Polycyclic Aromatic Hydrocarbons in Solvents Used in Extraction of Edible Oils

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A method has been developed for the isolation and determination of polycyclic aromatic hydrocarbons in commercial hexanes used in the solvent extraction of edible oils. The hydrocarbons are isolated by partition, column, and thin-layer chromatographic techniques and measured by ultraviolet and spectrophotofluorometric procedures. Average recoveries of benzo[*a*]pyrene, dibenz[*a*,*h*]anthracene, benz[*a*]anthracene, and benzo[*g*,*h*,*i*]perylene added to 500 grams of hexane solvents at levels of 2 p.p.b. ranged from 86 to 95%. Trace quantities of pyrene, fluoranthene, anthracene, phenanthrene, and various substituted phenanthrenes were isolated from 9 of the 15 solvents analyzed in this study. No known carcinogenic hydrocarbons were detected.

Commercial hexanes are the principal solvents used in the extraction of edible oils from cottonseed, soybeans, and peanuts to produce cooking oils, vegetable shortenings, and margarines. The meals obtained in the processing of the oils are also used as foods or feed. Because of the petroleum origin of these solvents, the potential presence of carcinogenic polycyclic aromatic hydrocarbons must be considered. At the present time, the only solvent specifications designed for solvent-edible oil extraction appear to be those adopted by the American Oil Chemists' Society for commercial hexane (A.O.C.S., 1946). The unsaturated and aromatic compound tests described under this specification are inadequate for assuring absence of tetracyclic and higher polycyclic compounds. There is also an ASTM specification (1966) for commercial hexanes (D1836-64) which may be used, since its scope is defined as covering "the range of products commonly referred to as hexanes which find uses..., and as solvents in various kinds of extraction operations." Its general requirements include the statement that the products "shall be free of extraneous or deleterious materials"; however, the tests do not provide assurance for the absence of higher polycyclic aromatic hydrocarbons. For example, the nonvolatile specification does not provide for use of fixative, and higher polycyclic hydrocarbons are known to be lost by volatilization at steambath temperature under such conditions.

Druckrey *et al.* (1959) observed the fluorescence characteristics of commercial hexanes and other organic solvents and suggested that higher aromatic compounds may be present. In further studies, they found that highly fluorescent solvent extracts were obtained when an optically pure solvent was shaken with some types of active carbons. Since these carbons are sometimes used in the further purification of distilled solvents, they were suspected of being a possible source of contamination. The report also indicates that metastasizing tumors were noted in several rats after the injection of a fluorescent residue obtained from the treatment of a solvent with a certain active carbon. No attempts were made to identify the fluorescent components. Druckrey *et al.* state that as a result of their studies, recommendations were made to vegetable oil producers to use only those solvents which did not fluoresce under ultraviolet light.

Lijinsky and Raha (1961) reported the presence of polycyclic aromatic hydrocarbons in various commercial solvents including toluene, benzene, isooctane, and several hexanes. However, the hexanes were technical grade, and Lijinsky (1962) reported no evidence that they were of the quality used in the processing of edible oils.

Ryder and Sullivan (1962) investigated the possible presence of carcinogenic hydrocarbons in a hexane product that meets the A.O.C.S. specification H16-56. Analyses were conducted on the product before and after shipment to the processing plants. No known carcinogens were isolated from the solvent with a method sensitive to 10 p.p.b.

During the past year, methods development work was initiated in this laboratory on refined vegetable oils and on commercial hexanes used in the processing of the oils to permit accumulation of analytical data for evaluation of the existing situation. The method developed for the determination of polycyclic aromatic hydrocarbons in refined vegetable oils (Howard *et al.*, 1966b) is sensitive to 2 p.p.b. or less. Analyses of various samples of refined oils (corn, soybean, cottonseed, olive, and peanut oils) obtained from local retail markets revealed the presence of trace quantities of the carcinogen, benzo[*a*]pyrene, and other polycyclic aromatic hydrocarbons. The purpose of the present investigation was to evaluate the extraction solvents as the source of these contaminants. This report summarizes the results obtained on pure (unused) and recycled hexanes collected from various edible oil processing plants and their solvent suppliers. The pure hexanes were those supplied by the solvent manufacturers while the recycled samples were collected from the recycling tanks at the processing plants. The latter are largely recovered after each extraction operation and are used repeatedly in the processing of the oils.

MATERIALS AND METHODS

The apparatus and reagents used in this study have been described in detail by Howard *et al.* (1966a). The solvents, isooctane, benzene, *n*-hexadecane, and methanol, were purified to meet rigid ultraviolet absorbance specifications to preclude the presence of polycyclic aromatic hydrocarbons. The adsorbent, Florisil (F-100, Fisher

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Scientific Co., Silver Spring, Md., or equivalent, 600- to 100-mesh), was pretreated with methanol, dried in a vacuum oven (24 to 26 inches vacuum) overnight at 50° C., and standardized with benzo[*a*]pyrene.

The ultraviolet spectra were recorded with a Cary 11 spectrophotometer, using 10 ± 0.05 mm. optical pathlength cells (1.5-ml. capacity, Optical Cell Co., Inc., Brentwood, Md., or equivalent). An Aminco-Bowman spectrophotofluorometer, equipped with a 1P28 multiplier phototube and slit arrangement No. 2, was used to record fluorescence spectra.

ANALYTICAL PROCEDURES

Analysis of Pure Hexanes. A 500-gram (approximately 750 ml.) sample of solvent was placed in a 1-liter evaporation flask (Howard *et al.*, 1966a) and evaporated under nitrogen and vacuum to a volume of approximately 5 ml. on the steam bath. The concentrate was transferred to a chromatographic column containing 60 grams of pretreated Florisil and 35 grams of anhydrous sodium sulfate. A 250-ml. evaporation flask was placed under the column. The 1-liter evaporation flask was washed three times with 10-ml. portions of isooctane which were passed individually through the column. A 175-ml. portion of benzene was then passed through the column. The eluate was evaporated on the steam bath under nitrogen and vacuum to 5 to 10 ml. The solution was transferred quantitatively to a 50-ml. glass-stoppered Erlenmeyer flask and concentrated carefully on the steam bath under nitrogen to a volume of 0.2 to 0.3 ml. (Caution: The solution should not be evaporated to dryness, since prolonged heating of dry polycyclic hydrocarbons will cause losses.) The solution was reserved for thin-layer chromatography on cellulose and cellulose acetate, respectively.

Analysis of Recycled Hexanes. A 500-gram sample of solvent was placed in a 1-liter evaporation flask, and 2 ml. of *n*-hexadecane was added. The solvent was evaporated to 2 ml. of residual hexadecane on the steam bath under nitrogen and vacuum as described above. A 10-ml. portion of isooctane was added, and the solvent was evaporated. This operation was repeated once. The 2 ml. of hexadecane was transferred quantitatively with 198 ml. of isooctane into a 500-ml. separatory funnel. The isooctane solution was washed three times with 200-ml. portions of 85% phosphoric acid; each wash was shaken for 1 minute, and after each wash, the layers were allowed to separate for 5 to 10 minutes. After drawing off the acid in the final wash, the funnel was swirled, and the residual acid was drawn off and discarded. The isooctane solution was extracted three times with 50-ml. portions of dimethyl sulfoxide (DMSO), and the individual extracts were washed in tandem with 25 ml. of isooctane pre-equilibrated with DMSO (Howard *et al.*, 1966a). The DMSO extracts were collected in a 1-liter separatory funnel containing 300 ml. of distilled water and 50 ml. of isooctane. After cooling, the contents of the funnel were shaken for 2 minutes, and the layers were allowed to separate. The lower aqueous layer was transferred to a second 1-liter separatory funnel, and the extraction was repeated with 50 ml. of isooctane. The lower aqueous layer was drawn off and discarded. Each of the isooctane extracts was washed twice with 75 ml. of distilled water, and the wash was discarded. The isooctane extracts were then passed individually through the Florisil column described above and discarded. A 250-ml. evaporation flask was placed under the column. The second and first separatory funnels were washed in that order successively with a 50-ml. portion of benzene which was passed through the column. An additional 125 ml. of benzene was passed through the column into the evaporation flask. The collected eluate was evaporated on the steam bath to 5 to 10 ml., transferred quantitatively to the 50-ml. flask, and evaporated to 0.2 to 0.3 ml. as described above. The concentrate was reserved for thin-layer chromatography on cellulose and cellulose acetate, respectively.

THIN-LAYER CHROMATOGRAPHY

The thin-layer chromatographic systems were described in detail in previous reports (Howard *et al.*, 1966b; White and Howard, 1967).

System 1. The entire concentrate with washings was streaked with a 50-μl. syringe on cellulose layers 500 microns thick. The plates were developed in a pre-equilibrated chamber for 1.25 hours (25° C.), using 20% *N*,*N*-dimethylformamide in ethyl ether as the stationary phase and isooctane as the mobile solvent. The fluorescent bands were outlined in a Chromato-Vue Cabinet and collected in a beaker; the polycyclic hydrocarbons were eluted 4 times with 5- to 10-ml. portions of hot methanol. The individual extracts of each band were filtered successively through a pressure filter under nitrogen and collected together in a 50-ml. glass-stoppered Erlenmeyer flask. The extract from each band was concentrated on the steam bath under nitrogen to a volume of 0.2 to 0.3 ml. and reserved for chromatography on cellulose acetate.

System 2. Each concentrated extract with washings was spotted on cellulose acetate layers 1000 microns thick. The plates were developed in a pre-equilibrated chamber for 1.5 hours (25° C.), using ethanol–toluene–water (17:4:4, v./v./v.) as the developer. The fluorescent spots were collected and eluted with methanol as described above. *n*-Hexadecane (1.0 ml.) was added to each extract, and the methanol was evaporated on the steam bath under nitrogen. Residual methanol was removed by two successive additions of 5 ml. of isooctane and re-evaporation. Each 1.0-ml. hexadecane residue was transferred to a 1-cm. path length cell (1.5-ml. capacity), and the ultraviolet spectrum was recorded against isooctane in the reference cell. The observed maxima were compared with those in the spectra of known polycyclic aromatic hydrocarbons obtained under the same instrumental conditions. Estimation of the quantity of the identified hydrocarbon was made by the base line technique in conjunction with spectra of these hydrocarbons. The identification was confirmed by spectrophotofluorometry.

RECOVERY STUDIES

Solutions of polycyclic hydrocarbons, benz[*a*]anthracene, benzo[*a*]pyrene, dibenz[*a*,*h*]anthracene, and benzo[*g*,*h*,*i*]perylene, were prepared at concentrations of 1 to 2 μg. per ml. in isooctane. An appropriate aliquot of each solution was placed in the 1-liter evaporation flask containing the 2 ml. of hexadecane and 500 grams of hexane

solvent. In most instances, three of the above hydrocarbons were added, alternating the dibenz[*a*,*h*]anthracene and benzo[*g*,*h*,*i*]perylene in each recovery study. The analysis was then carried out as previously described. Prior to conducting the recovery studies, the solvents were analyzed for the polycyclic hydrocarbons to establish that they were free of the compounds used in the investigation.

RESULTS AND DISCUSSION

The recoveries of representative polycyclic hydrocarbons (including 4-, 5-, and 6-ring types) added at a level of 2 p.p.b. to 500 grams of pure and recycled hexane solvents are summarized in Table I. Average recoveries ranged from 86 to 95%.

Ultraviolet and fluorescence spectra obtained for benzo[*a*]pyrene added to and recovered from a hexane solvent at the 2-p.p.b. level are presented in Figures 1 and 2. Spectra of equal definition were also obtained for the other three compounds used in the investigation.

The procedure described above has been applied to 15 hexane samples obtained from 11 different plants involved in the processing of edible oils and related products in the United States. Six of the solvents were pure hexanes as obtained from the manufacturer while the remaining nine solvents were collected from the recycling tanks at the processing plants. As would be expected, the latter solvents contained considerably more background material than the pure hexanes, and additional purification steps were necessary to obtain satisfactory ultraviolet and fluorescence spectra.

The results of the analysis of the pure and recycled hexanes are summarized in Table II. The hydrocarbons were identified by comparing their R_f values with those of known compounds and by their ultraviolet and fluorescence spectra. No known carcinogenic polycyclic hydrocarbons were found in any of the samples examined. Trace quantities of pyrene, fluoranthene, anthracene, phenanthrene, and various substituted phenanthrenes were isolated from nine of the solvents analyzed in this study. Levels of the isolated hydrocarbons ranged from 0.6 p.p.b. for pyrene to 35 p.p.b. for phenanthrene. The substituted phenanthrenes were only tentatively identified because reference polycyclic hydrocarbons were not available; estimations were based on phenanthrene as a reference.

The ultraviolet and fluorescence spectra for anthracene and phenanthrene isolated from 500 grams of pure hexane solvent are shown in Figures 3 to 6. Repeated chromatography on the cellulose adsorbent was necessary to obtain separation of these hydrocarbons. They may also be readily identified in the presence of each other by selective choice of fluorescence excitation and emission wavelengths (Thommes and Leininger, 1961) (Figure 7).

Estimations of the admixed individual compounds were made from the ultraviolet spectrum using the base line technique and the absorbance maxima at 374 mμ for anthracene and 293 mμ for phenanthrene. The foregoing also holds true for pyrene and fluoranthrene which as previously discussed (Howard *et al.*, 1966b) can be determined either by the base line technique or by the variable reference procedure.

Table I. Average Recoveries of Polycyclic Hydrocarbons Added to 500 Grams of Solvent at a Level of 2 P.P.B.

Polycyclic Hydrocarbon	No. of Runs	Recovery, % Range	Recovery, % Average
Benzo[*a*]pyrene	5	80–100	89
Benz[*a*]anthracene	4	80–90	86
Dibenz[*a*,*h*]anthracene	4	80–100	95
Benzo[*g*,*h*,*i*]perylene	3	87–100	95

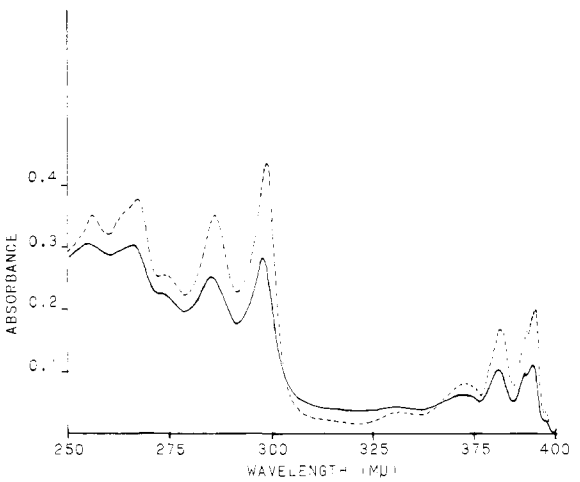

Figure 1. Ultraviolet absorption spectrum of benzo[*a*]pyrene recovered from a 500-gram sample of hexane solvent at the 2-p.p.b. level

——— Recovery
------- Reference (reference standard, 1.7 mg./liter)

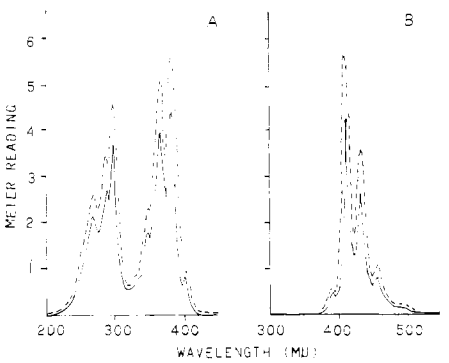

Figure 2. Fluorescence spectra of benzo[*a*]pyrene recovered from hexane

A. Excitation spectra at emission 410 mμ, 0.001 MM
B. Emission spectra at excitation 390 mμ
——— Recovery
------- Reference (reference standard, 1.0 mg./liter)

A number of unidentifiable fluorescent bands were noted in the thin-layer chromatograms of some of the solvent extracts. However, ultraviolet spectra analyses of these bands did not reveal spectra characteristic of condensed-ring polycyclic aromatic hydrocarbons. No maxima were obtained for the majority of these bands; therefore the

Table II. Polycyclic Aromatic Hydrocarbons Found in Hexane Solvents Used in the Extraction of Vegetable Oils

Solvent	Vegetable Oil Extracted	Pyrene, P.P.B.	Fluoranthene, P.P.B.	Anthracene, P.P.B.	Phenanthrene, P.P.B.	Substituted Phenanthrene, P.P.B.
		PURE HEXANES				
Hexane	Soybean	...	...	...	4.6	...
Hexane	Cottonseed	...	...	...	...	...
Hexane	Cottonseed	0.8	0.8	4.2	15.0	3.0
Skellysolve-B	Cottonseed	...	...	...	...	...
Hexane	Soybean	...	...	12.0	35.0	7.6
Hexane	Soybean	...	...	...	...	...
		RECYCLED HEXANES				
Hexane	Soybean	...	...	...	...	...
Hexane	Soybean	...	...	...	...	0.6
Hexane	Soybean	...	...	...	...	...
Hexane	Cottonseed	2.6	2.3	2.4	14.4	...
Hexane	Soybean	0.7	1.5	...	6.0	...
Hexane	Soybean	...	...	...	4.0	...
Skellysolve-B	Cottonseed	...	...	...	2.0	0.8
Skellysolve-B	Cottonseed	...	...	...	...	2.0
Skellysolve-B	Peanut	...	...	...	...	...

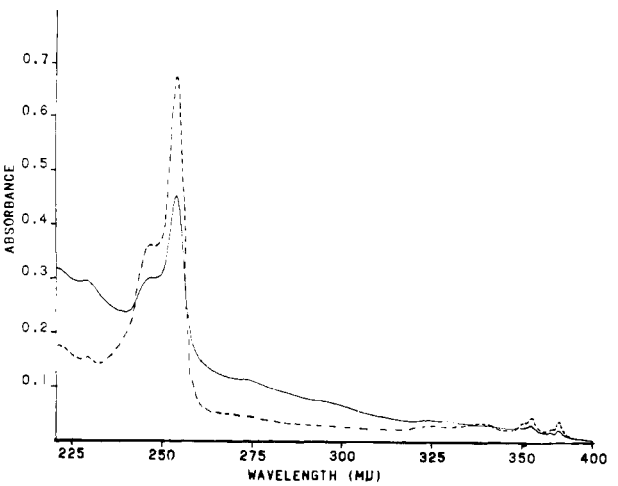

Figure 3. Ultraviolet absorption spectrum of anthracene isolated from 500 grams of a hexane solvent

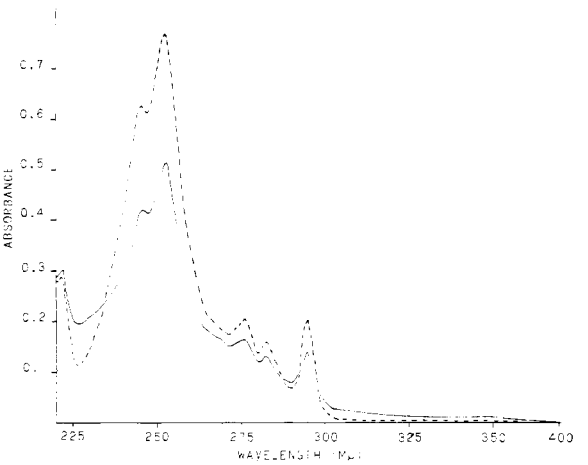

Figure 5. Ultraviolet absorption spectrum of phenanthrene isolated from 500 grams of a hexane solvent

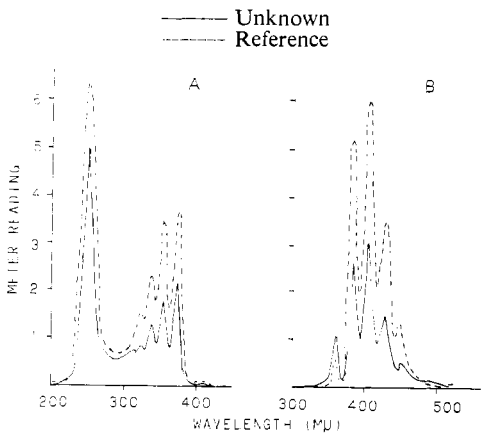

Figure 4. Fluorescence spectra of anthracene isolated from 500 grams of a hexane solvent

A. Excitation spectra at emission 385 mμ, 0.001 MM
B. Emission spectra at excitation 360 mμ
——— Unknown
------- Reference (reference standard, 1.0 mg./liter)

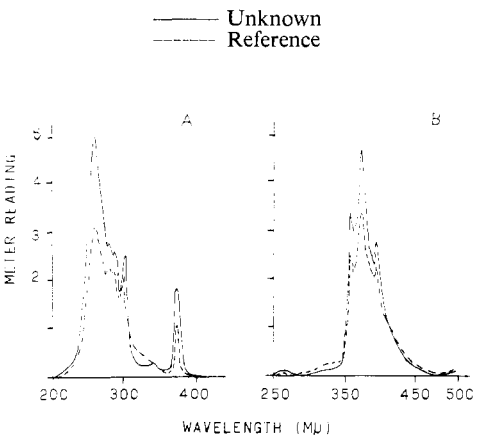

Figure 6. Fluorescence spectra of phenanthrene isolated from 500 grams of a hexane solvent

A. Excitation spectra at emission 370 mμ, 0.003 MM
B. Emission spectra at excitation 255 mμ
——— Unknown
------- Reference (reference standard, 1.0 mg./liter)

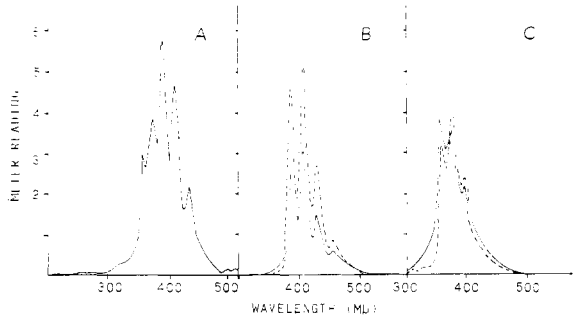

Figure 7. Fluorescence emission spectra

A. Admixture of anthracene and phenanthrene isolated from hexane solvent. Emission at excitation 255 mμ, 0.003 MM
B. Anthracene from anthracene–phenanthrene admixture. Emission at excitation 360 mμ, 0.03 MM
——— Unknown
- - - - - - Reference (reference standard, 1.0 mg./liter)
C. Phenanthrene from anthracene–phenanthrene admixture. Emission at excitation 295 mμ, 0.001 MM
——— Unknown
- - - - - - Reference (reference standard, 1.0 mg./liter)

compounds probably were present in quantities below the sensitivity of the method. Other components isolated from several of the solvents exhibited broad maxima at 235 and 300 mμ (similar to those obtained for antioxidants or other phenolic types). No attempt was made to identify these compounds since their spectra were not consistent with the higher condensed-ring aromatic types and accordingly not relevant to the objectives of this study.

Trace quantities of polycyclic aromatic hydrocarbons were isolated from hexane solvents used in the processing of edible oils; however, no known carcinogenic types were found. The results of this study indicate that hexane extraction solvents are not the source of the carcinogenic trace contaminants found in refined vegetable oils.

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