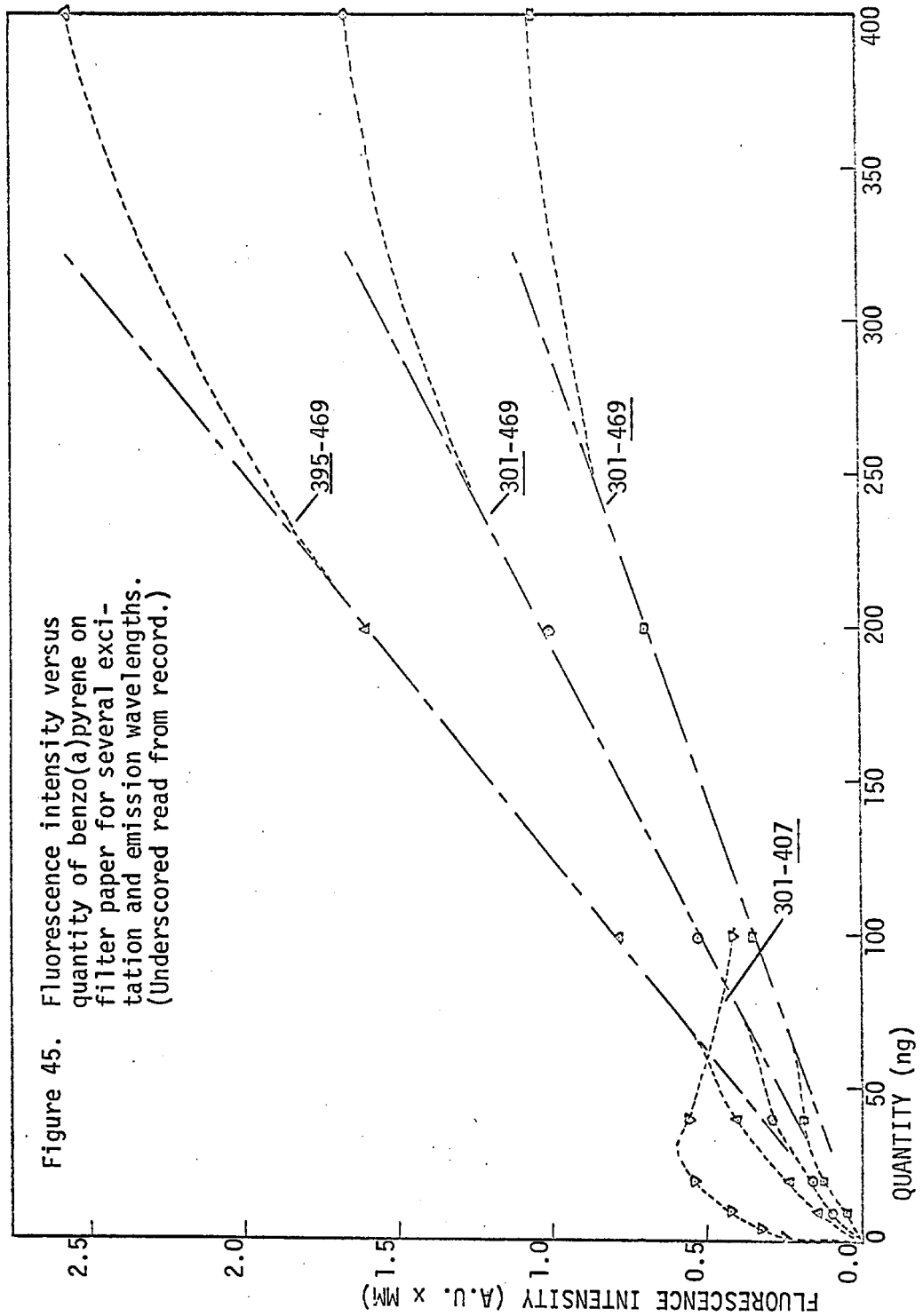


Figure 45. Fluorescence intensity versus quantity of benzo(a)pyrene on filter paper for several excitation and emission wavelengths. (Underscored read from record.)

to 275 ng (Figure 45), a linear range about 25 times larger than the one for the 407 nm peak. The excitation peak at 301 nm, for emission wavelength 469 nm, is distinct for quantities as small as 10 ng (Figure 7) and, even though this peak shifts with quantity, it may provide a suitable means for the analysis of benzo(a)pyrene on chromatographic media. The height of this peak for emission wavelength of 469 nm is also linearly related to quantity over a large quantity range. Background scatter for an excitation wavelength of 395 nm causes the 469 nm emission peak to appear as a shoulder on the scatter peak (Appendix C, Figure 55), but the excitation peak at approximately 395 nm for this emission wavelength is intense, distinct, and relatively free from scatter (Figure 7). This 395 nm peak shifts with quantity, but its height can be measured from records of the spectra. The linear range for this peak is from about 70 ng to about 240 ng, and the departure from linearity for quantities less than 70 ng is not great (Figure 45). The 469 nm peak does decay during exposure of the compound to ultraviolet radiation, but it does not increase.

The difference between using the 407 nm and the 469 nm peaks, both excited at 301 nm, for quantitative analysis is shown in Figure 42. The 407 nm peak height changes little for samples between the extremes, whereas the 469 nm peak height shows a symmetric surface-concentration distribution about an eccentric point. The 469 nm peak is considered the better measurement of the surface concentration for these samples.

5.3 BzPer ON PAPER

The emission spectrum from benzo(ghi)perylene exhibited spectral

changes similar to those in the emission spectrum for benzo(a)pyrene for increasing quantities of the compounds on paper. The benzo(ghi)-perylene spectrum appears to be composed of two components: Component I, having peaks at 408 nm, 420 nm, and 433 nm, is dominant for small quantities; and Component II, having a peak at 469 nm, is dominant for larger quantities. Component I of benzo(ghi)perylene exhibited the same height reversal with increasing quantity and the same peak growth during exposure to ultraviolet radiation that were noted for Component I for benzo(a)pyrene. There was no shift of peak position with quantity increases for the spectrum from benzo(ghi)perylene as there was for the spectrum from benzo(a)pyrene since Component II for benzo-(ghi)perylene had no peaks coincident with Component I peaks (Figure 12).

The excitation spectra for the two compounds also changed similarly for increasing quantities. For an emission wavelength setting at the monomer peak, the monomer excitation peak experienced a height reversal with increasing quantity (Figures 6 and 14), and, for an emission wavelength of 469 nm, the excitation peaks shifted (Figures 7 and 15) but increased linearly with quantity (Figures 13 and 45).

The intensity of the fluorescence from one compound, relative to the intensity of the second, was different for the compounds in solution, as Species I on paper, and as Species II on paper. The ratio of the major peak heights (BzPy/BzPer) for one ug/ml cyclohexane solutions of benzo(a)pyrene and benzo(ghi)perylene are given

below*

		Benzo(ghi)perylene	
		(301-420)	(386-420)
Benzo(a)-Pyrene	(386-405)	9.1	7.3
	(298-405)	5.8	4.6

These values are less than the ratio of 15 calculated from reported K_Q values** (Sawicki et al., 1960c) for benzo(a)pyrene's (381-403) peak to benzo(ghi)perylene's (280***-419) peak for one ug/ml concentrations in pentane.

The intensity of the fluorescence from benzo(a)pyrene relative to the intensity of the fluorescence from benzo(ghi)perylene is less for Species I of the compounds on paper than for the compounds in solution, and is much less for Species II of the compounds on paper than for the compounds in solution, as shown below.

Species I:

Benzo(a)pyrene peak = (301-407)

Benzo(ghi)perylene peak = (305-408)

Amount each compound (ng) ⟶	1	2	10	20
Peak-height ratio (BzPy/BzPer) ⟶	2.1	3.1	2.9	3.1

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* The first number in parenthesis is the excitation wavelength and the second is the emission wavelength.

** $K_Q = F_x C_s / F_s C_x$, where: C = concentration of compound (ug/ml);
F = fluorescence intensity of compound at its excitation and emission maxima;
Subscript x stands for compound; and
Subscript s stands for standard.

*** In the same report Sawicki et al. do not report an excitation peak for benzo(ghi)perylene at this excitation wavelength used for the K_Q-value determination.

Species II:

Benzo(a)pyrene peak = (395*-469)

Benzo(ghi)perylene peak = (375*-469)

Amount each compound (ng) ⟶	100	200
Peak-height ratio (BzPy/BzPer) ⟶	0.38	0.40

5.4 BzPy AND BzPer ON MYLAR

5.4.1 Excitation Wavelength Selection

These studies on the fluorescence from benzo(a)pyrene and benzo-(ghi)perylene on Mylar were conducted preliminary to the analysis of mixtures on Mylar. For the following reasons, 325 nm was selected as the excitation wavelength for the analysis of mixtures.

1. The excitation wavelength should not be in a region where the excitation spectrum has a large slope. In such a region small errors in setting the excitation wavelength would result in large changes in the intensity of the emission spectrum. At 325 nm benzo(ghi)perylene has a broad shoulder and benzo(a)pyrene has a wide pit.
2. The excitation wavelength must be far enough from the 425 nm peak to prevent the 425 nm peak from being annihilated by scatter radiation. The 425 nm peak is the only characteristic of the emission spectrum

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* These excitation peaks shift with quantity.

that distinguishes benzo(a)pyrene from benzo(ghi)-perylene on Mylar.

3. A Mylar background peak occurring at 310 nm has a pit at 325 nm.

5.4.2 BzPy and BzPer Spectra on Mylar

The fluorescence from benzo(a)pyrene and benzo(ghi)perylene on Mylar resembles that portion of the fluorescence from the compounds on paper designated as Component II (Figures 25 and 39). The benzo-(a)pyrene fluorescence has peaks at 425 nm and 469 nm, and benzo(ghi)-perylene has only a 469 nm peak; both compounds display shoulders at 500 nm. No monomer fluorescence is apparent for either compound on Mylar, indicating that few, if any, molecules are free of interactions with other molecules on Mylar.

The excitation spectra for large quantities of benzo(a)pyrene on paper (Appendix C, Figure 56) and on Mylar (Appendix G, Figure 72) are similar to each other for emission wavelength 469 nm. This is also true for benzo(ghi)perylene (Appendix E, Figure 68; and Figure 25).

5.4.3 Spectra Changes with Quantity

The emission spectra for benzo(a)pyrene and benzo(ghi)perylene on Mylar excited at 325 nm do not change appreciably for quantities increasing to 400 and to 200 ng respectively (Appendix G and Figure 25). Over much of these quantity ranges the emission peak heights increase linearly with quantity (Figures 33 and 34). For quantities larger than 400 ng, there are changes in the emission spectrum for benzo(a)pyrene that are most easily seen in the spectrum corrected for background

(Figure 46).

The emission spectrum for 400 ng benzo(a)pyrene, corrected for background (Figure 46), exhibits not only the 425 nm and the 469 nm peaks noted earlier but also a 439 nm peak which is not apparent in the uncorrected spectrum. For 800 ng the 439 nm peak is higher than the 425 nm peak. The 469/439 peak-height ratio changes little with increasing quantities (1.18 for 400 ng; 1.19 for 800 ng; and 1.21 for 1200 ng), indicating atrophy of the 425 nm peak rather than a disproportionate increase of the 439 nm peak.

5.4.4 Decay of Fluorescence with U.V. Exposure

The decaying spectra from benzo(a)pyrene and benzo(ghi)perylene on Mylar do not display peak shifts or significant changes in peak-height ratios (Appendices H and I) for either exposure condition. This is true also for the 1200 ng benzo(a)pyrene spectrum, which displays a quantity effect (Figure 24).

The compounds differ in their stability during exposure to ultraviolet radiation, as determined from the decrease in the 469 nm peak height with time. For quantities of 100 ng and greater, the 469 nm peak from benzo(a)pyrene is decreased about six times more than the 469 nm peak from benzo(ghi)perylene by 40 minutes of continuous exposure.

No monomer fluorescence appears for the compounds on Mylar as the excimer fluorescence decays, unlike the decay of the fluorescence from the compounds on paper. The quenching reaction, which is presumed to be oxidation, must therefore either affect both molecules forming the fluorescing species, permitting neither to fluoresce as a monomer, or affect only one molecule of the pair, permitting the second molecule to

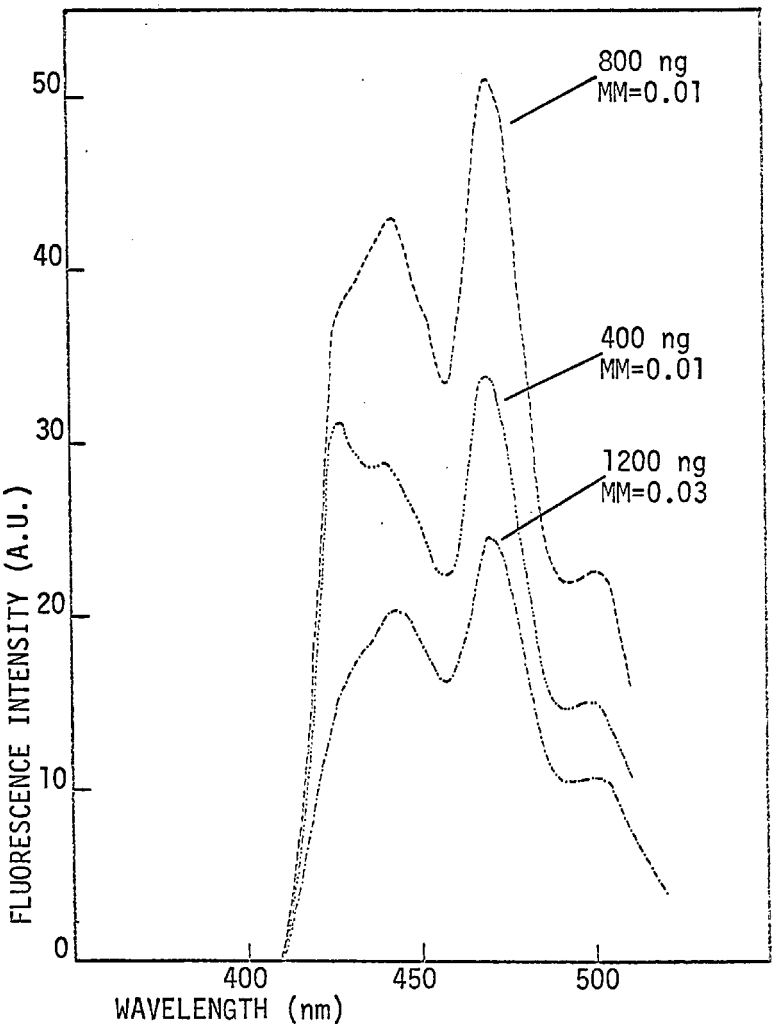


Figure 46. Emission spectra for 400, 800 and 1200 ng of benzo-(a)pyrene on Mylar corrected for background for excitation wavelength 325 nm.

interact with a third to continue the excimer fluorescence. The less rapid decay of the fluorescence from the compounds on Mylar than from the compounds on paper suggests the latter. That is, oxidation of a single molecule on paper destroys the fluorescing pair, but on Mylar the oxidation of a single molecule only reduces the fluorescing quantity by one molecule.

5.4.5 Intensity of Fluorescence from Compounds on Mylar

Both benzo(a)pyrene and benzo(ghi)perylene, excited at their main excitation peaks, yield smaller 469 nm peaks on Mylar than on paper. The 469 nm peak-height ratio (paper/Mylar) for 100 ng of benzo(a)pyrene is 6.2 and for 100 ng benzo(ghi)perylene is 3.6. The fluorescence intensities of the compounds, relative to each other, are also different for the compounds on paper and on Mylar. The 469 nm peak-height ratio (BzPy/BzPer) for 100 ng of each compound on Mylar excited at its main excitation wavelength is 0.22, as compared with 0.38 for the compounds on paper. (For excitation wavelength 325 nm, the ratio for the compounds on Mylar is 0.13, indicating a greater sacrifice in the fluorescence from benzo(a)pyrene than in the fluorescence from benzo(ghi)-perylene to get an excitation wavelength having the desired requirements (Section 5.4.1).) Thus, depositing solutions of the compounds on Mylar changed the maximum peak-height ratio for the compounds from 5/1 (BzPy/BzPer) for the compounds in solution to about 1/5 for the compounds on Mylar.

5.4.6 Comparison with Reported Spectra

The emission spectra for benzo(a)pyrene and benzo(ghi)perylene on Mylar do not agree with reported excimer fluorescence spectra (Birks and Cameron, 1959; and Stevens, 1962). The spectrum for benzo(a)-pyrene has a peak at 469 nm and, depending upon quantity, a peak at 425 nm or 439 nm. Birks and Cameron (1959) report the excimer spectrum as being unstructured with a broad peak at about 480 nm and a shoulder at 430 nm. Stevens (1962) reports unstructured spectra with a broad peak at about 506 nm for microcrystals and at 460 nm for the sublimate. Although there are several similarities between this present study and that by Birks and Cameron (1959), differences in the conduct of the studies may be partially responsible for the differences in the reported spectra.

Birks and Cameron (1959) evaporated cyclohexane solutions of benzo(a)pyrene on a glass plate to deposit the microcrystalline samples. In this present study the samples were deposited by evaporation of cyclohexane solutions on Mylar. The thickness of Birks and Cameron's (1959) deposits was 0.1 micron, which is thinner than the samples used in this present study. A second difference between the studies is that Birks and Cameron observed the spectra in transmission through the sample and glass, and in this present study the spectra were observed from the surface of the sample. The absence of a 430 to 440 nm peak in the spectrum reported by Birks and Cameron (1959) may be due to the difference in the methods that were employed for observing the spectrum since both the specimen and the glass could effect a reduction in the 430 nm fluorescence, relative to the 480 nm peak. The wavelength

spread between the peak and the shoulder in the reported study and between the peaks in this study would contradict a statement of good agreement between the spectra, even though the difference in shape can be explained.

Benzo(ghi)perylene on Mylar has a single peak at 469 nm and a shoulder at 500 nm. Stevens (1962) presents an unstructured spectrum for microcrystals of benzo(ghi)perylene with a peak at about 500 to 505 nm and a shoulder at about 480 nm. For the range of quantities used in this study, the spectra are in disagreement.

5.5 ANALYSIS OF MIXTURES ON MYLAR

The preliminary studies on the fluorescence from benzo(a)pyrene and benzo(ghi)perylene on Mylar uncovered two spectral differences, either of which would have been a promising base for the analysis of mixtures. The fluorescence quenching that occurs in mixtures of the compounds on mylar forced a reapprasial of these differences for analytical exploitation.

The spectral difference that would have afforded the most direct resolution of mixtures is the presence of a 425 nm peak in the spectrum for benzo(a)pyrene and its absence from the spectrum for benzo(ghi)-perylene. In the absence of fluorescence quenching, the amount of benzo(a)pyrene present could have been determined from this peak height, and the amount of benzo(ghi)perylene present could have been determined by difference using the height of the common 469 nm peak. Fluorescence quenching, though, prevented the use of this difference for the analysis.

The use of the second spectral difference for the analysis, the difference in stability between the spectra from the two compounds during ultraviolet irradiation, is not precluded by the fluorescence quenching in mixtures. If quenching had not occurred, the decrease of the 469 nm peak during exposure could have been used to determine the ratio of the compounds (Figure 35), and this ratio and the 469 nm peak height could have been used to determine the quantity of each compound. Since the ratio of the compounds determines the amount by which the 469 nm peak is quenched (Table 10), in addition to determining its decay rate, once this ratio has been determined from the decrease of the 469 nm peak during exposure, it can be used to correct the 469 nm peak height for quenching.

The calculations for determining the amount of benzo(a)pyrene and benzo(ghi)perylene in a binary mixture are outlined below:

1. Determine the ratio of the compounds (R) present from the decay rate of the 469 nm peak (Figure 35).
   ($R = ng_{BzPy}/ng_{BzPer}$)
2. From (R) above, determine the amount by which the 469 nm peak is quenched (Q) (Table 10).
3. Calculate the corrected 469 nm peak height using (Q):
   $P.H._{measured}/(1-Q) = P.H._{corrected}$
4. Since: $P.H._{corrected} = p.h._{BzPy} + p.h._{BzPer}$
   where: $p.h._{BzPer} = (4.83 \times 10^{-3})(ng_{BzPer}) - 3.65 \times 10^{-2}$
   for the portion of Figure 34 between 20 and 100 ng where the curve is linear; and
   where: $p.h._{BzPy} = 6.4 \times 10^{-4} (ng_{BzPy})$
   for 0 to 200 ng (Figure 34);

then: $P.H._{corrected} = 10^{-3} (4.8\ ng_{BzPer} + 0.64\ ng_{BzPy} - 36.5)$

5. Substitute: $ng_{BzPy} = (R)(ng_{BzPer})$;

replace: $P.H._{corrected} = P.H._{measured}/(1-Q)$;

and rearranging: $$ng_{BzPer} = \frac{(10^3\ P.H._{measured}/(1-Q)) + 36.5}{4.8 + (0.64\ R)}$$

and: $ng_{BzPy} = (R)(ng_{BzPer})$

The main problem revealed by the application of this analytical method to several trial mixtures was the poor precision in the results for the benzo(a)pyrene determination (Table 18). This poor precision in the results for benzo(a)pyrene is attributed to the low fluorescence intensity of the compound on Mylar excited at 325 nm radiation. The 469 nm peak height for benzo(a)pyrene is approximately one-tenth the 469 nm peak height for benzo(ghi)perylene for equal quantities, and the relative reduction in the 469 nm peak height for benzo(a)pyrene, resulting from 30 minutes of ultraviolet irradiation, is six times greater than the relative reduction in the 469 nm peak for benzo(ghi)-perylene. Therefore, a small error in determining the reduction in the 469 nm peak for a mixture could cause a large error in the benzo-(a)pyrene determination. For example, a positive error of three per cent in the 30-minute peak-height determination for Run No. 3, Mixture 1 (Table 18) would increase the ratio of the net peak heights from 0.825 to 0.860, but this increase would change the mass ratio determination from 1.8 to 1.2 and the peak-height quenching determination from 57 per cent to 50 per cent. This three per cent error would result in benzo(a)pyrene and benzo(ghi)perylene determinations of 111 ng and 93 ng respectively, instead of 180 ng and 100 ng. A

Table 18. Results from the analysis of trial BzPy-BzPer mixtures.

Mixture No.	1				2			
Mixture	200 ng BzPy - 100 ng BzPer				200 ng BzPy - 20 ng BzPer			
Run No.	1	2	3	4	1	2	3	4
Net 469 nm P.H.								
at T=0 min	0.242	0.222	0.240	0.201	0.080	0.068	0.049	0.071
at T=30 min	0.201	0.191	0.198	0.173	0.048	0.046	0.029	0.042
P.H. Ratio*	0.831	0.860	0.825	0.860	0.60	0.68	0.59	0.59
Mass Ratio**	1.7	1.2	1.8	1.2	6.2	4.8	6.3	6.3
% Q***	0.56	0.50	0.57	0.50	0.46	0.62	0.45	0.45
ng BzPer	100	86	100	79	21	27	14	19
ng BzPy	169	104	180	94	131	131	90	118
Mixture No.	3				4			
Mixture	80 ng BzPy - 100 ng BzPer				80 ng BzPy - 40 ng BzPer			
Run No.	1	2	3	4	1	2	3	4
Net 469 nm P.H.								
at T=0 min	0.197	0.202	0.237	0.276	0.074	0.069	0.062	0.069
at T=30 min	0.168	0.179	0.216	0.245	0.065	0.067	0.063	0.069
P.H. Ratio*	0.853	0.886	0.911	0.888	0.88	0.97	1.0	1.0
Mass Ratio**	1.3	0.88	0.62	0.86	0.96	0	0	0
% Q***	0.52	0.46	0.38	0.45	0.47	0	0	0
ng BzPer	80	77	81	101	33	22	21	22
ng BzPy	103	67	50	89	31	0	0	0

* $P.H._{30}/P.H._{0}$

** From Figure 35

*** From plot of Table 10 on semilog paper.

three per cent change in 30 minutes was unfortunately probable in the equipment used.

The precision in the results for the benzo(ghi)perylene determinations was better than for the benzo(a)pyrene determinations, and the standard deviations in the results for the larger quantities of benzo(ghi)perylene, about 10 per cent, approximately agree with the standard deviations in the peak-height measurements for the compounds on mylar (Table 13).

The accuracy of the results may have been influenced by possible changes in the operating characteristics of the instrument in the time from when all previous data were recorded and when the trial mixtures were analyzed. Between these uses of the instrument it was relocated and modified for solution analysis. The readings for the uranium-glass bead were within the same range for the two uses of the instrument, but this agreement in the readings for the standard does not rule out the possibility of compensating changes in the excitation energy flux and the performance of the photomultiplier tube. Before the accuracy of the method can be established, though, the precision of the method must be greatly improved.

It is believed that the precision of the method can be improved by using a longer excitation wavelength for the analysis. Excitation wavelength 325 nm was selected for this study instead of a longer wavelength because scatter from this 325 nm excitation radiation did not prevent the measurement of the 425 nm peak for benzo(a)pyrene, but this 425 nm peak was used only to calculate the results given in Table 12 and is not needed in the calculation of the component quantities for the mixtures. Using a 375 nm excitation wavelength instead

of this 325 nm wavelength would increase the 469 nm peak heights by 71 per cent for benzo(a)pyrene and by 33 per cent for benzo(ghi)perylene, thereby, not only increasing the 469 nm peak heights for the mixtures, but also increasing the contribution from benzo(a)pyrene to the peak of the mixtures. The relative and absolute changes in the peak heights resulting from exposure to ultraviolet radiation would be increased, and the influence from such a three per cent error, mentioned above, would be reduced, provided that the compounds display the same sensitivity to 375 nm radiation that they display to 325 nm radiation.

Trial Mixture No. 2 (Table 18) illustrates the importance of a large contribution from benzo(a)pyrene to the 469 nm peak. The 469 nm peak height for this mixture was reduced most by irradiation, and, despite the fact that the height of this peak was very small, the precision in the benzo(a)pyrene determination was better for this mixture than for the other mixtures. The improved precision believed possible using the longer excitation wavelength will not exceed the reproducibility of the peak-height determinations, which should be of fundamental concern in any further exploration of the method for its potential use.

The greater height of the 469 nm peak and the greater contribution from benzo(a)pyrene to the peak should improve the detection limit of the method for benzo(a)pyrene in addition to improving the precision of the benzo(a)pyrene determination. For Mixture No. 4, 80 ng of benzo(a)pyrene could not be detected in the presence of 40 ng of benzo(ghi)perylene. The 469 nm peak height for this mixture was approximately the same as for Mixture No. 2, but the change in the peak height during exposure was too small to measure. The detection of this quantity of benzo(a)pyrene might have been possible if the peak-height change

had been larger; a longer peak-height change for this mixture is believed possible using 375 nm excitation radiation. Similarly, smaller quantities of benzo(a)pyrene should be detectable in the presence of constant quantities of benzo(ghi)perylene, thereby lowering the mass ratio of 0.5 (BzPy/BzPer), which is considered the minimum ratio for which the 469 nm peak from a mixture has a noticeably different decay rate from the benzo(ghi)perylene 469 nm peak.

It is interesting to note that 20 ng of benzo(ghi)perylene were detected in the presence of 200 ng of benzo(a)pyrene. In solution, the intense fluorescence from benzo(a)pyrene usually prevents the detection of the other polycyclic aromatic hydrocarbons present. In this analysis on Mylar, the problem in the benzo(a)pyrene determination is reversed.

6. CONCLUSIONS

The following conclusions are drawn from the results of this research.

1. The analysis of benzo(a)pyrene on chromatographic media using the 407 nm peak is complicated by the following.
   a. The 407 nm peak increases or decreases during exposure of benzo(a)pyrene to ultraviolet radiation, depending on the quantity of the compound present.
   b. The peak-height continues to change in the dark after an initial exposure to ultraviolet radiation.
   c. The 407 nm peak height decreases with increasing quantity of benzo(a)pyrene beyond a short region over which the peak height is linearly related to quantity of compound.
   d. For large quantities of the compound the 407 nm peak height becomes a small shoulder on the 425 nm peak.
   e. The scatter background is changed by the presence of the compound on the media and is not the same for samples of different quantities.
2. Most of the above problems in using the 407 nm peak are not present in using the 469 nm emission peak or one of its excitation peaks. Although the excitation peaks for emission wavelength 469 nm shift with quantity, they are distinct, uninfluenced by scatter, and are linearly related to quantity for a range larger than that for the 407 nm peak. The 469 nm peak is decreased by exposure of the compound to ultraviolet radiation, but it is not increased by the exposure.
3. The analysis of benzo(ghi)perylene on chromatographic media using

either the 408 nm or the 420 nm peaks, would be complicated by the peak-height changes and scatter-background changes given above in (1) for benzo(a)pyrene, but the peak-height changes would not be as pronounced as for benzo(a)pyrene. The use of the 469 nm benzo(ghi)perylene peak for the analysis would have advantages similar to those given in (2) for benzo(a)-pyrene.

4. The fluorescence from benzo(a)pyrene and benzo(ghi)perylene on filter paper has two components, one contributed by the monomer and the other believed to be contributed by the excimer. The monomer fluorescence, after first increasing, decreases with increasing quantities of the compounds; the excimer fluorescence increases continuously with quantity. The decrease in monomer fluorescence with increasing quantity is believed caused by the presence of fewer monomers in the sample rather than by self-absorption of fluorescence by the sample.
5. The existence of a surface-concentration threshold for the formation of the fluorescing species believed to be the excimer could not be shown.
6. During and after exposure of the compounds on paper to ultraviolet radiation, chemical reactions, presumed to be oxidation reactions, result in a decrease in the excimer fluorescence and an increase in the monomer fluorescence. These reactions appear to affect only one molecule in an interacting pair, freeing the second from the irteraction and permitting the second to fluoresce as a monomer. Reactions occurring simultaneously to those which convert the

excimer to the monomer, convert the monomer to a non-fluorescing molecule.

7. Both of the reactions given in (6) occur during exposure of the compounds to ultraviolet radiation and procede in dark following an initial exposure to ultraviolet radiation.

8. The fluorescence from benzo(a)pyrene and benzo(ghi)perylene on Mylar is similar to the excimer fluorescence from these compounds on filter paper. No monomer fluorescence is initially apparent nor is any apparent after ultraviolet irradiation of the compounds on Mylar.

9. The benzo(ghi)perylene excimer, on filter paper and on Mylar, is more stable to ultraviolet radiation than is the benzo(a)-pyrene excimer.

10. The chemical reactions causing the decrease of excimer fluorescence from compounds on Mylar appear to affect one molecule of the interacting pair, permitting the second to interact with another molecule to continue the excimer fluorescence.

11. Under the conditions described in this study, recylcled exhaust from the xenon lamp in the spectrophotofluorometer is not sufficient to induce chemiluminescence in benzo(a)pyrene on filter paper by an ozone-benzo(a)pyrene reaction.

12. The fluorescence intensity of benzo(a)pyrene and benzo(ghi)-perylene, relative to each other, are altered considerably by evaporating solutions of the compounds on Mylar. The ratio of the main peak heights for the compounds in cyclohexane solution is approximately 5/1 (BzPy/BzPer) and for the compounds

on Mylar is approximately 1/5, the fluorescing species being different for each of the two conditions.

13. The maximum peak heights for benzo(a)pyrene and benzo(ghi)perylene decrease for the sequence: monomer on paper, excimer on paper, excimer on Mylar.

14. The decrease in the maximum peak heights is less for benzo(ghi)-perylene than for benzo(a)pyrene at each step in the sequence given above.

15. The reproducibility in peak heights for samples deposited on Mylar is not good. The most important errors causing the poor reproducibility are believed to be those occurring during the deposition of the sample on Mylar.

16. The ratio of the compounds determines the amount by which the fluorescence from a mixture is quenched.

17. The ratio of the compounds also determines the amount by which the 469 nm peak for a mixture is decreased by a period of ultraviolet irradiation.

18. The method described for the quantitative analysis of benzo-(a)pyrene - benzo(ghi)perylene mixtures is capable of determining nanogram quantities of each compound in the presence of the other, and it provides a means for analyzing smaller quantities than can be determined by the presently used ultraviolet absorption method. It is believed that the method can be improved by using 375 nm excitation radiation, and exploration of the method using this wavelength is recommended.

APPENDIX A

POLYCYCLIC AROMATIC HYDROCARBONS PRESENT IN POLLUTED AIR

Table 19. Concentration of polycyclic aromatic hydrocarbons in ambient air.

Location	Time Period	Conc. (ug/100 m3) High[a]	Low[a]	Ave.	Reference
Anthracene-3618					
Salford, Eng.	1/53	--	--	2	Cooper, 1954
Oslo, Norway	2/55-1/56	--	--	0.01	Campbell and Kreyberg, 1956
Fluoranthene-4799					
Salford, Eng.	2/53	--	--	20	Cooper, 1954
21 Ontario Cities	11/61-6/63	1.1	0.03	0.24	Moore et al., 1965
11 Ontario Cities	11-12/62[b]	4.1	0.06	1.67	"
Oslo, Norway	2/55-1/56	--	--	0.21	Campbell and Kreyberg, 1956
Pyrene-5262					
Salford, Eng.	11/52-3/53	35.0	18.0	25.5	Cooper, 1954
Los Angeles	8/52-6/53	--	--	0.5	Kotin et al., 1954
12 U.S. Cities	Winter,'59	--	--	1.56	Sawicki et al., 1962b
9 U.S. Cities	Summer,'58	--	--	0.12	"
21 Ontario Cities	5/61-6/63	1.2	0.02	0.2	Moore et al., 1965
11 Ontario Cities	11-12/62[b]	5.4	0.1	1.88	"
Oslo, Norway	2/55-1/56	--	--	0.25	Campbell and Kreyberg, 1956

a Monthly averages unless otherwise specified.
b Inversion period.
c Average for the time period for the highest and lowest sites.
d Geometric mean.
e Three month average.

Table 19. Continued.

Benzo(k)fluoranthene-6075					
12 U.S. Cities	Winter,'59	--	--	0.98	Sawicki et al., 1962b
9 U.S. Cities	Summer,'58	--	--	0.23	"
21 Ontario Cities	4/62-6/63	0.5	0.01	0.15	Moore et al., 1965
10 Ontario Cities	11-12/62[b]	1.4	0.13	0.53	"
Benzo(a)pyrene -6399					
London	6/49-5/50	14.7	1.4	6.1	Waller, 1952
Sheffield, Eng.	5/49-4/50	7.8	2.0	4.6	"
Cannock, Eng.	7/49-6/50	3.2	0.4	1.9	"
Salford, Eng.	11/52-3/53	29.0	19.7	23.6	Cooper, 1954
Los Angeles	8/52-6/53	--	--	3.3	Kotin et al., 1954
Milan, Italy	1/58-10/58	23.1	0.3	5.4	D'Ambrosio et al.,1958
21 Ontario Cities	5/61-6/63	2.2	0.06	0.51	Moore et al., 1965
11 Ontario Cities	11-12/62[b]	4.2	0.62	2.36	"
Atlanta	7/58-6/59	1.5	0.16	0.62	Sawicki et al., 1962a
Birmingham	7/58-6/59	7.4	0.59	2.19	"
Cincinnati	7/58-6/59	2.6	0.13	1.10	"
Detroit	7/58-6/59	3.1	0.34	1.50	"
Los Angeles	7/58-6/59	1.3	0.04	0.30	"
Nashville	7/58-6/59	5.5	0.14	1.92	"
New Orleans	7/58-6/59	0.6	0.2	0.38	"
Philadelphia	7/58-6/59	1.9	0.25	0.77	"
San Francisco	7/58-6/59	0.75	0.025	0.19	"
94 Urban Sites in U.S.	1/59-3/59	6.1[c]	0.011[c]	0.66[d]	"
28 Non-urban Sites in U.S.	1/59-3/59	0.19[c]	0.001[c]	0.04[d]	"
Copenhagen, Denmark	1/56-12/56	1.54[e]	0.54[e]	1.04	Campbell and Clemmesen, 1956
Oslo, Norway	2/55-1/56	--	--	0.56	Campbell and Kreyberg, 1956
12 U.S. Cities	Winter, '59	--	--	1.48	Sawicki et al., 1962b
9 U.S. Cities	Summer, '58	--	--	0.28	"

Table 19. Concluded.

Benzo(e)pyrene-6400					
8 U.S. Cities	Winter, '59	--	--	1.04	Sawicki et al., 1962b
9 U.S. Cities	Summer, '58	--	--	0.27	"
21 Ontario Cities	11/61-6/63	1.07	0.03	0.26	Moore et al., 1965
11 Ontario Cities	11-12/62[b]	4.2	0.43	1.61	"
Perylene-6401					
12 U.S. Cities	Winter, '59	--	--	0.30	Sawicki et al., 1962b
9 U.S. Cities	Summer, '58	--	--	0.07	"
Dibenzo(def,mno)chrysene-7033					
12 U.S. Cities	Winter, '59	--	--	0.12	Sawicki et al., 1962b
9 U.S. Cities	Summer, '58	--	--	0.01	"
Benzo(ghi)perylene-7036					
Salford, Eng.	12/52-3/53	35.0	16.1	23.5	Cooper, 1954
Los Angeles	8/52-6/53	--	--	3.6	Kotin et al 1954
21 Ontario Cities	4/62-6/63	1.4	0.05	0.42	Moore et al., 1965
11 Ontario Cities	11-12/62[b]	4.6	0.63	1.79	"
Oslo, Norway	2/55-1/56	--	--	0.40	Campbell and Kreyberg, 1956
12 U.S. Cities	Winter, '59	--	--	1.5	Sawicki et al., 1962b
9 U.S. Cities	Summer, '58	--	--	0.5	"
Coronene-7392					
12 U.S. Cities	Winter, '59	--	--	0.78	Sawicki et al., 1962b
9 U.S. Cities	Summer, '58	--	--	0.22	"
21 Ontario Cities	11/61-6/63	0.47	0.02	0.15	Moore et al., 1965
11 Ontario Cities	11-12/62[b]	1.69	0.34	0.69	"

APPENDIX B

FLUORESCENT SPECTRA FOR BENZO(a)PYRENE AND BENZO(ghi)PERYLENE IN SOLUTION

Table 20. Reported peaks for benzo(a)pyrene and benzo(ghi)perylene in solution.

Solvent	Excitation Peaks (nm)	Emission Peaks (nm)	Reference
Benzo(a)pyrene:			
Hexane	***	403,408,428,457	Chaudet and Kaye, 1961
*	382**	403**	Dubois et al., 1965
Cyclohexane	***	405**	Monkman et al., 1962
Benzene	***	406,428	Morlin and Saringer, 1961
95% EtOH	364**	404**	Mukai et al., 1964
Pentane	381**	403,428,453	Sawicki et al., 1960d
Pentane	295,345,361,380	403,427,454	Sawicki et al., 1960c
*	383**	403	Thomas et al., 1965
Cyclohexane	380**	405,428,457,485	Van Duuren, 1960
Cyclohexane	363**	403,426,452	Thomas et al., 1962
***	***	403,426,454	Lyons, 1962
Benzene	***	407,430**	Kutscher et al., 1966
Benzo(ghi)perylene:			
*	300,385**	422**	Dubois et al., 1965
Cyclohexane	***	420**	Monkman et al., 1962
Pentane	358**	397,405,419,443	Sawicki et al., 1960b
Pentane	292,324,340,354,374	393,404,419,440	Sawicki et al., 1960c
*	300,385**	422**	Thomas et al., 1965
Cyclohexane	385**	405,425,450,475	Van Duuren, 1960
***	***	408,430	Lyons, 1962
***	305,375,395	430	American Instrument Co., 1966

* Probably cyclohexane
** Main peak(s) given
*** Not given

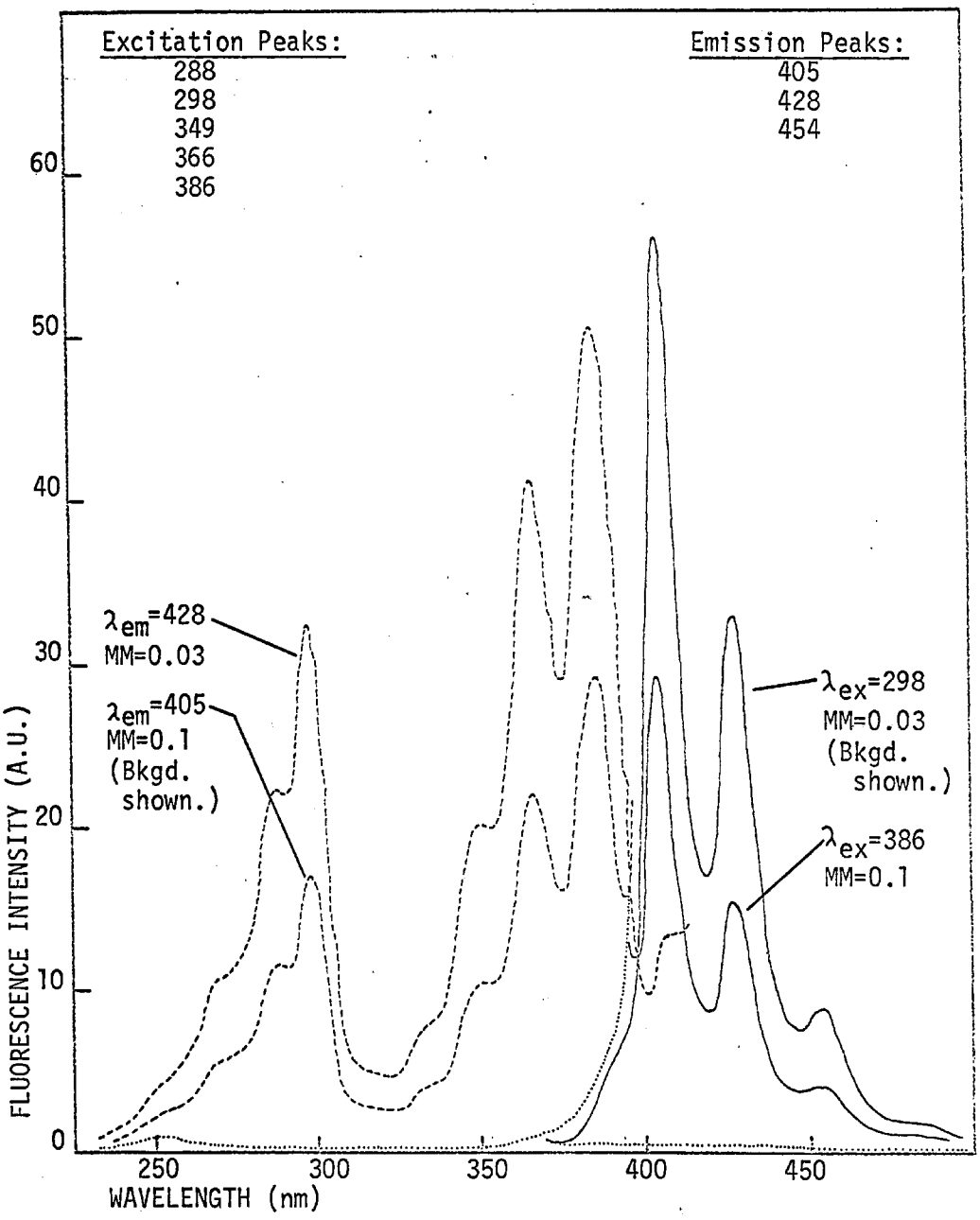


Figure 47. Excitation and emission spectra for benzo(a)pyrene in cyclohexane, one ug/ml. **(Solid-sample accessory** used.)

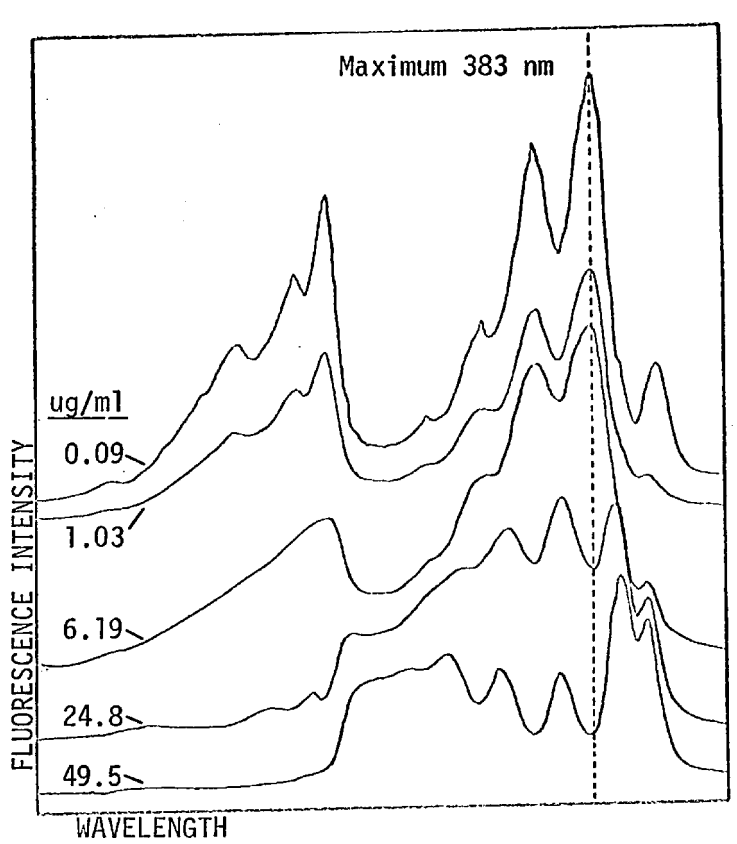


Figure 48. (Left) Reported excitation spectra for benzo(a)pyrene in cyclohexane. (From Thomas et al., 1965.) (Reproduced with permission from Mr. J.L. Monkman and Pergamon Press, Inc.)

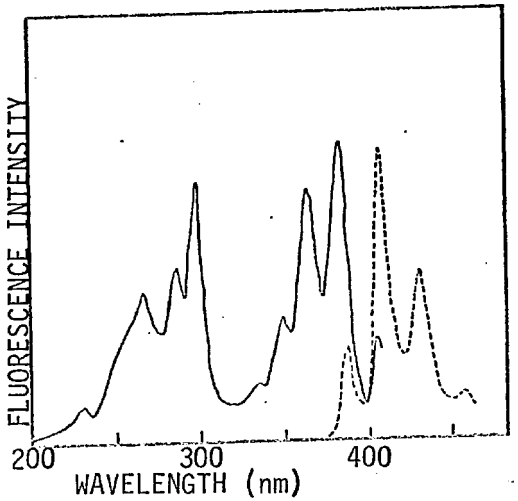


Figure 49. (Above) Reported excitation and emission spectra for benzo(a)pyrene in cyclohexane, 0.1 ug/ml. (From Monkman et al., 1962.) (Reproduced with permission from Mr. J.L. Monkman and the American Industrial Hygiene Association.)

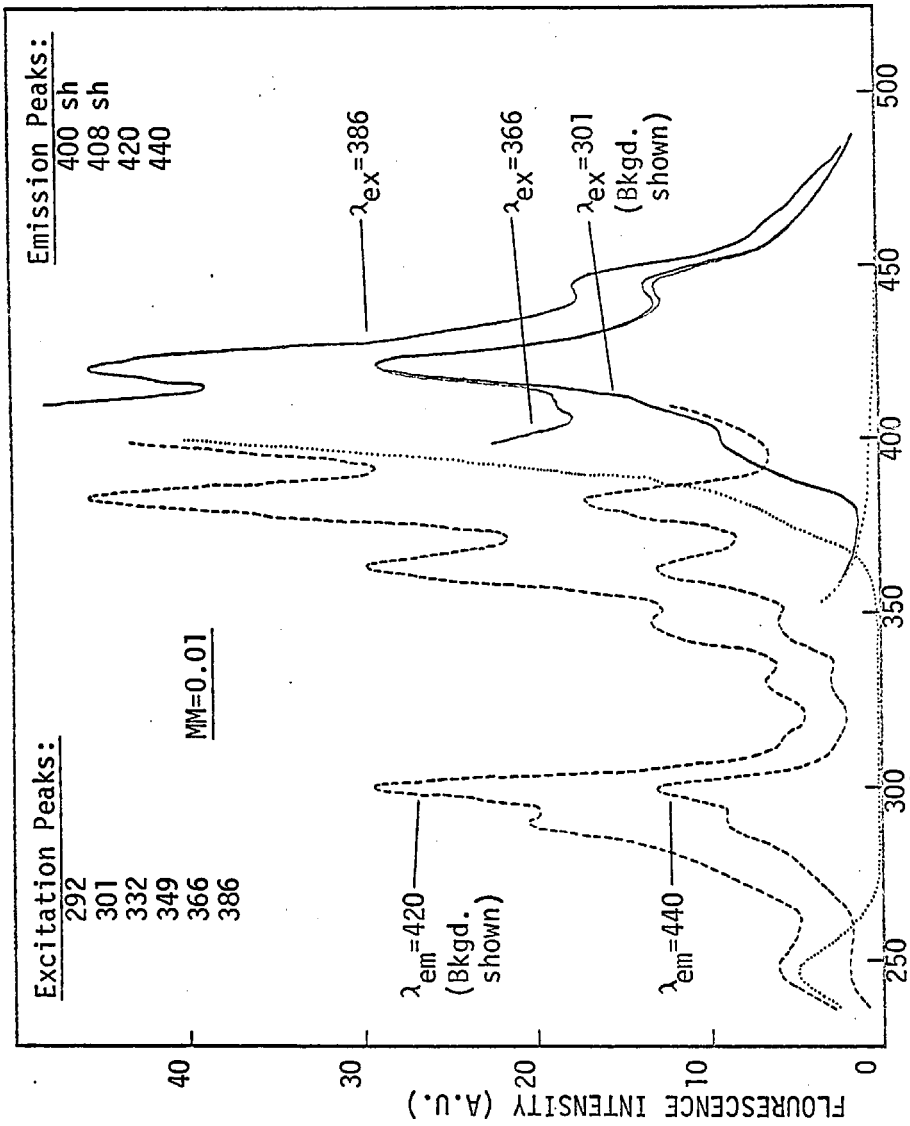


Figure 50. Excitation and emission spectra for one ug/ml benzo(ghi)perylene in cyclohexane. **(Solid-sample accessory used.)**

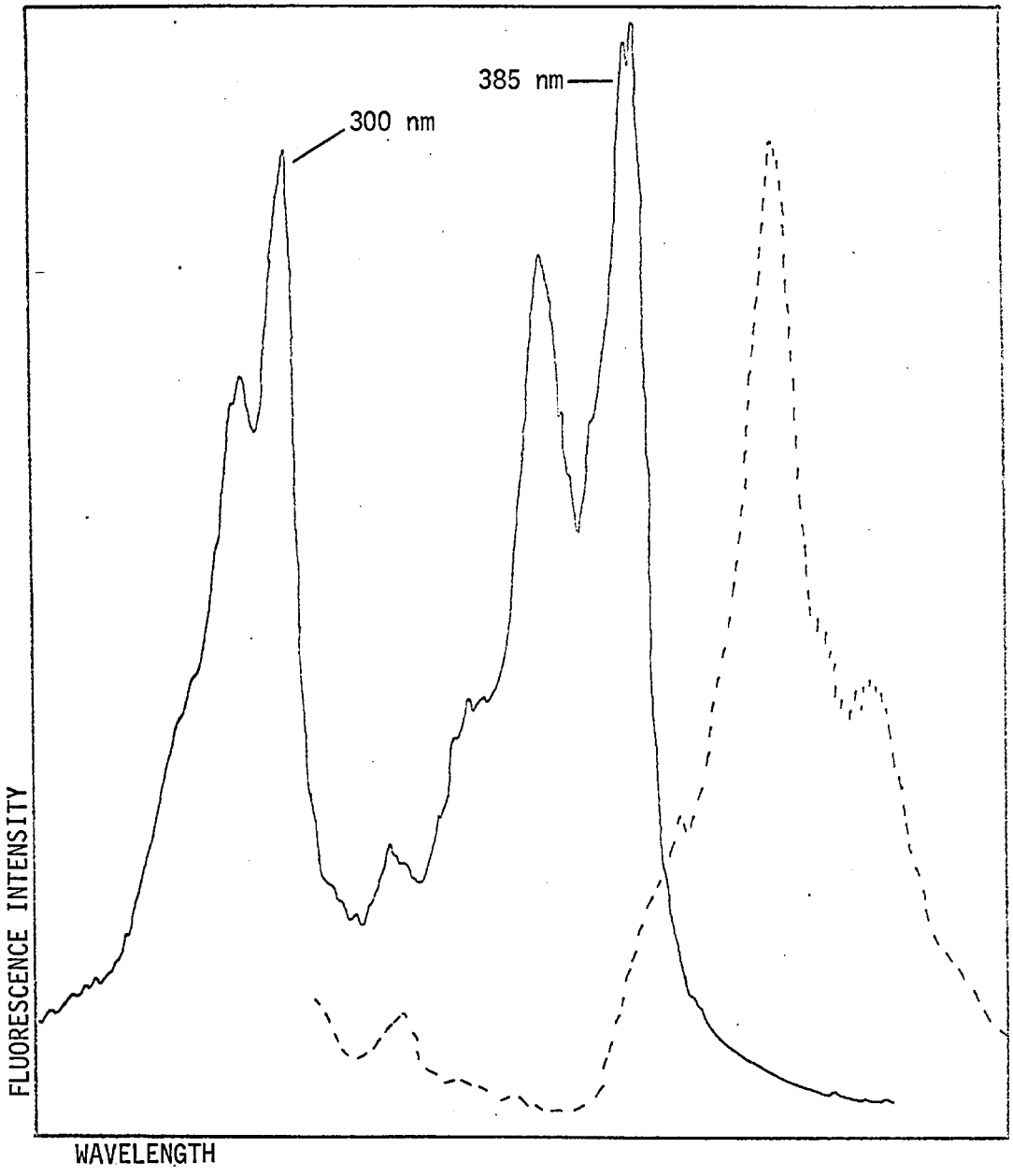


Figure 51. Reported excitation and emission spectra for benzo(ghi)-perylene in solution. (From Dubois et al., 1965) (Reproduced with permission from Mr. J.L. Monkman.)

APPENDIX C

FLUORESCENT SPECTRA FOR BENZO(a)PYRENE ON FILTER PAPER

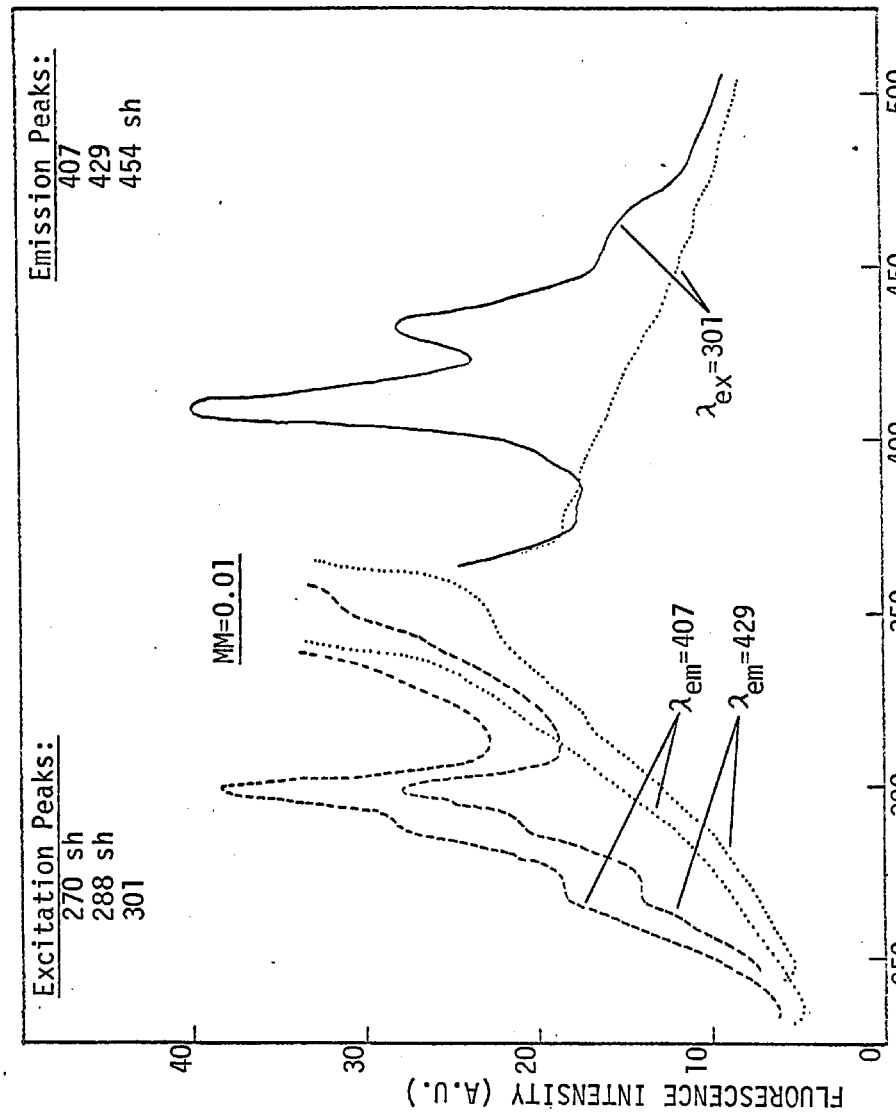


Figure 52. Excitation and emission spectra for one ng benzo(a)pyrene on filter paper.

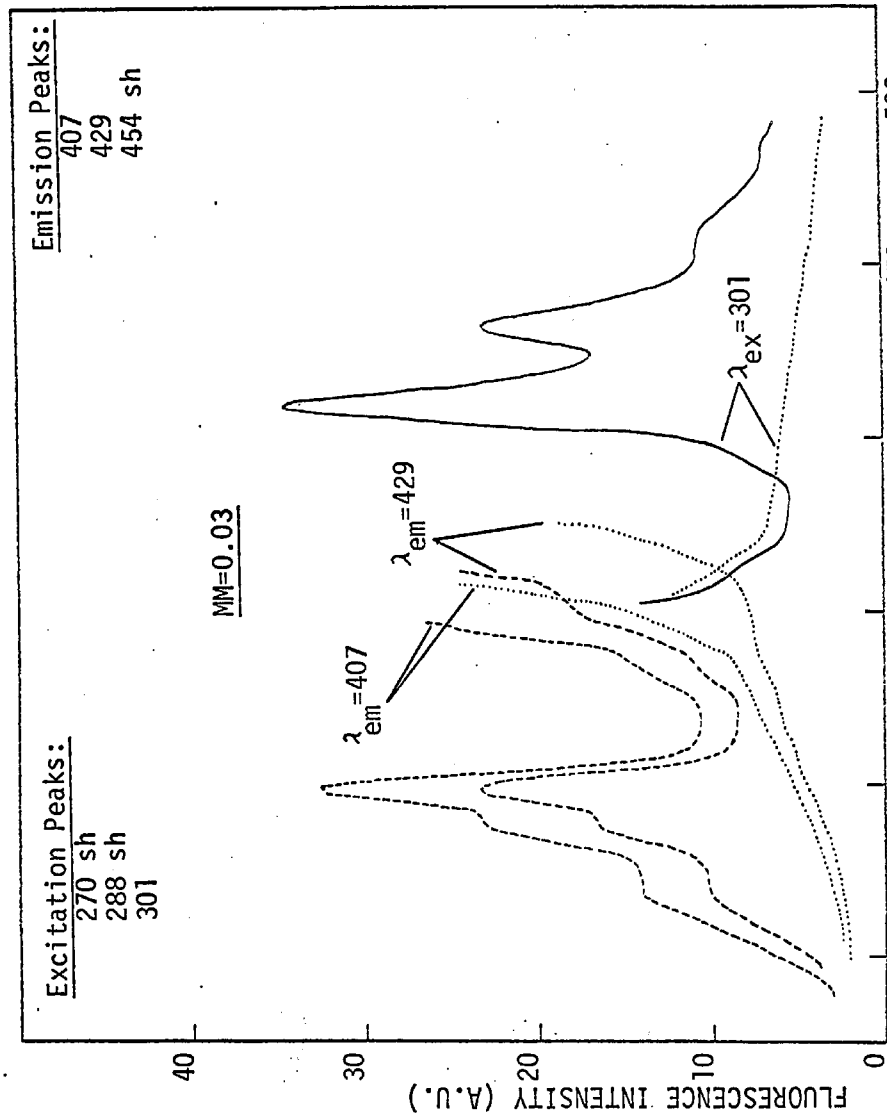


Figure 53. Excitation and emission spectra for 10 ng benzo(a)pyrene on filter paper.

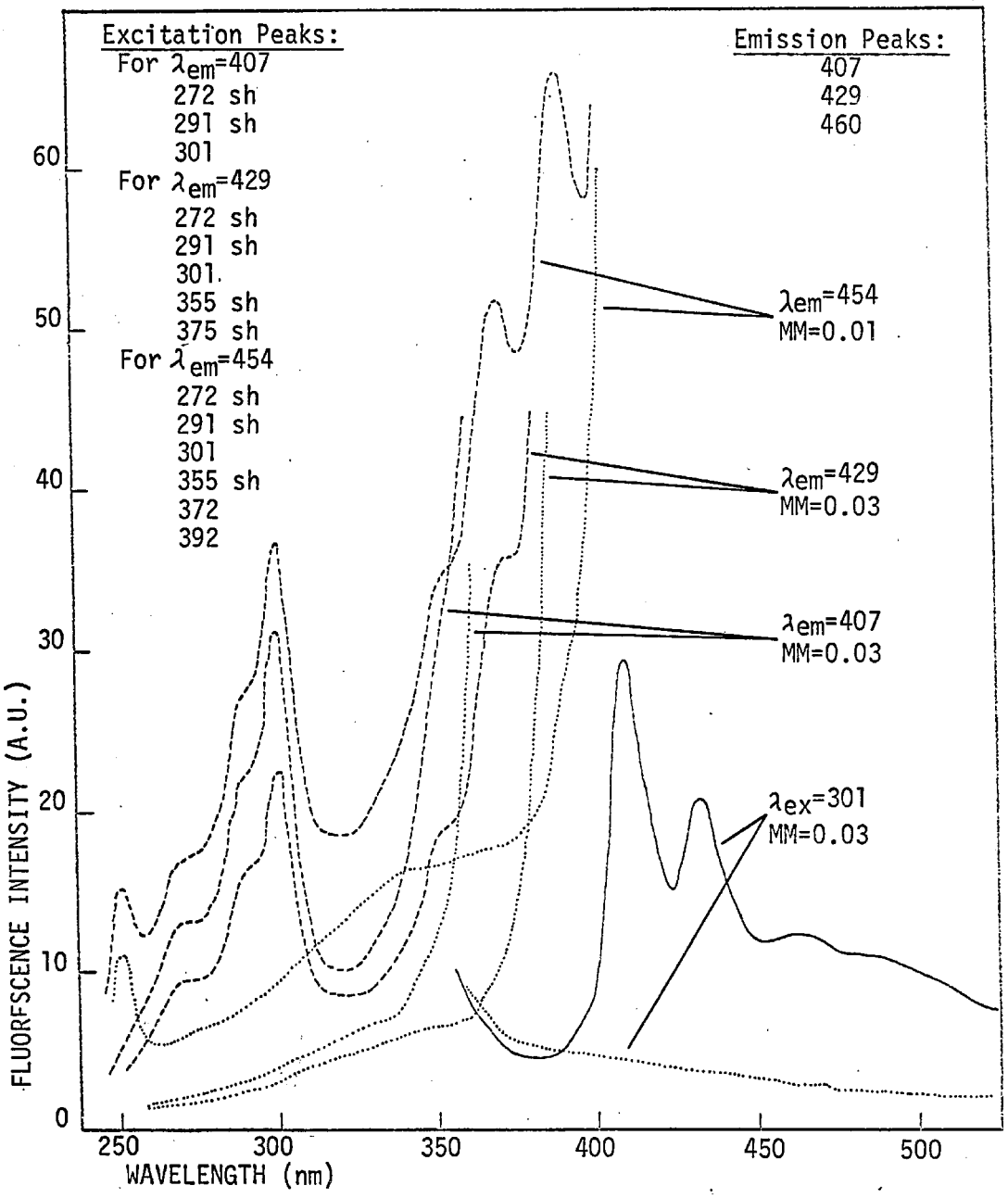


Figure 54. Excitation and emission spectra for 40 ng benzo(a)pyrene on filter paper.

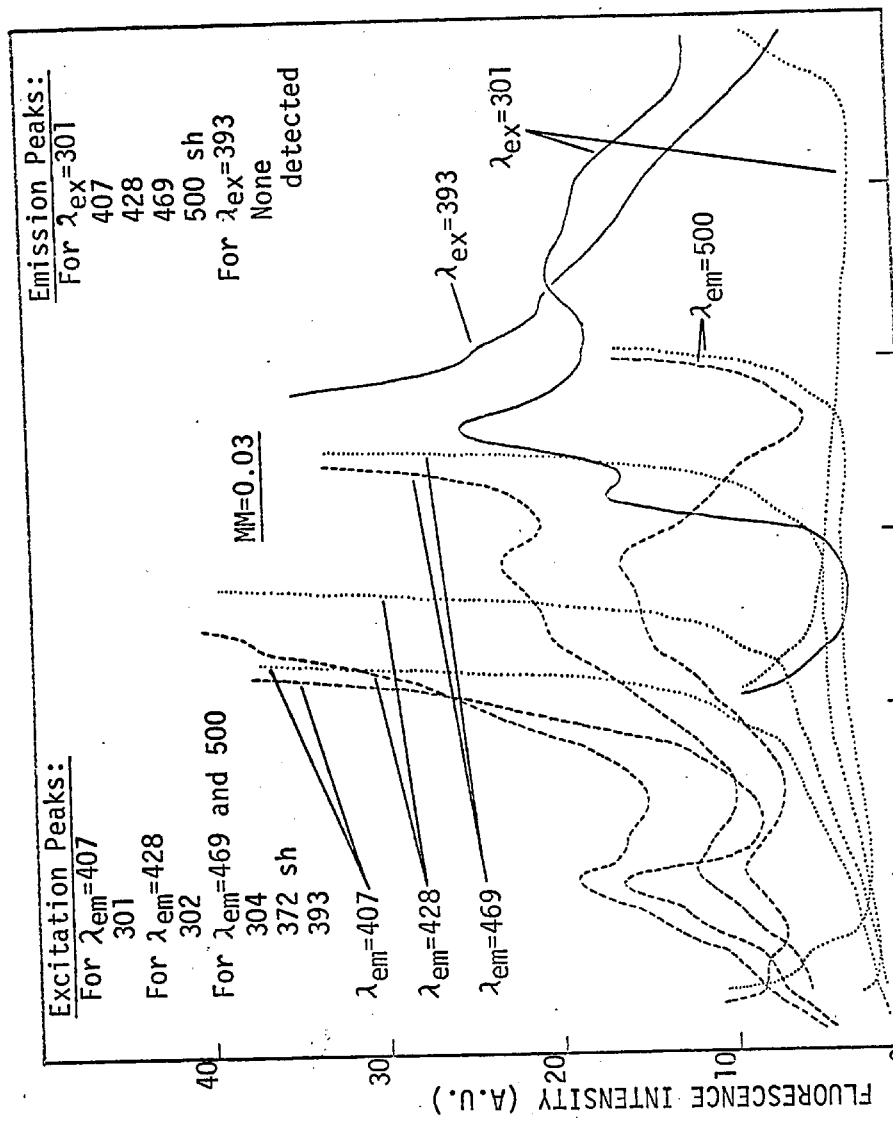


Figure 55. Excitation and emission spectra for 200 ng benzo(a)pyrene on filter paper.

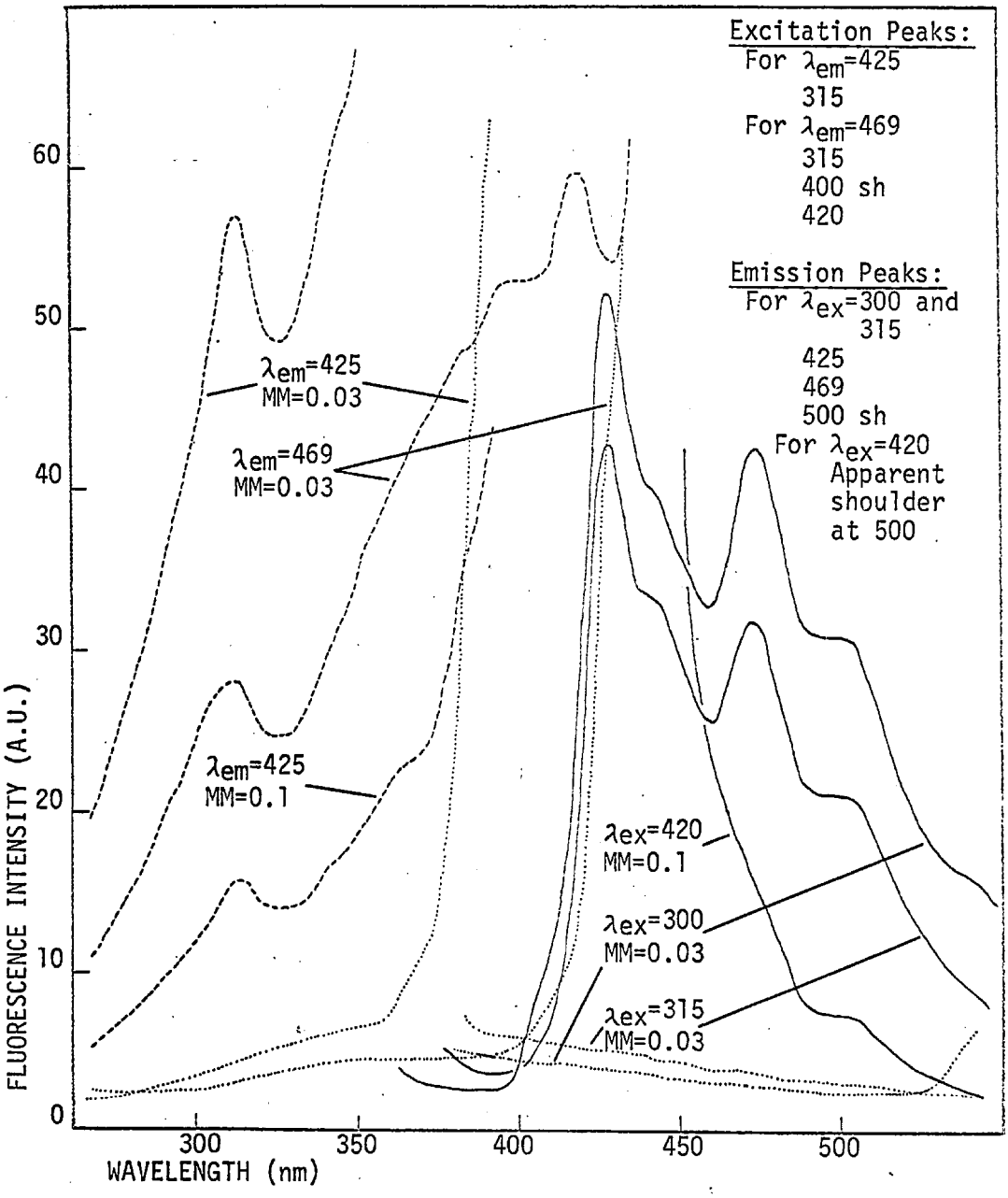


Figure 56. Excitation and emission spectra for 400 ng of benzo(a)-pyrene on filter paper.

APPENDIX D

RELATIVE EMISSION-PEAK HEIGHTS FOR BENZO(a)PYRENE ON FILTER PAPER EXPOSED TO ULTRAVIOLET RADIATION

Table 21. Relative peak heights for benzo(a)pyrene on filter paper after various times of continuous and limited exposure to 301 nm radiation. (In per cent of peak height at time 0.)

Quantity (ng)	1				10			
Peak (nm)	407		429(425)		407		429(425)	
Condition of Exp.	Cont.	Lim.	Cont.	Lim.	Cont.	Lim.	Cont.	Lim.
Time (min):								
0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
1	104.7	106.0	104.8	108.7	104.2	109.3	103.5	110.0
3	108.1	114.1	106.1	117.2	107.7	119.6	110.1	119.8
5	107.9	119.4	106.1	123.0	109.8	125.2	111.1	126.4
8	107.9	124.0	107.5	126.9	108.9	131.5	111.1	133.7
12	99.8	130.0	103.3	132.1	106.3	141.1	108.5	143.5
17	93.1	134.0	102.9	138.2	101.9	146.5	105.0	148.3
25	86.4	138.4	90.6	143.5	95.3	151.8	98.0	155.7
40	72.3	142.1	76.4	147.0	83.6	160.8	85.8	171.2
60	63.1	144.3	69.8	148.5	71.6	168.0	73.6	177.0

Quantity (ng)	40				100			
Peak (nm)	407		429(425)		407		429(425)	
Condition of Exp.	Cont.	Lim.	Cont.	Lim.	Cont.	Lim.	Cont.	Lim.
Time (min):								
0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
1	120.5	111.8	116.7	110.4	109.5	107.8	94.2	99.3
3	128.0	124.5	123.0	120.8	112.0	111.2	88.6	96.7
5	126.6	131.6	123.0	128.6	110.8	111.7	84.4	94.1
8	121.6	139.0	118.1	135.8	108.1	114.2	81.0	91.5
12	116.7	154.0	112.9	150.1	105.5	115.0	79.6	90.0
17	108.8	156.9	104.3	152.2	101.1	114.0	73.0	87.6
25	100.8	162.6	96.3	157.1	96.0	114.7	69.3	85.7
40	87.5	172.2	84.7	168.0	86.0	116.2	61.0	85.7
60	73.6	184.8	69.5	179.6	75.6	116.2	51.7	83.8
70	----	185.0	----	181.0	----	----	----	----
80	----	184.0	----	179.6	----	----	----	----
90	----	182.5	----	178.9	----	----	----	----

Table 21. Continued

Quantity (ng)	200					
Peak (nm)	407		429(425)		469	
Condition of Exp.	Cont.	Lim.	Cont.	Lim.	Cont.	Lim.
Time (min):						
0	100.0	100.0	100.0	100.0	100.0	100.0
1	101.1	103.5	75.0	82.3	68.3	79.8
3	99.4	103.5	59.2	71.0	46.8	66.9
5	96.6	107.0	52.2	65.5	37.3	60.0
8	93.2	103.5	45.8	59.2	28.9	52.8
12	86.8	101.1	39.7	54.2	22.6	46.8
17	83.4	102.9	35.5	50.8	18.1	43.7
25	76.0	100.6	30.7	46.4	13.6	38.4
40	66.8	98.3	25.5	41.8	9.1	34.4
60	59.4	94.3	20.5	37.1	6.3	29.4

Quantity (ng)	400					
Peak (nm)	407		429(425)		469	
Condition of Exp.	Cont.	Lim.	Cont.	Lim.	Cont.	Lim.
Time (min):						
0	100.0	100.0	100.0	100.0	100.0	100.0
1	89.5	87.6	70.9	77.8	69.7	79.4
3	84.2	86.4	52.9	65.7	49.0	68.5
5	79.0	83.9	44.2	58.2	38.6	59.7
8	77.2	81.5	37.2	51.3	31.1	52.6
12	70.2	80.2	32.1	46.5	24.8	47.3
17	64.8	79.0	27.2	42.4	19.9	43.0
25	57.9	77.7	22.0	38.6	15.3	38.4
40	49.1	76.5	16.9	33.8	10.8	33.8
60	40.3	72.8	12.4	28.9	6.9	29.2

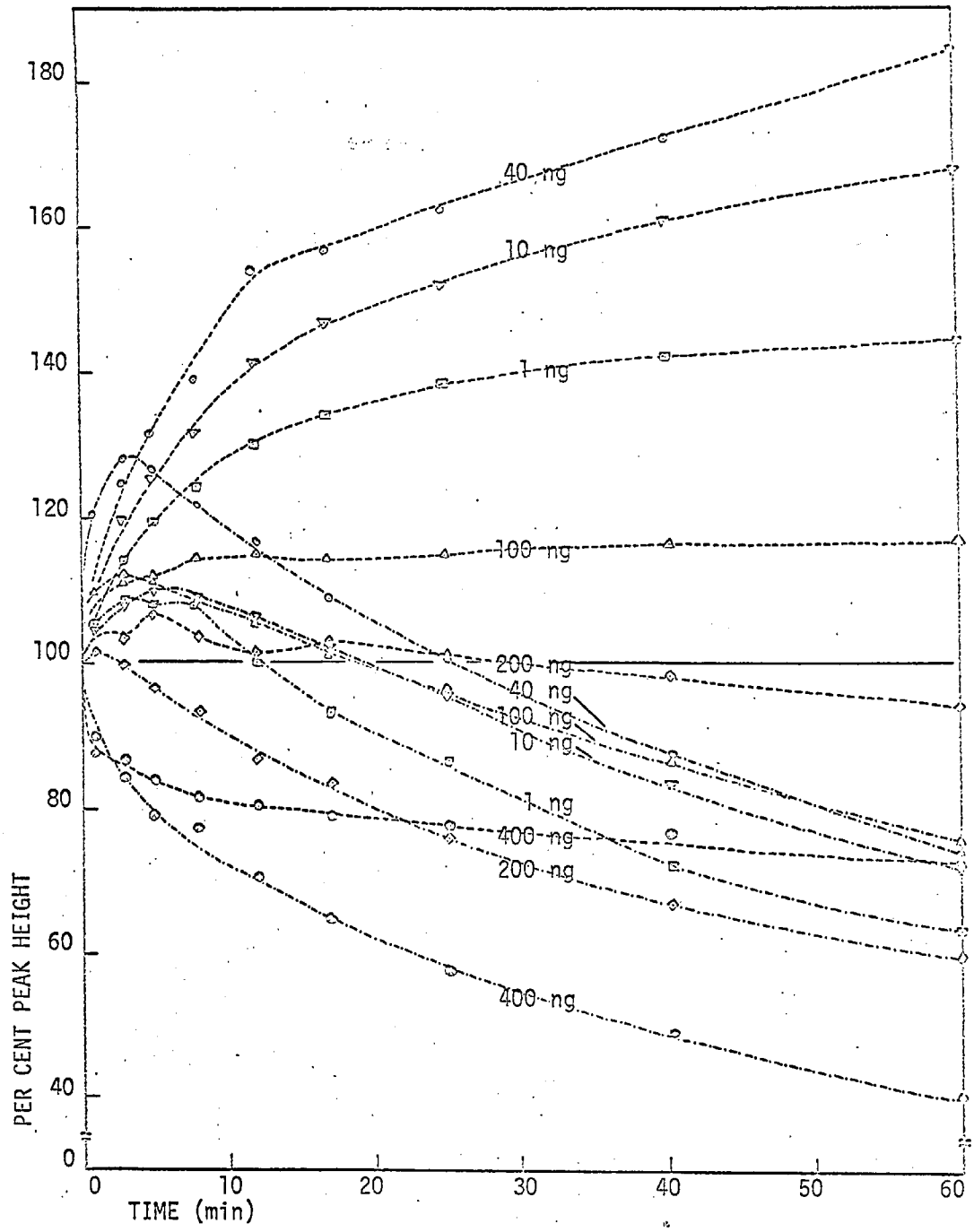


Figure 57. Change in the 407 nm benzo(a)pyrene peak height with time during continuous (-.-.-.-) and limited (------) exposure for excitation and exposure wavelength 301 nm.

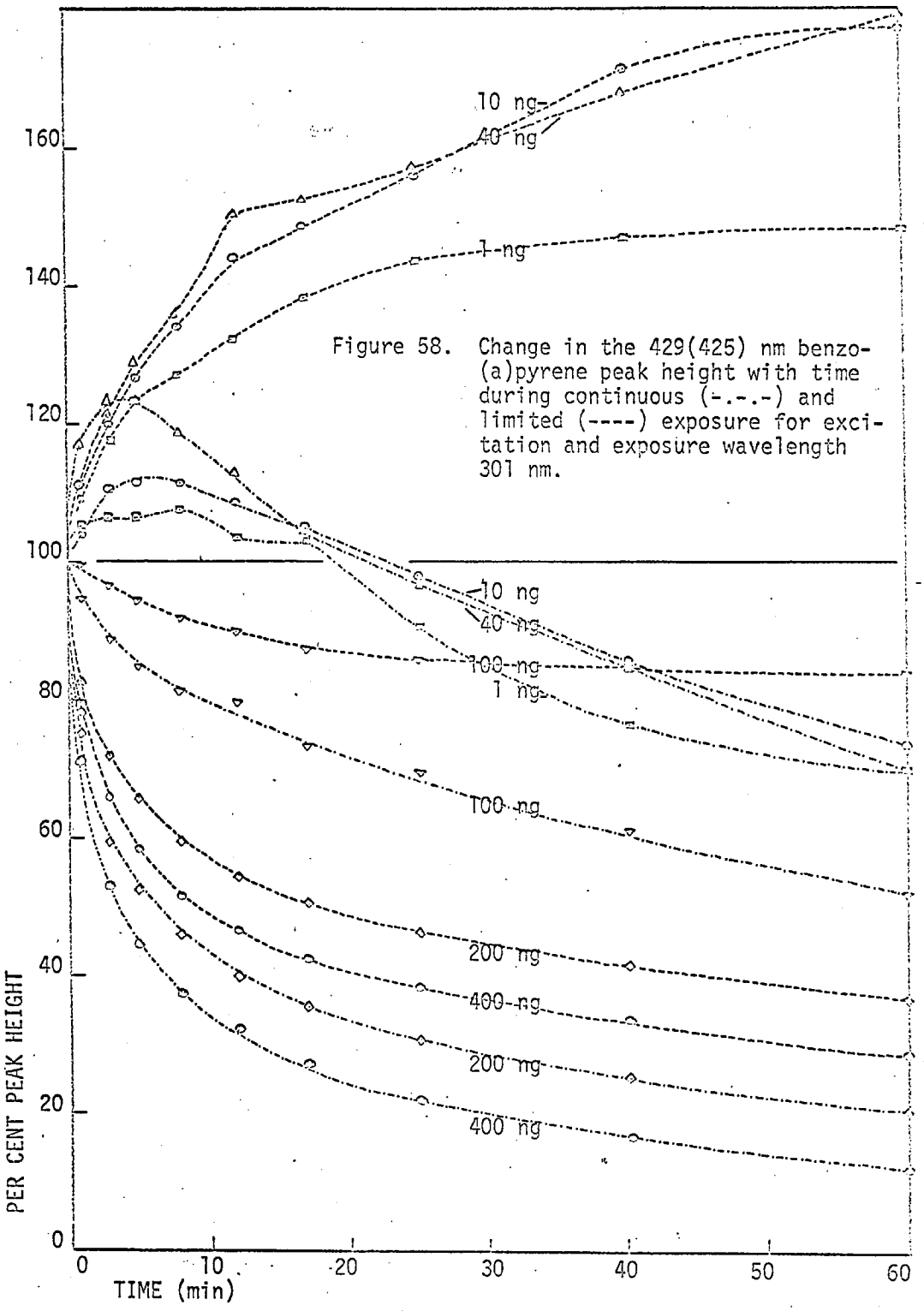


Figure 58. Change in the 429(425) nm benzo-(a)pyrene peak height with time during continuous (-.-.-) and limited (----) exposure for excitation and exposure wavelength 301 nm.

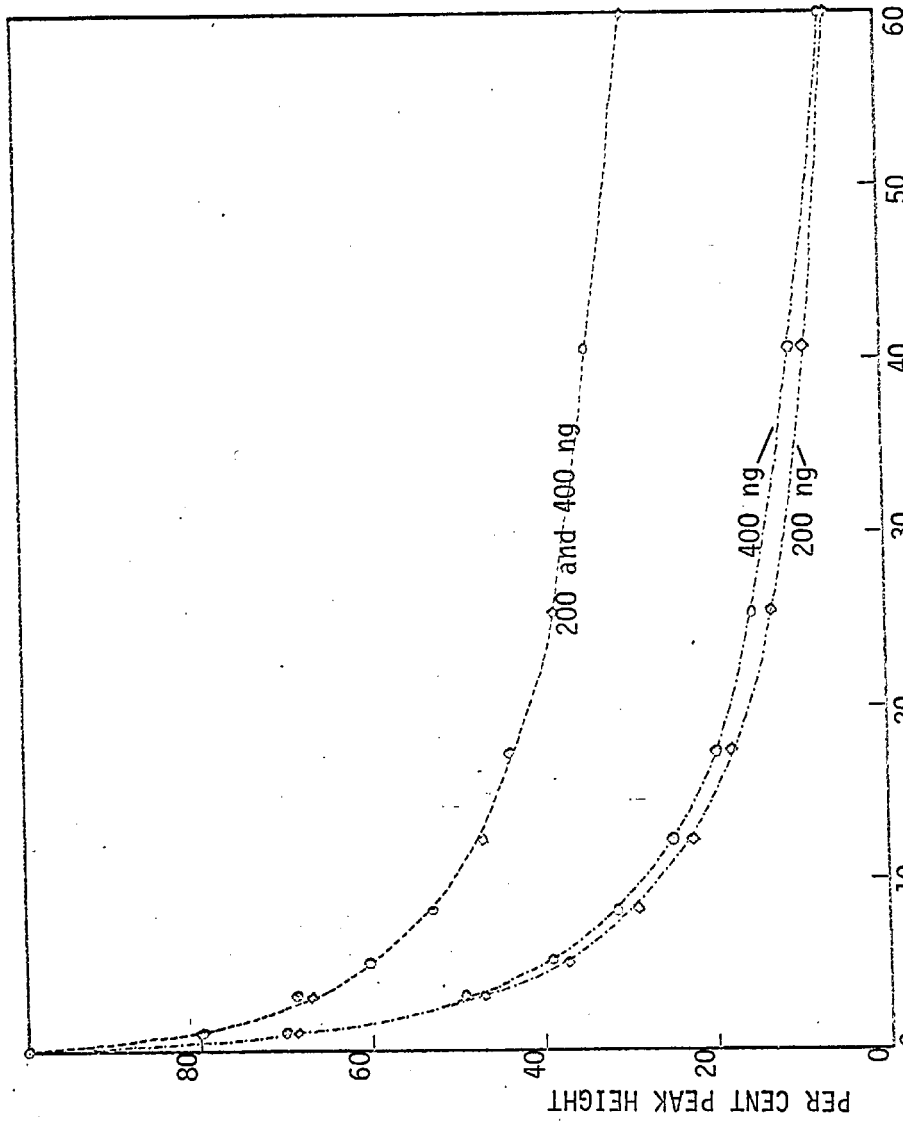


Figure 59. Change in the 469 nm benzo(a)pyrene peak height with time during continuous (-.-.-) and limited (-----) exposure for excitation and exposure wavelength 301 nm.

XERO COPY XERO COPY XERO COPY XERO COPY

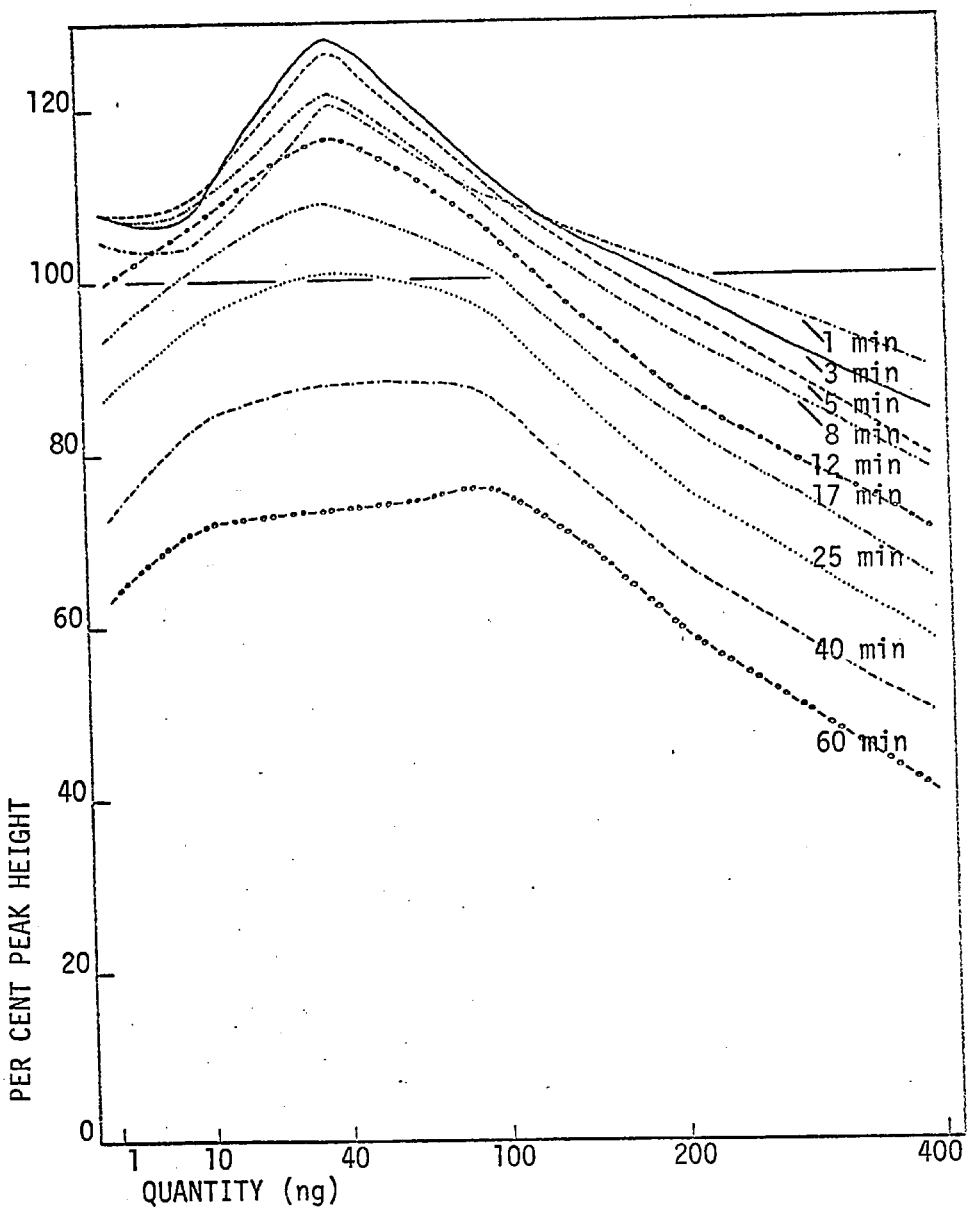


Figure 60. Change in the 407 nm benzo(a)pyrene peak height with quantity for various times of continuous exposure for excitation and exposure wavelength 301 nm.

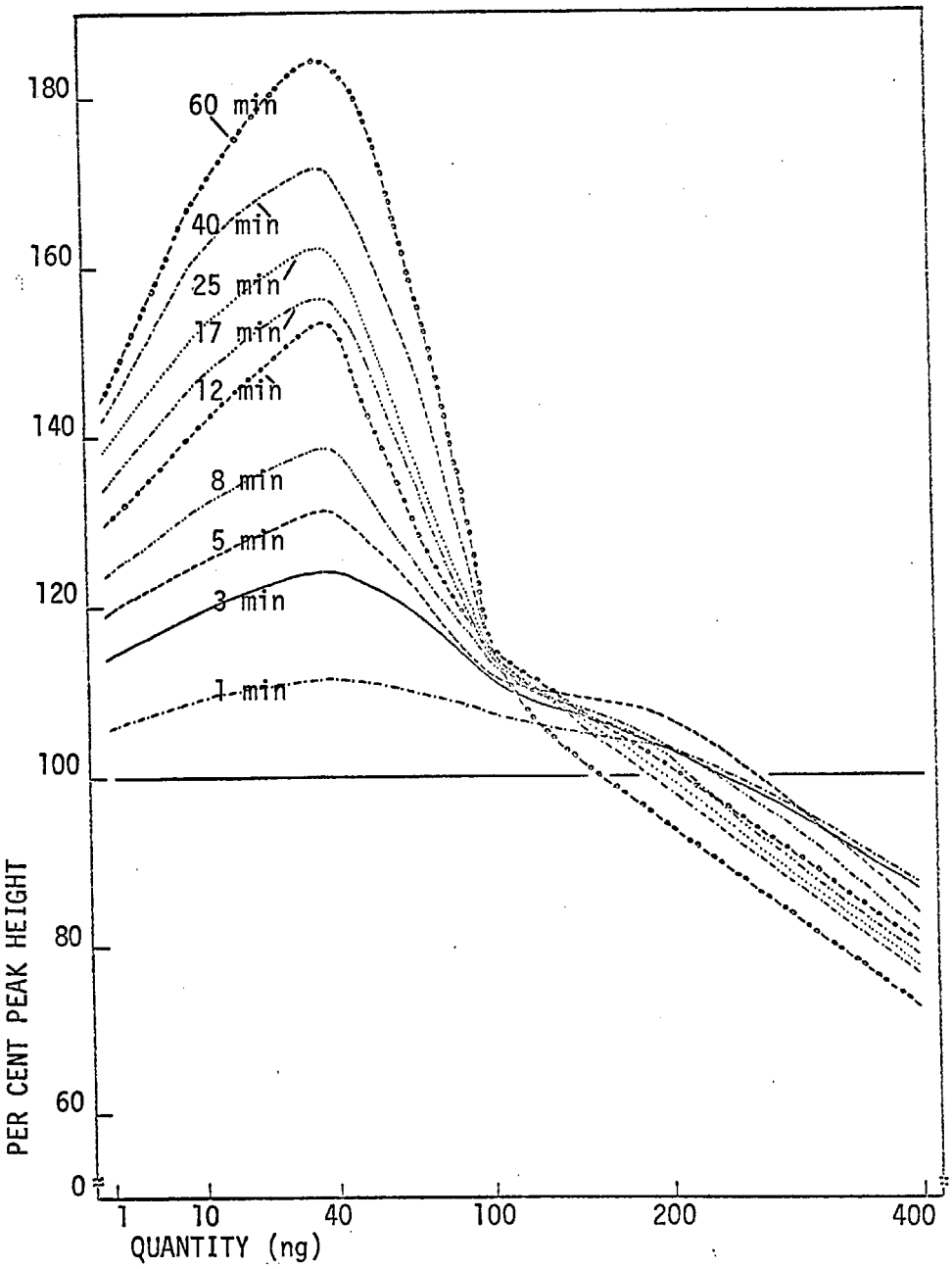


Figure 61. Change in the 407 nm benzo(a)pyrene peak height with quantity for various times of limited exposure for excitation and exposure wavelength 301 nm.

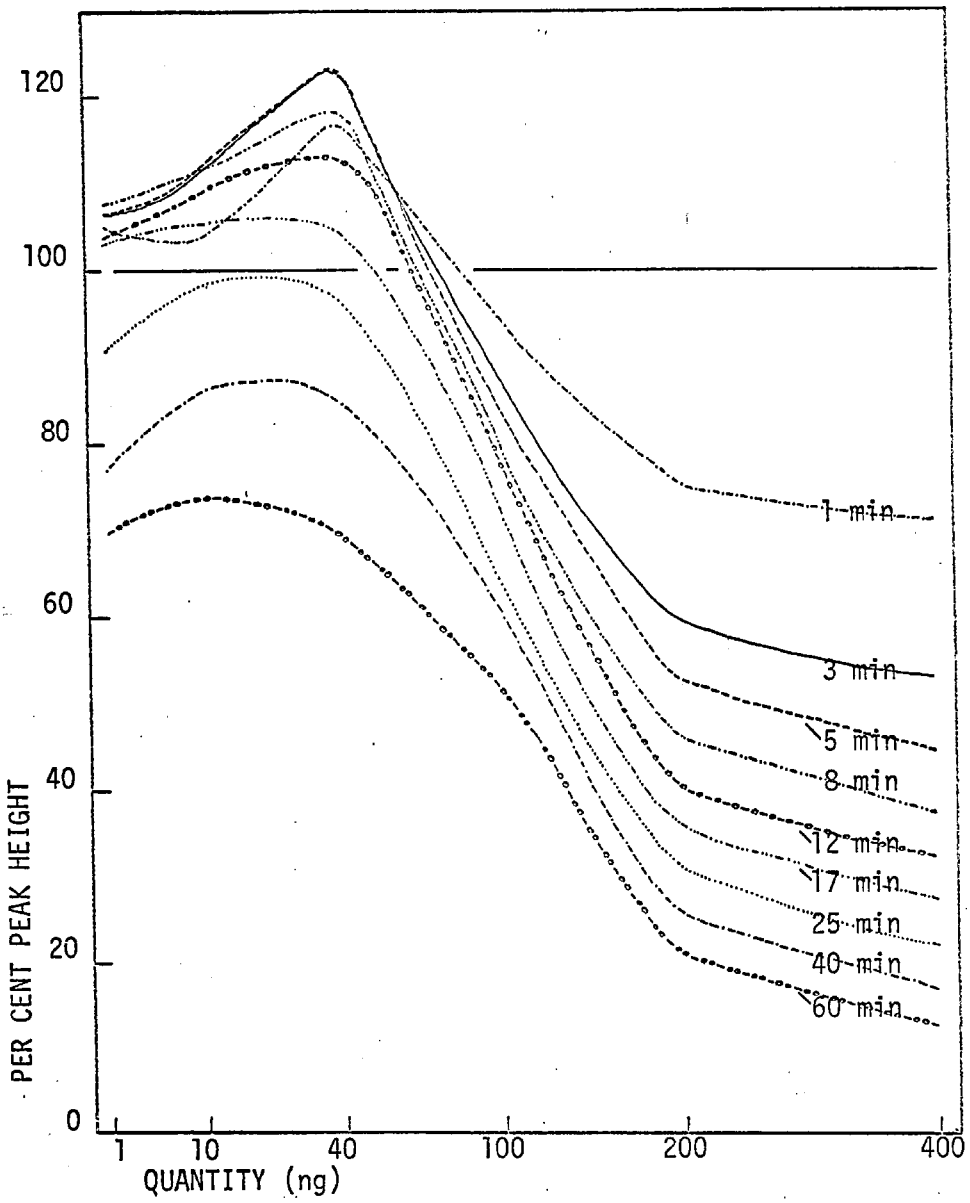


Figure 62. Change in the 429(425) nm benzo(a)pyrene peak height with quantity for various times of continuous exposure for excitation and exposure wavelength 301 nm.

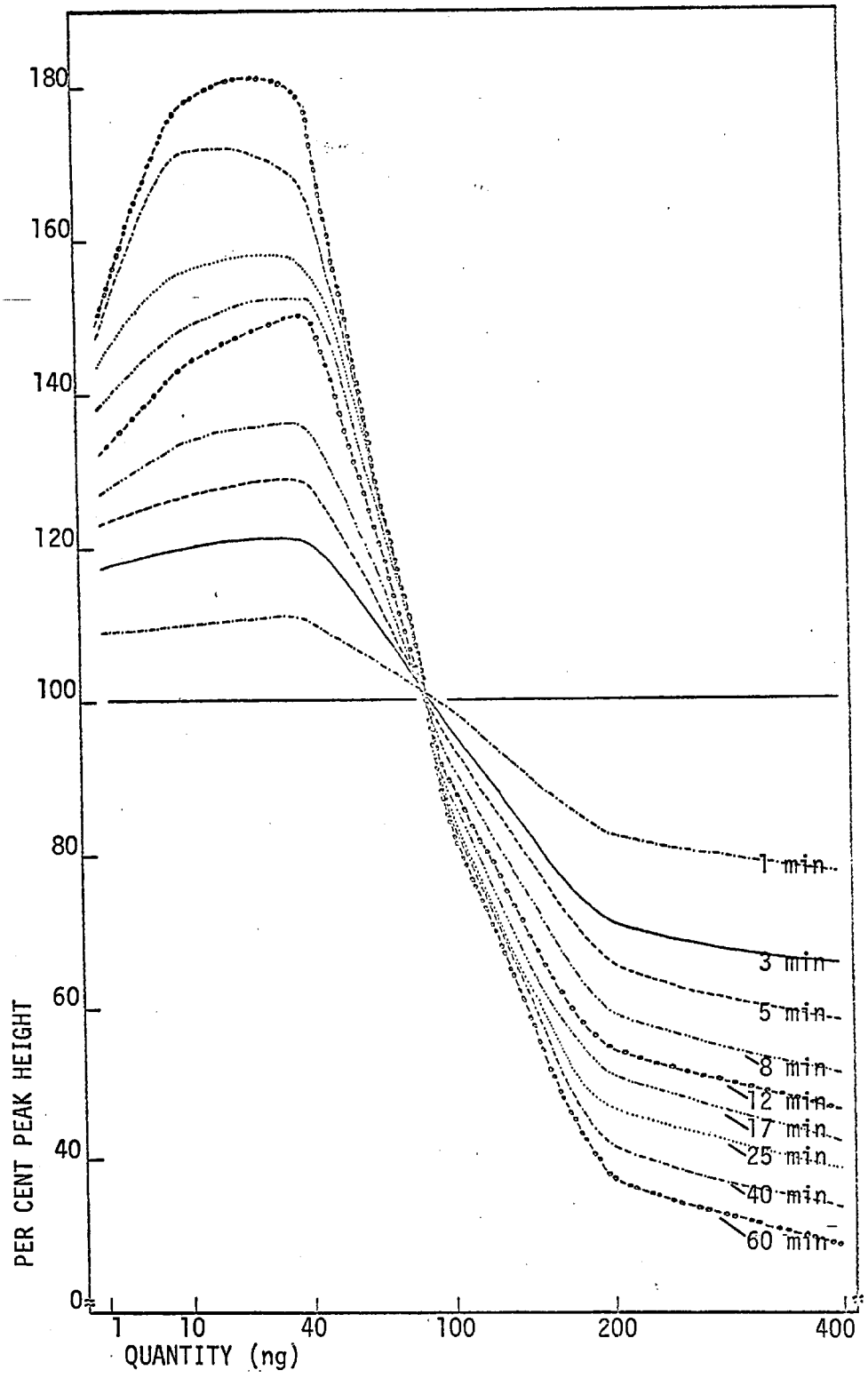


Figure 63. Change in the 429(425) nm benzo(a)pyrene peak height with quantity for various times of limited exposure for excitation and exposure wavelength 301 nm.

APPENDIX E

FLUORESCENT SPECTRA FOR
BENZO(ghi)PERYLENE ON FILTER PAPER

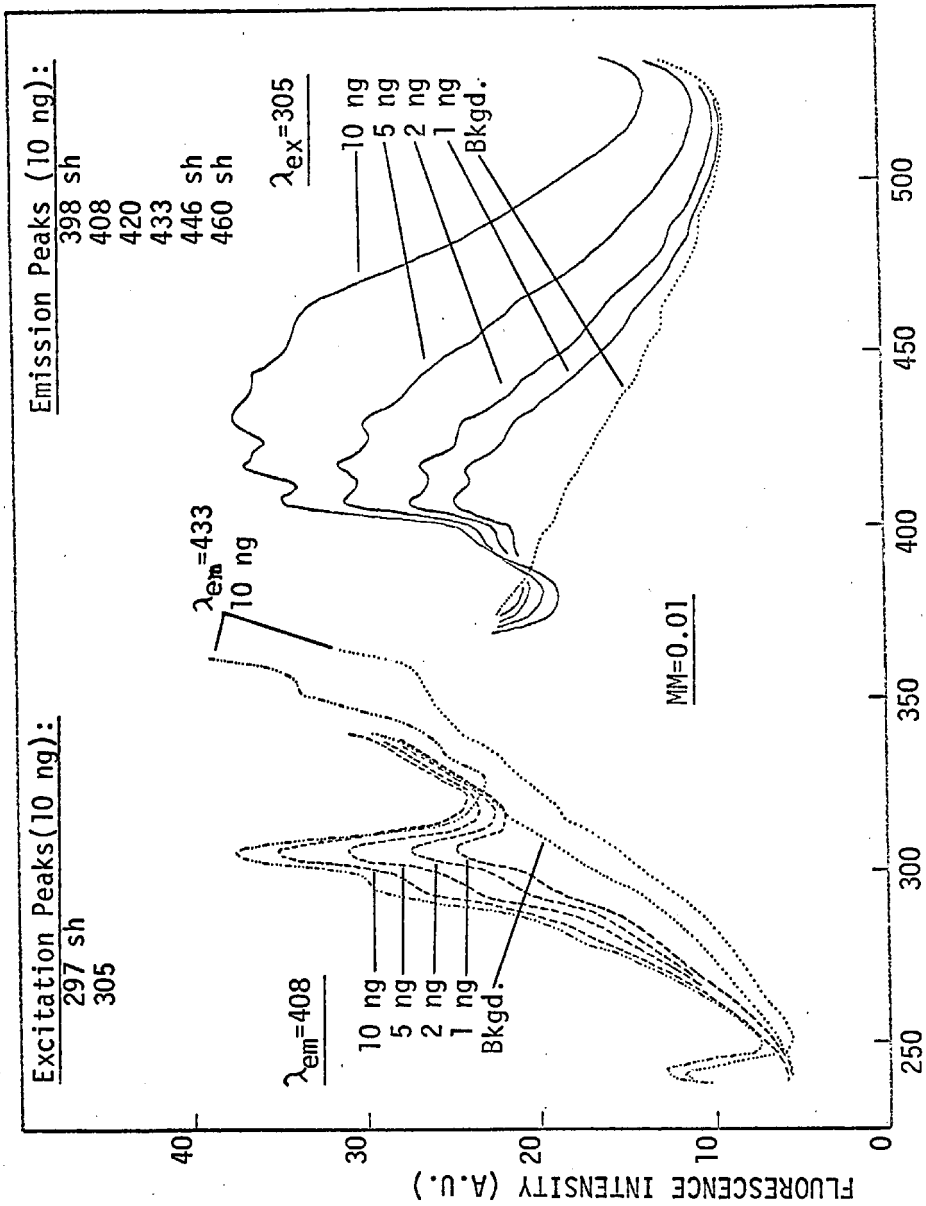


Figure 64. Excitation and emission spectra for one, two, five and 10 ng of benzo-(ghi)perylene on filter paper.

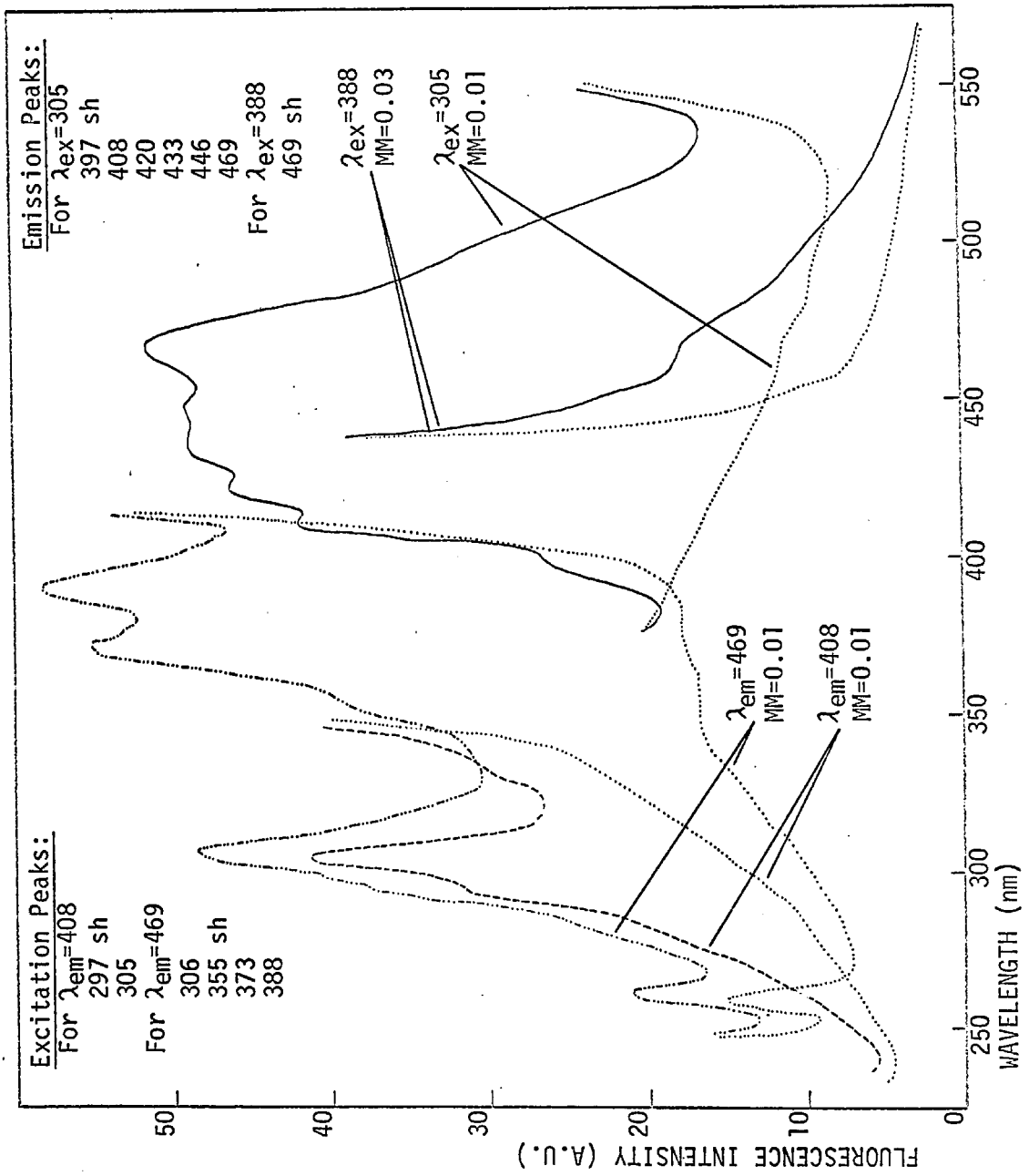


Figure 65. Excitation and emission spectra for 20 ng benzo(ghi)perylene on filter paper.

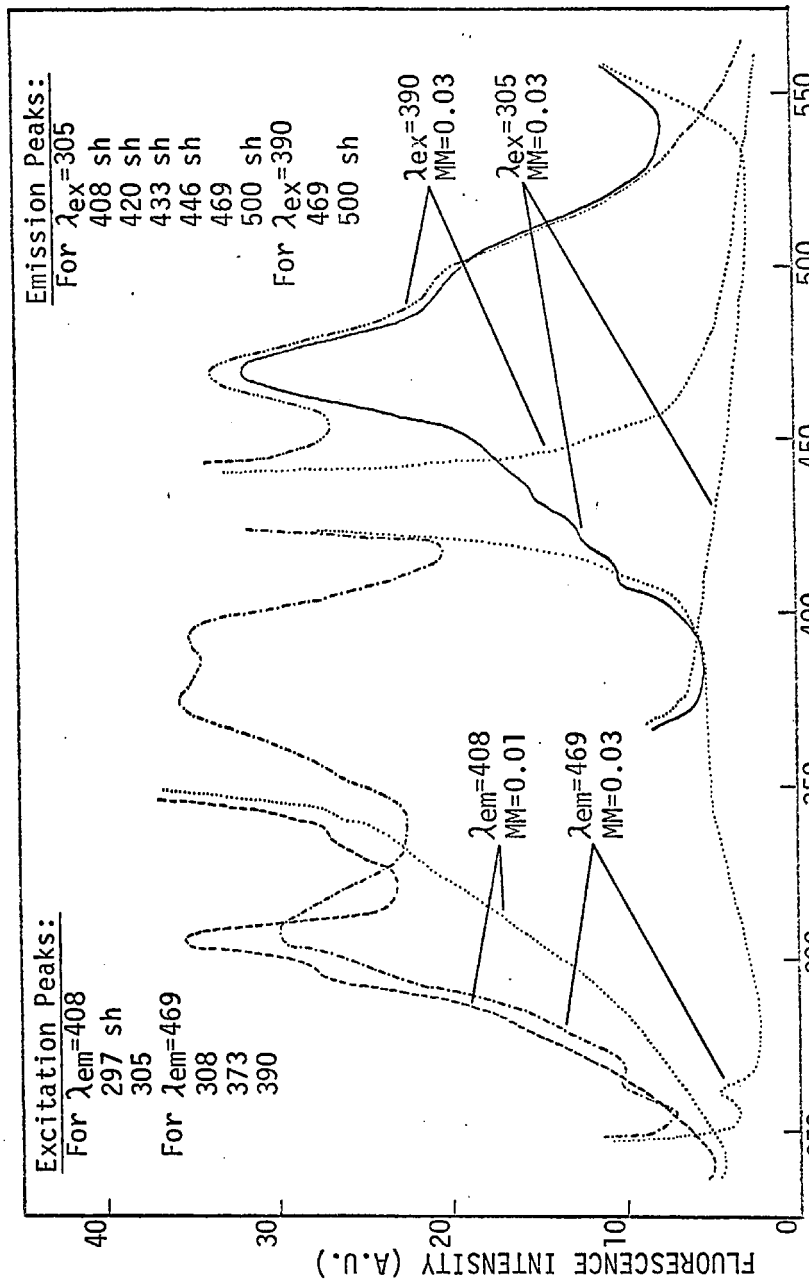


Figure 66. Excitation and emission spectra for 50 ng benzo(ghi)perylene on filter paper.

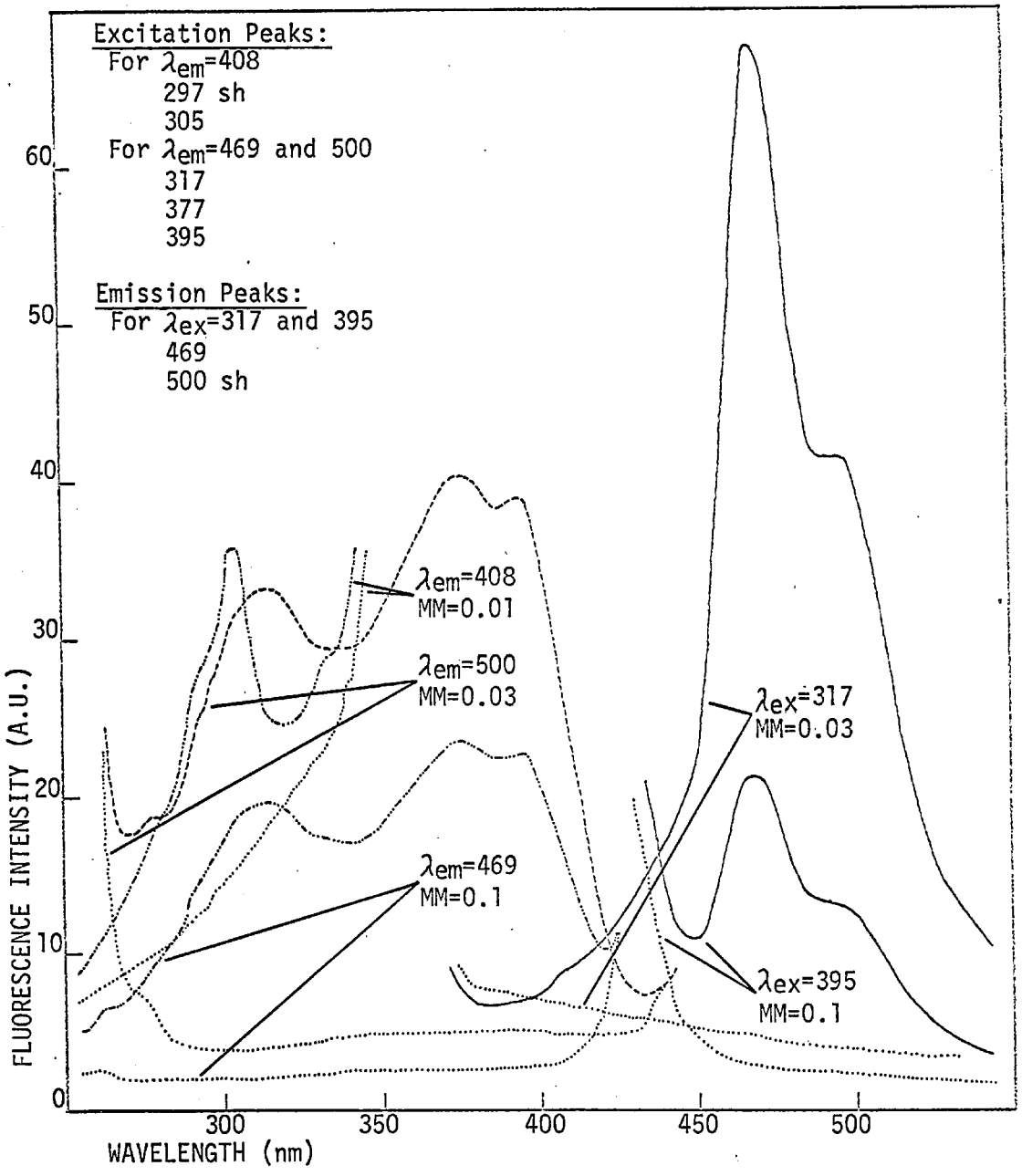


Figure 67. Excitation and emission spectra for 100 ng benzo(ghi)perylene on filter paper.

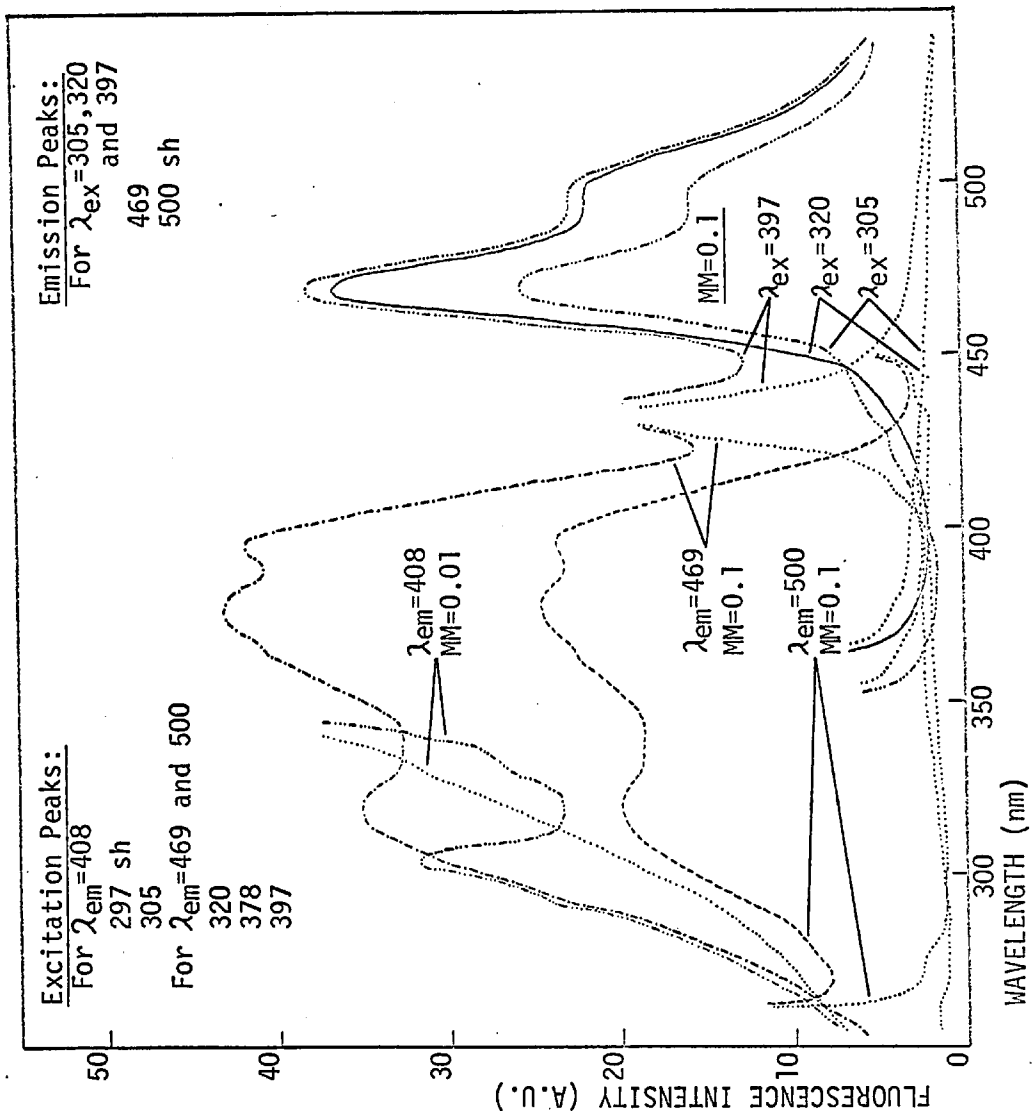


Figure 68. Excitation and emission spectra for 200 ng benzo(ghi)-perylene on filter paper.

APPENDIX F

RELATIVE EMISSION-PEAK HEIGHTS FOR BENZO(ghi)PERYLENE ON FILTER PAPER EXPOSED TO ULTRAVIOLET RADIATION

Table 22. Relative peak heights for benzo(ghi)perylene on filter paper after various times of continuous and limited exposure to 305 nm radiation. (In per cent of peak height at time 0.)

Quantity (ng)	1		20		20		50	
Peak (nm)	408		408		420		433	
Condition of Exp.	Cont.	Lim.	Cont.	Lim.	Cont.	Lim.	Cont.	Lim.
Time (min):								
0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
1	101.2	100.5	103.8	105.4	103.5	104.3	101.5	101.0
3	99.4	102.6	101.7	110.2	104.9	106.7	98.0	102.8
5	94.8	99.5	104.4	114.2	105.8	110.4	94.9	103.2
8	92.9	103.7	99.0	119.0	103.5	116.0	90.6	105.0
12	84.5	102.6	95.7	121.0	101.2	116.5	84.8	106.9
17	81.4	105.2	90.3	122.3	95.1	117.5	78.6	106.9
25	76.1	108.0	82.0	124.4	88.0	120.2	71.1	106.9
40	60.6	109.0	74.5	126.5	80.4	121.5	63.4	107.9
60	45.2	111.5	65.2	130.0	70.6	125.2	54.0	108.8

Quantity (ng)	20		50		100		200	
Peak (nm)	469		469		469		469	
Condition of Exp.	Cont.	Lim.	Cont.	Lim.	Cont.	Lim.	Cont.	Lim.
Time (min):								
0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
1	96.5	95.2	90.5	94.8	91.2	92.6	92.3	92.6
3	85.6	90.0	78.2	90.4	81.2	87.8	83.3	88.1
5	76.1	86.1	69.8	84.9	74.8	83.0	77.5	84.0
8	67.0	82.2	61.6	81.1	68.0	78.0	71.4	81.2
12	58.6	76.4	54.0	76.5	62.2	75.8	65.6	78.2
17	49.2	71.8	47.6	72.8	56.4	73.0	60.1	75.5
25	40.9	68.3	39.6	69.4	50.0	70.0	55.5	72.0
40	33.0	65.1	32.5	65.8	42.6	66.2	48.0	67.5
60	27.3	----	----	63.0	36.5	63.4	42.9	66.0

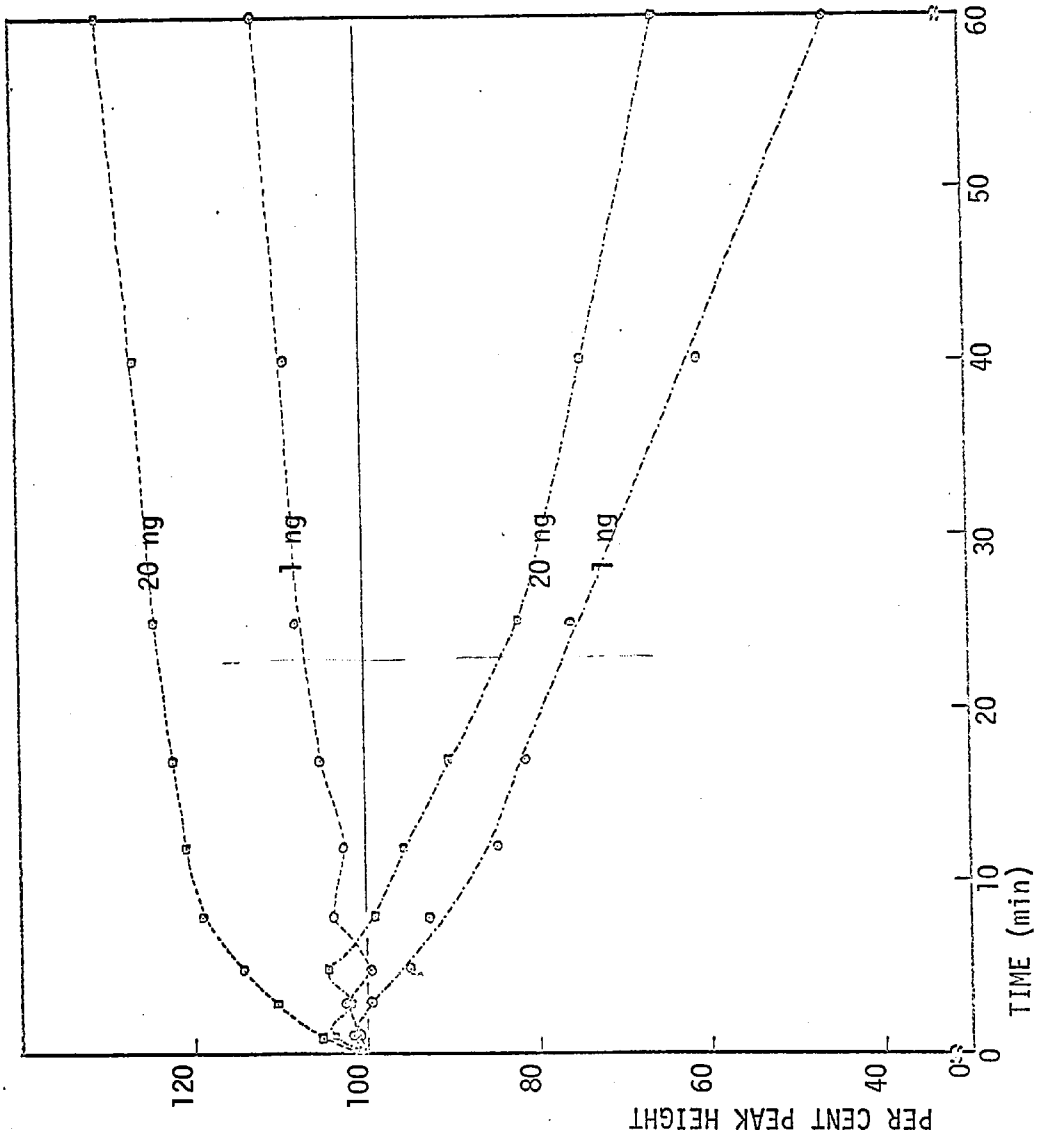


Figure 69. Change in the 408 nm benzo(ghi)perylene peak height with time during continuous (-.-.-) and limited (-----) exposure for excitation and exposure wavelength 305 nm.

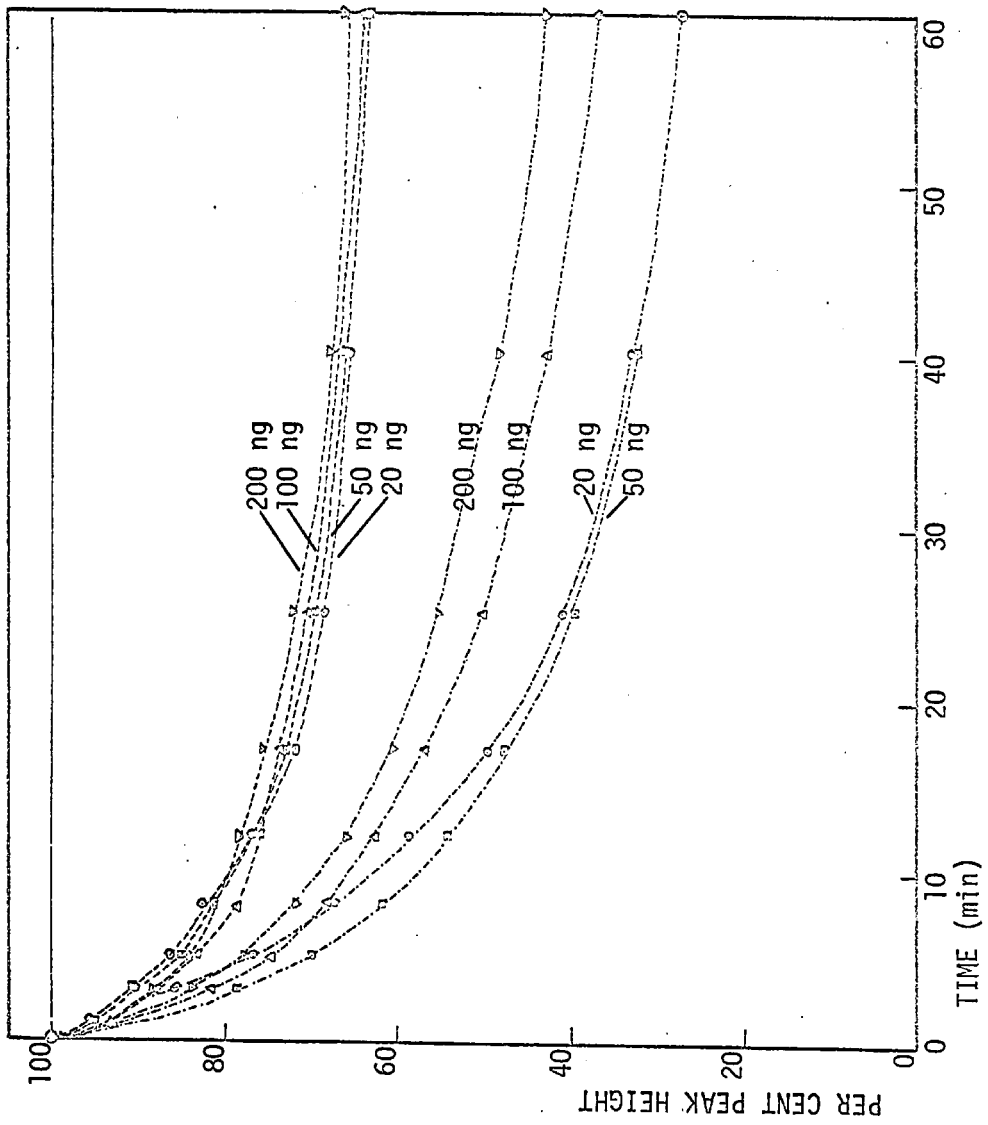


Figure 70. Change in the 469 nm benzo(ghi)perylene peak height with time during continuous (-.-.-) and limited (-----) exposure for excitation and exposure wavelength 305 nm.

APPENDIX G

FLUORESCENT SPECTRA FOR BENZO(a)PYRENE ON MYLAR

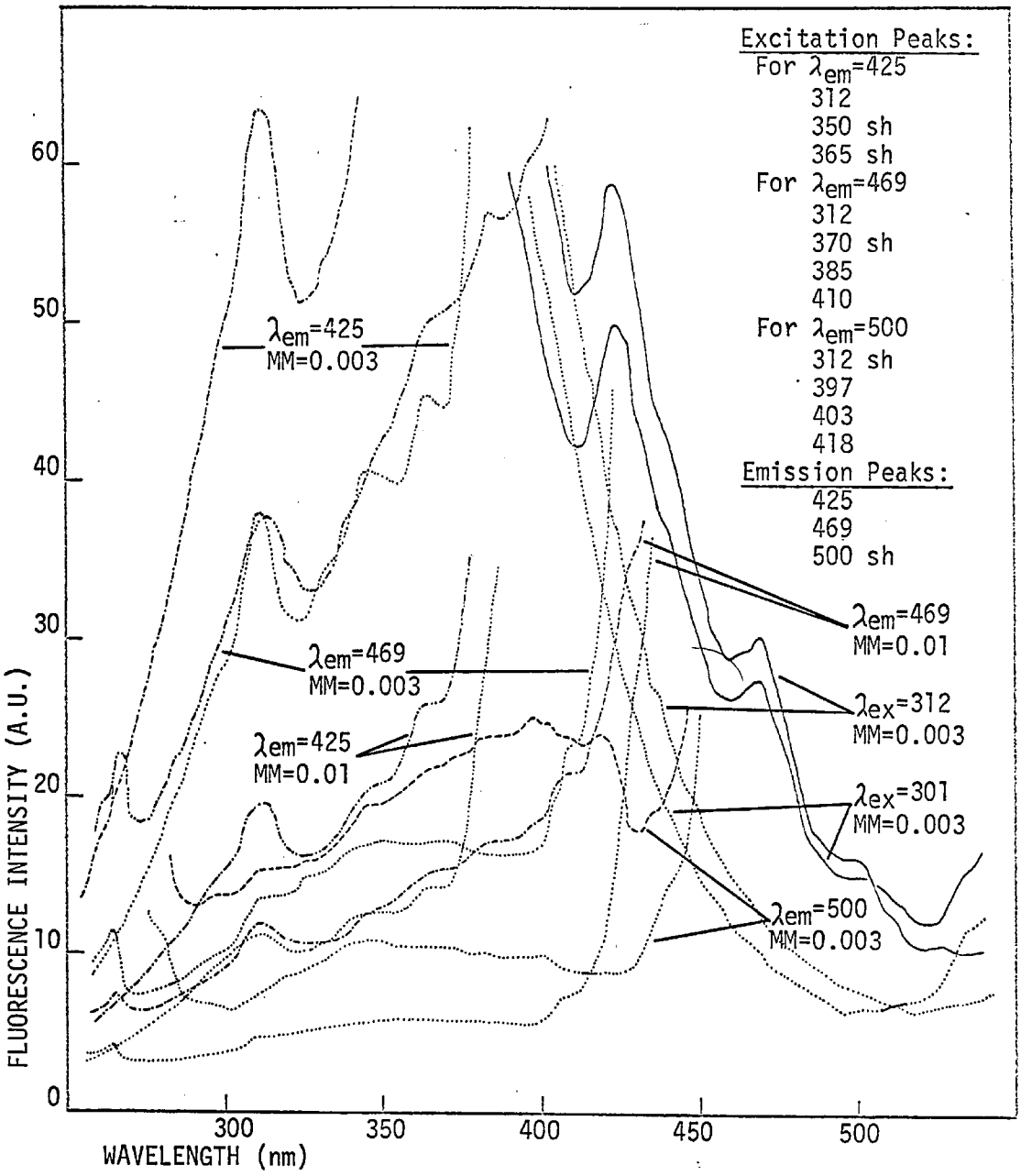


Figure 71. Excitation and emission spectra for 100 ng benzo(a)pyrene on Mylar.

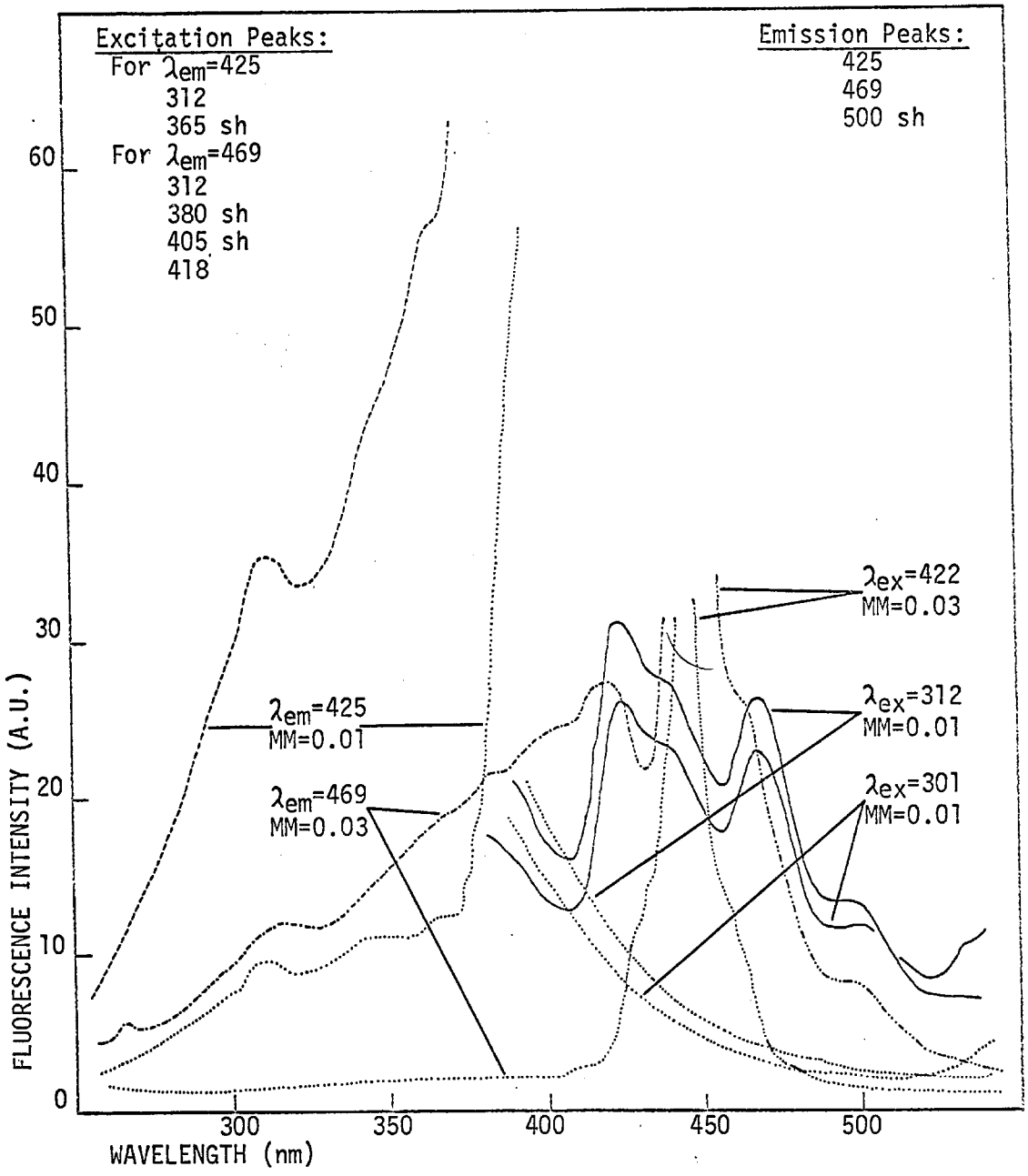


Figure 72. Excitation and emission spectra for 400 ng benzo(a)pyrene on Mylar.

APPENDIX H

RELATIVE EMISSION-PEAK HEIGHTS FOR BENZO(a)PYRENE ON MYLAR EXPOSED TO ULTRAVIOLET RADIATION

Table 23. Relative peak heights for benzo(a)pyrene on Mylar after various times of continuous and limited exposure to 325 nm radiation. (In per cent of peak height at time 0.)

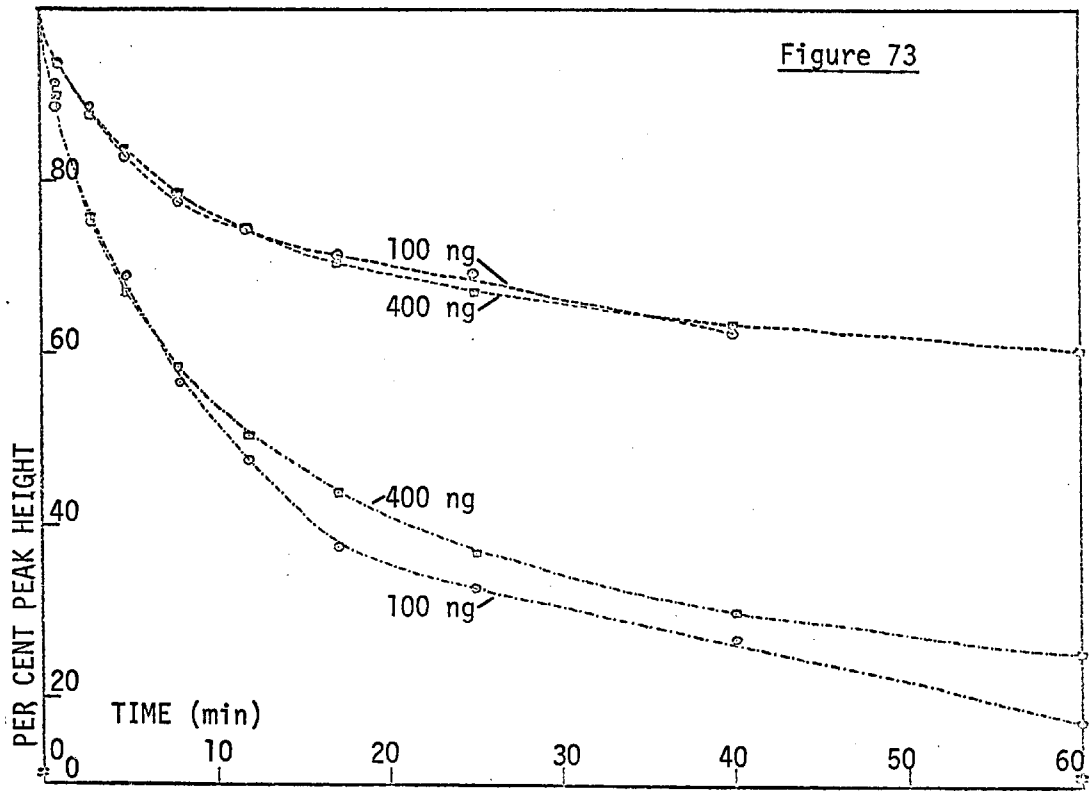
Quantity (ng)	100				400			
Peak (nm)	425		469		429		469	
Condition Of Exp.	Cont.	Lim.	Cont.	Lim.	Cont	Lim.	Cont.	Lim.
Time (min):								
0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
1	88.8	91.6	88.0	95.0	90.0	93.5	90.5	93.0
3	75.1	88.6	76.0	88.0	75.4	87.9	76.0	87.4
5	69.0	82.5	66.7	83.0	67.2	83.4	67.5	82.4
8	56.4	77.6	59.4	80.6	58.5	78.6	58.4	78.5
12	47.4	74.5	50.5	75.1	50.1	74.4	50.4	74.5
17	37.1	71.1	44.8	71.9	43.8	70.3	44.5	70.5
25	32.8	69.2	38.5	71.0	36.8	67.2	37.2	67.1
40	26.8	62.0	31.8	66.4	29.8	63.0	30.8	63.2
60	16.4	----	24.5	----	24.6	60.1	25.2	60.9

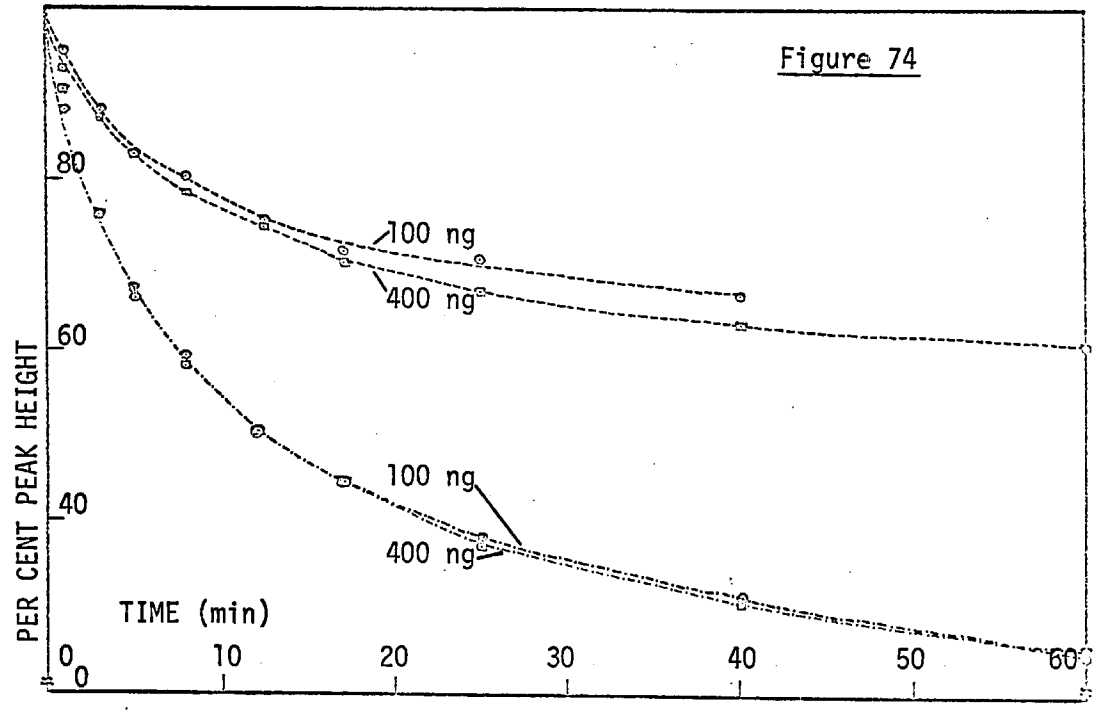
Quantity (ng)	40		40		100		200	
Peak (nm)	469		469		469		469	
Condition of Exp.	Cont.		Cont.		Cont.		Cont.	
	Ave.*	S.D.	Ave.**	S.D.	Ave.*	S.D.	Ave.*	S. D.
Time (min):								
0	100.0		100.0		100.0		100.0	
2.5	79.0	2.5	77.4	1.4	81.4	3.2	84.1	7.7
5	66.8	12.3	61.8	4.2	69.2	1.4	73.5	7.4
7.5	55.8	7.2	50.8	0.8	62.0	1.9	65.0	6.3
10	50.6	5.3	44.7	2.3	57.7	1.7	60.2	5.9
12.5	42.4	12.1	34.2	3.9	54.1	1.7	55.6	5.4
15	33.7	12.0	25.6	4.4	49.9	2.2	52.7	5.0
17.5	30.7	13.8	21.1	2.9	48.1	1.6	49.2	4.8
20	32.7	12.8	18.6	3.4	45.5	2.7	46.7	5.1
22.5	22.4	14.3	13.1	7.0	40.7	3.1	43.9	2.7
25	18.7	16.2	7.5	4.5	41.3	2.8	41.3	3.8
27.5	17.6	18.3	5.0	5.0	38.9	3.5	40.5	3.8
30	15.0	18.5	2.0	2.0	36.9	4.7	38.7	3.4

* Three determinations.
** Two determinations.

Figure 73. Change in the 425 nm benzo(a)pyrene peak height with time during continuous (-.-.-) and limited (-----) exposure for excitation and exposure wavelength 325 nm.

Figure 74. Change in the 469 nm benzo(a)pyrene peak height with time during continuous (-.-.-) and limited (-----) exposure for excitation and exposure wavelength 325 nm.





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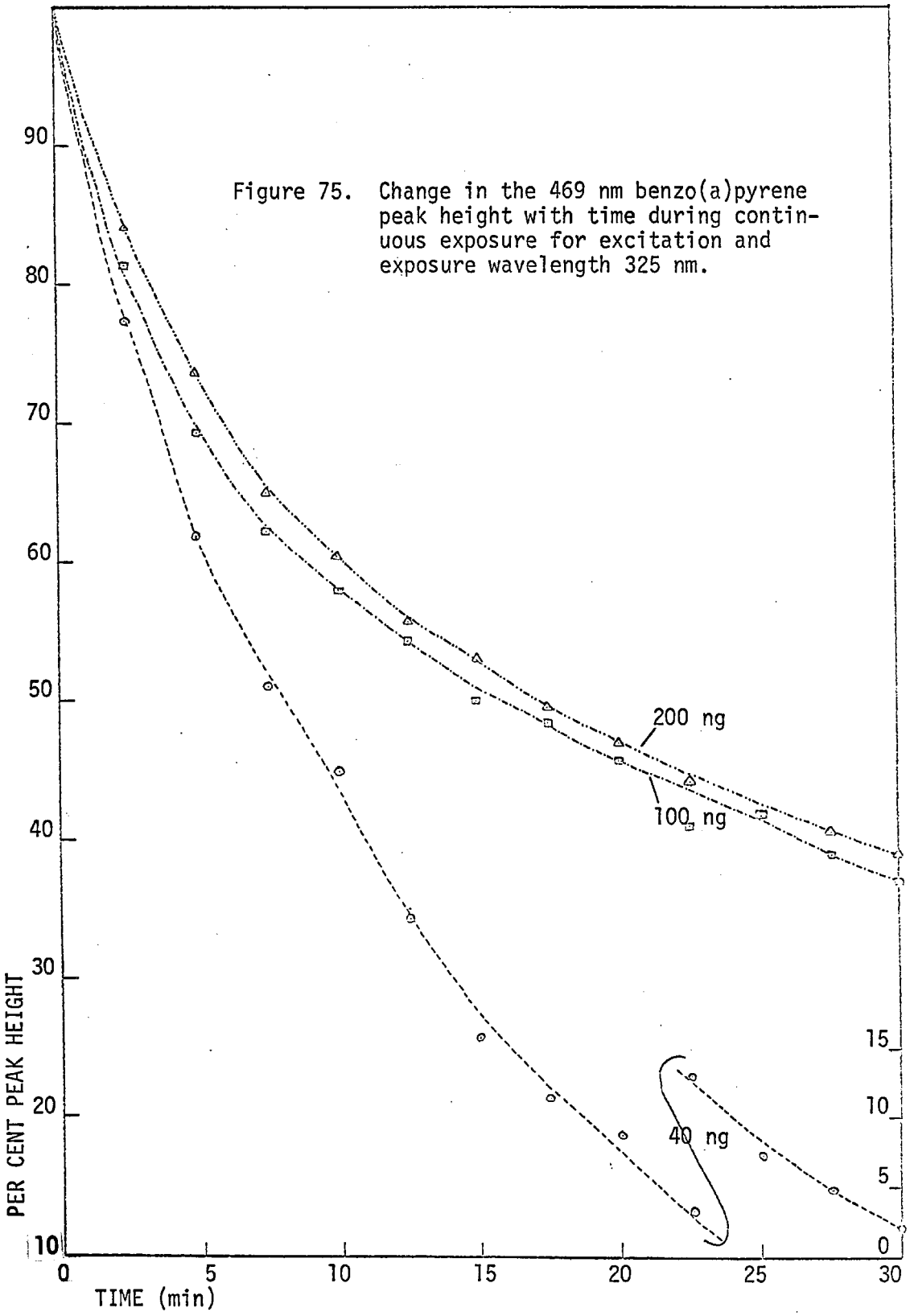


Figure 75. Change in the 469 nm benzo(a)pyrene peak height with time during continuous exposure for excitation and exposure wavelength 325 nm.

APPENDIX I

RELATIVE EMISSION-PEAK HEIGHT FOR BENZO(ghi)PERYLENE ON MYLAR EXPOSED TO ULTRAVIOLET RADIATION

Table 24. Relative 469 nm peak height for benzo(ghi) perylene on Mylar after various times of continuous and limited exposure to 325 nm radiation. (In per cent of peak height at time 0.)

Quantity (ng)	100		200	
Condition of Exp.	Cont.	Lim.	Cont.	Lim.
Time (min):				
0	100.0	100.0	100.0	100.0
1	99.0	99.0	99.9	99.2
3	97.5	97.8	98.6	98.5
5	96.2	97.1	96.5	97.1
8	95.2	96.7	95.3	97.0
12	93.9	95.2	93.5	96.5
17	90.6	95.2	91.7	96.2
25	90.3	93.4	90.6	94.2
40	88.4	92.0	88.5	93.3
60	88.4	91.0	85.0	92.5

Quantity (ng)	10		20		50		100	
Condition of Exp.	Cont.		Cont.		Cont.		Cont.	
	Ave.*	S.D.	Ave.*	S.D.	Ave.*	S.D.	Ave.*	S.D.
Time (min):								
0	100.0		100.0		100.0		100.0	
2.5	92.1	3.9	96.8	2.4	92.5	1.5	97.9	0.4
5	91.1	3.0	92.0	2.7	90.8	0.9	97.1	1.0
7.5	89.1	2.5	88.1	2.3	88.4	0.5	95.9	0.6
10	84.1	3.1	87.8	3.4	86.6	0.7	94.6	0.9
12.5	79.3	3.0	86.2	3.5	85.2	0.7	94.3	0.9
15	75.9	2.6	85.0	3.6	84.8	1.0	94.0	1.0
17.5	70.7	2.6	83.2	3.4	83.7	1.4	93.8	0.9
20	71.0	1.9	81.1	2.8	83.4	0.7	93.3	0.4
22.5	67.5	5.4	81.8	2.9	81.6	1.0	93.0	0.8
25	67.3	2.0	81.0	3.9	81.0	0.8	93.0	0.4
27.5	67.4	4.7	79.0	2.5	80.3	1.5	92.5	0.9
30	67.0	4.6	77.0	3.6	80.4	0.7	91.9	0.9

* Three determinations.

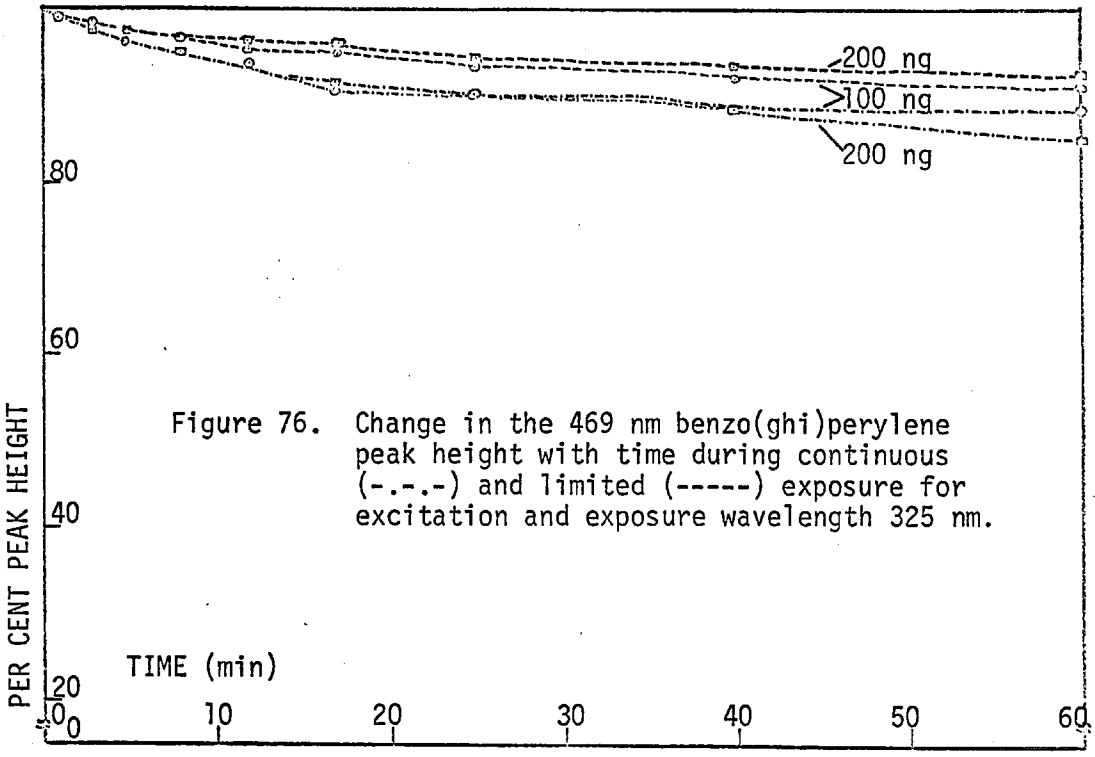


Figure 76. Change in the 469 nm benzo(ghi)perylene peak height with time during continuous (-.-.-) and limited (-----) exposure for excitation and exposure wavelength 325 nm.

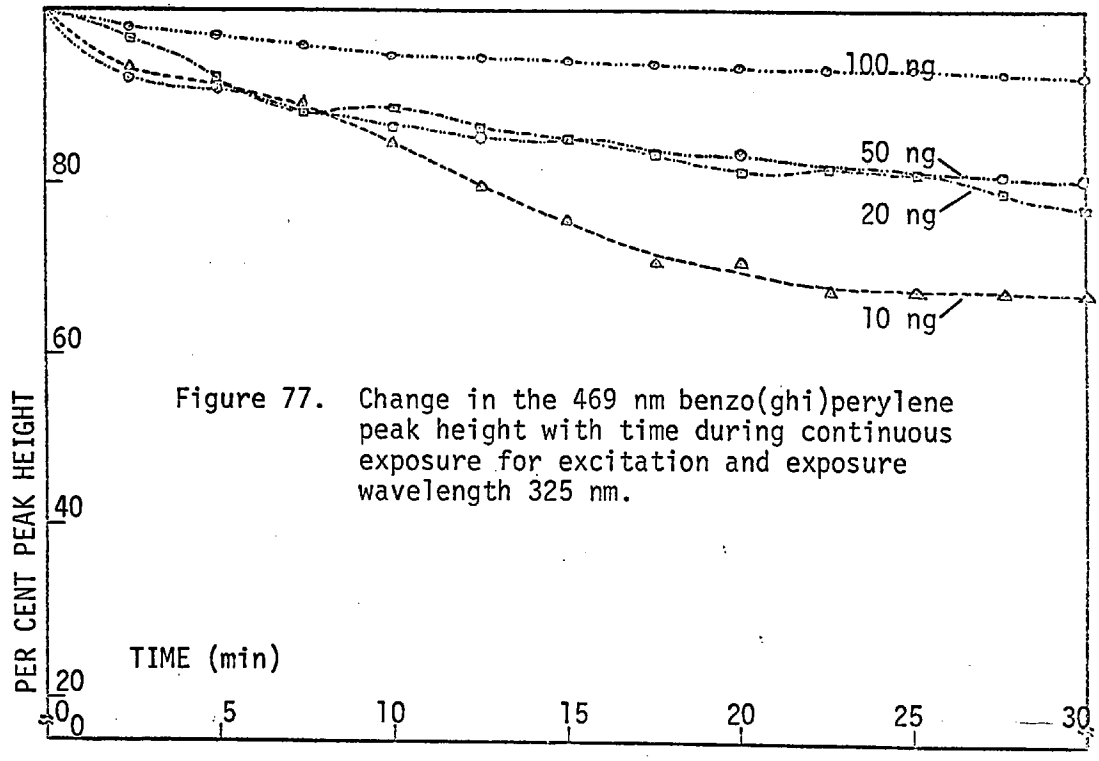


Figure 77. Change in the 469 nm benzo(ghi)perylene peak height with time during continuous exposure for excitation and exposure wavelength 325 nm.

APPENDIX J

EMISSION-PEAK HEIGHTS FOR MIXTURES OF BENZO(a)PYRENE AND BENZO(ghi)PERYLENE ON MYLAR

Table 25: 425 nm and 469 nm peak heights for BzPy-BzPer mixtures on Mylar excited at 325 nm.

Mass Ratio (BzPy/BzPer)	BzPy (ng)	BzPer (ng)	425 nm P.H. (A.U. x MM) Ave.	S.D.	469 nm P.H. (A.U. x MM) Ave.	S.D.	No. Det.
20/1	200	10	0.129	0.007	0.151	0.009	3
8/1	200	25	0.116	0.007	0.150	0.004	3
	100	12.5	0.046	0.002	0.060	0.001	3
4/1	200	50	0.031	0.004	0.087	0.005	3
	100	25	0.008	0.007	0.032	0.005	4
	40	10	0.006	0.003	0.013	0.003	3
2/1	200	100	0.045	0.008	0.233	0.015	3
	100	50	0.015	0.001	0.109	0.007	3
	40	20	0.009	0.003	0.035	0.007	4
1/1	100	100	0.026	0.003	0.337	0.021	4
	50	50	0.012	0.007	0.107	0.018	5
	20	20	0.002	0.001	0.031	0.002	3
0.5/1	50	100	0.037	0.001	0.284	0.005	3
	25	50	*	*	0.162	0.002	3
0.2/1	20	100	*	*	0.407	0.031	5

* Could not be distinguished from background.

APPENDIX K

RELATIVE EMISSION-PEAK HEIGHTS FOR BzPy-BzPer MIXTURES ON MYLAR EXPOSED TO ULTRAVIOLET RADIATION

Table 26. Relative peak heights for BzPy-BzPer mixtures after various times of continuous exposure to 325 nm radiation. (In per cent of peak height at time 0.)

Mass Ratio (BzPy/BzPer)	20/1		8/1		4/1		2/1	
BzPy (ng)	200		200		200		200	
Peak (nm)	425	469	425	469	425	469	425	469
Time (min):								
0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
2.5	74.8	78.6	81.4	87.4	83.7	94.6	85.1	97.9
5	58.7	64.5	66.2	73.2	73.0	91.8	81.2	94.3
7.5	52.6	58.5	60.4	68.8	71.9	89.0	74.8	92.7
10	45.2	48.4	55.3	64.4	67.4	86.0	69.6	91.1
12.5	40.8	46.8	50.9	61.6	62.9	83.5	68.4	88.5
15	37.4	44.3	48.4	58.0	61.2	83.0	64.5	89.1
17.5	31.3	41.5	46.2	55.8	57.8	80.4	61.9	86.5
20	29.5	39.9	43.6	54.4	56.7	80.6	58.0	85.9
22.5	26.9	36.3	40.5	51.0	54.5	77.4	58.6	85.2
25	25.2	35.9	37.3	50.2	52.8	76.5	57.4	84.0
27.5	24.3	33.5	35.4	48.7	51.6	75.7	54.2	81.6
30	20.8	30.6	35.4	48.7	50.5	73.7	55.4	81.1

Mass Ratio (BzPy/BzPer)	1/1		0.5/1		0.2/1	
BzPer (ng)	100		50		20	
Peak (nm)	425	469	425	469	425	469
Time (min):						
0	100.0	100.0	100.0	100.0	100.0	100.0
2.5	77.8	97.1	92.5	99.2	87.5	100.0
5	58.4	95.9	91.3	97.8	82.5	98.3
7.5	55.5	95.4	86.3	97.8	75.0	97.3
10	52.8	93.5	85.0	97.3	77.5	97.0
12.5	47.2	91.8	82.5	97.3	72.5	96.0
15	44.4	91.5	81.3	96.0	75.0	95.2
17.5	47.2	90.6	81.3	96.0	70.0	94.5
20	44.4	90.6	81.3	95.8	70.0	94.1
22.5	41.7	89.1	80.0	95.6	70.0	93.6
25	41.7	87.9	80.0	94.2	65.0	93.2
27.5	41.7	87.0	77.5	93.8	65.0	92.9
30	22.2	83.8	77.5	92.8	65.0	92.7

APPENDIX L

CALCULATION OF AVERAGE SURFACE CONCENTRATION PER ANNULUS AND TOTAL MASS PER ANNULUS

The average surface concentration per annulus and the total mass per annulus were calculated as follows:

Assumptions:

1. The surface-concentration distribution is normal across a diameter of the deposited spot.
2. The entire mass of the compound is contained in a circle, radius three length units (LU), where one LU represents one standard deviation.

1. The area of the annuli, one-fourth LU thick, were calculated (Table 27, columns 2 and 3).
2. From tabled values for the cumulative normal distribution (column 4) the areas between the curve and the annular plane were determined (column 5). (Note: These values are for two times the width of the annulus since a two-sided table was used.)

   (The height of the curve above the plane has surface-concentration units of MU/LU^2 since it is the surface concentration that is assumed to be normally distributed. Therefore, the volume between the annulus and the "bell" formed by rotating the normal curve around its Y-axis has units of mass.)

3. The average relative surface concentration per annulus (column 6) was calculated by dividing the area (column 5) by two times the thickness of the annulus.

4. The relative mass per annulus (column 7) was calculated by multiplying the average relative surface concentration (column 6) by the area of the annulus (column 3).
5. The relative masses were converted to total 100 MU (column 8), and a cumulative mass distribution calculated by summation (column 9).
6. The average surface concentration per annulus (column 10) was calculated for 100 MU by dividing the mass per annulus (column 8) by the area of the annulus (column 3).

Table 27. Calculated values for surface-concentration distribution and mass distribution for 100 MU.

(1) Diameter	(2) Area of Circle	(3) Area of Annulus	(4) Cum. Area Under Normal Distrib. Curve	(5) Area Under Curve In Annulus	(6) Ave. Rel. Surface Conc. Per Annulus	(7) Rel. Mass Per Annulus	(8) Mass Per Annulus for 100 MU	(9) Cum. Mass Per Annulus for 100 MU	(10) Ave. Surface Conc. Per Annulus for 100 MU
0.0	0.0000		0.0000					100.02	
		0.1964		0.1974	0.3948	0.0775	3.11		15.84
0.25	0.1964		0.1974					96.91	
		0.5891		0.1855	0.3710	0.2185	8.78		14.91
0.50	0.7854		0.3829					88.13	
		0.9818		0.1638	0.3276	0.3216	12.92		13.16
0.75	1.767		0.5467					75.21	
		1.374		0.1360	0.2720	0.3739	15.02		10.93
1.00	3.142		0.6827					60.19	
		1.767		0.1060	0.2120	0.3746	15.04		8.51
1.25	4.909		0.7887					45.15	
		2.160		0.0777	0.1554	0.3356	13.48		6.24
1.50	7.069		0.8664					31.67	
		2.553		0.0535	0.1070	0.2731	10.97		4.30
1.75	9.621		0.9199					20.70	
		2.945		0.0346	0.0692	0.2038	8.18		2.78
2.00	12.57		0.9545					12.52	
		3.338		0.0211	0.0422	0.1409	5.66		1.70
2.25	15.90		0.9756					6.86	
		3.731		0.0120	0.0240	0.0895	3.59		0.96
2.50	19.64		0.9876					3.27	
		4.123		0.0064	0.0128	0.0515	2.07		0.50
2.75	23.76		0.9940					1.20	
		4.516		0.0033	0.0066	0.0298	1.20		0.27
3.00	28.27		0.9973			2.4903	100.02		

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