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ABSTRACT

Benzo(a)pyrene Ingested from Composite Meals: Analysis and Method Development Related to Total Human Exposure

by
Che-Han Hsu
Master of Science in Environmental Science
(Toxics option)
January, 1990

Thesis Directed by Dr. Arthur Greenberg
Professor of Chemistry, NJIT

Polycyclic aromatic hydrocarbons including benzo[a]pyrene (BaP) have been known for many years to be environmental carcinogen. Moreover, B(a)P has often been used as an indicator for the carcinogenic potency of environmental matter. One of its exposure pathways, ingestion, may possibly be more important than other routes, conventionally studied such as inhalation. The purposes of this thesis are 1) to analyze the B(a)P concentrations in weekly composite and some daily meals obtained in the Total Human Exposure to Environmental Substances (THEES) study, 2) to develop techniques which can measure the levels of B(a)P in foods accurately and efficiently, 3) to compare exposure to B(a)P by inhalation vs ingestion. The analytical method developed is a hybrid of the previous recommended analytical procedures which involve

food digestion with saponification, extraction by liquid-liquid partition steps, column chromatography on Florisil, solvent evaporation and concentration. Final analysis of B(a)P employs TLC separation and fluorescence detection techniques.

For the subjects of the THEES Study, the average daily exposure to B(a)P via food is 0.146 ug and it almost 5 times larger than inhalation exposure. Therefore, B(a)P content of foods should be studied and is a significant source of exposure.

BENZO(a)PYRENE INGESTED FROM COMPOSITE MEALS: ANALYSIS AND METHOD DEVELOPMENT RELATED TO TOTAL HUMAN EXPOSURE

by
Che-Han Hsu

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January, 1990

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APPROVAL PAGE

Benzo(a)pyrene Ingested from Composite Meals: Analysis and Method Development Related to Total Human Exposure

by
Che-Han Hsu

Date

Dr. Arthur Greenberg, Thesis Advisor
Professor of Chemistry, NJIT

Date

Dr. Richard Trattner, Committee Member
Professor of Chemistry and Environmental Science, Associate Chairperson and Graduate Advisor for Environmental Science, NJIT

Date

Dr. Barbara B. Kebbekus, Committee Member
Professor of Chemistry, Associate Chairperson of the Department Chemical Engineering, Chemistry, and Environment Science and Co-Director of the Air Pollution Research Laboratory, NJIT

BIOGRAPHICAL SKETCH

Author: Che-Han Hsu

Degree: Master of Science in Environmental Science
(Toxics Option)

Date: January, 1990

Date of Birth:

Place of Birth:

Undergraduate and Graduate Education:

- Master of Science in Environmental Science (Toxics Option), New Jersey Institute of Technology, Newark, NJ, January 1990
- Diploma of Chemical Engineering, Ming-Chi Institute of Technology, Taipei, Taiwan, R.O.C., June 1986

Major: Environmental Science

Publications:

Arthur Greenberg, Suyi Luo, Che-Han Hsu, and Peter Creighton "Benzo[a]pyrene in Composite Meals: Analytical Modifications and Data for a Human Exposure Study", Polycyclic Aromatic Compounds, submitted.

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CHAPTER I

INTRODUCTION

Pollutant contamination in both community and occupational environments is a serious public health concern in the United States and throughout the world. It has long been of concern to environmental scientists that certain occupations carry a high risk for cancer, and there have been correlations between cancer incidence and exposure to specific chemical substances in the work environment. Polycyclic aromatic hydrocarbons (PAH) are a well-known class of environmental carcinogens, and one member of this class, benzo(a)pyrene [B(a)P] has often been used as an indicator for their presence and even as a numerical parameter for the carcinogenic potency of environmental matter.

1.1 Historical Review

Studies of occupational hazards related to PAH in particular have a long history. Ever since the British surgeon Sir Percival Pott, in 1775, reported[1] that chimney sweeps in Britain often developed cancer of the scrotum, there has been an awareness of the harmful effects of soot, tar, and pitch. The association of these compounds with the development of cancer has, thus, been recognized for more than 200 years. In the early part of the twentieth century researchers discovered that PAH were major carcinogens in

chimney soot and tar. By 1976, more than 30 parent PAH compounds and several hundred alkyl derivatives of PAH were reported to have some carcinogenic effects.[2,3] This established the PAH as the largest single class of chemical carcinogens known today. Table 1 is a listing of the carcinogenic activities of selected PAH.[4]

1.2 PAH Formation Mechanism

Analytical chemical studies show that PAH appear in a large number of industrial processes, and can be formed by thermal decomposition of any organic materials containing carbon and hydrogen. The formation is based on two major mechanisms:

(1) pyrolysis or incomplete combustion

(2) carbonization processes.

In addition, some publications suggest a biological route of formation of PAH. Once formed, PAH may undergo further pyrolytic reactions to large PAH by intermolecular condensation and cyclization. The amount of PAH formed depends on both the type of the organic material and temperature. The relative distribution of PAH components depends mainly on the temperature.[5]

Table 1 Carcinogenic Activities of Some Selected PAH[4]

Compound	Carcinogenic[a.] potential	Bioactivity[b.]	Ref.
2-Methylnaphthalene	0	TP	7
Fluoranthene	0	CC	6
2-Methylfluoranthene	+	C,TI	6
3-Methylfluoranthene	?	TI	6
Pyrene	0	CC	6
Benz[a]anthracene	+	TI	6
Chrysene	+	TI	6
Benzo[c]phenanthrene	+++	C	2
3-Methylchrysene	+	TI	8
5-Methylchrysene	+++	C,TI	8
7,12-Dimethyl benz[a]anthracene	++++	C,TI	9
Benzo[b]fluoranthene	++	C,TI	6
Benzo[j]fluoranthene	++	C,TI	6
Benzo[a]pyrene	+++	C,TI	6
Dibenz[a,h]anthrancene	+++	C,TI	6
Indeno[1,2,3-cd]pyrene	+	TI	6
Benzo[ghi]perylene	0	CC	6
Picene	+	TI	10

[a] Key: ? = uncertain; 0 = inactive; + to ++++ = active.

[b] CC = cocarcinogenic with BaP; TP = tumor promoter; TI = tumor initiator; C = complete carcinogen.

(Table 1 Adapted From Ref. 4, Bjorseth, A.)

1.3 Study of PAH in Food

Dating from the 1960s, with the development of reliable analytical procedures, a large volume of data on the presence of PAH in foodstuffs has been reported on a worldwide basis. The early work on the PAH content of foods has been reviewed by Haenni (1968),[11] and by Howard and Fazio (1969).[12] Many of the initial investigations were concerned with BaP alone, or the "benzopyrene fraction"(Benzo(a)pyrene, Benzo(e)pyrene, Benzo(k)fluoranthene and perylene). Staring in the late 1960s, recommended methods have been recognized by the Association of Official Analytical Chemists (AOAC) and International Union of Pure and Applied Chemistry (IUPAC) for the determination procedure of PAH in foodstuffs.[12,13] The Food Section of Applied Chemistry Division of IUPAC accepted the procedure for a recommended method in 1972.[14,15] This procedure employs several complicated steps based upon saponification with ethanolic KOH, solvent partitioning, column chromatography on Florisil, and thin-layer chromatography with UV and fluorescence. Other procedures has been recently recommended by IUPAC, although they are oriented to smoked food and do not involve saponification, since most of the PAH are found on the surface.[16,17]

It is obvious that possible sources of PAH contamination of foodstuffs in the modern human environment are numerous, varied and extremely widespread. There are several pathways which contribute B(a)P to food including atmospheric

deposition onto crops, absorption through roots, endogenous synthesis, smoking of meat and cheese, pyrolysis during cooking and processing, smoking and contamination during cooking (especially charbroiling), and food processing.

PAH have been reported in many kinds of food including smoked fish and meats, grilled and roasted foods, root and leaf vegetables, vegetable oils, grains, plants, fruits, seafoods and whiskies. In Table 2, there is a list of reported B(a)P levels in a variety of foods.

As indicated above, the occurrence of PAH in food should be viewed as a part of the much larger problem of the presence of these compounds in the environment. It is also apparent that the findings of PAH in air, water, and soil are being given much more attention with respect to their contamination of the food supply. The purpose of this thesis is to update and to develop techniques and measure B(a)P levels in food, and to assess this route of exposure compared to other exposure routes.

From the present study, it is shown that, in this small sampling, diet is one of the major routes of exposure to B(a)P and, on average, exceeds inhalation exposure by a factor of 4 to 5. Moreover, the estimated dose from some previous reports shown that dietary exposure may exceeds inhalation by a factor of 10 (assumes the absorption of dietary B(a)P and inhaled B(a)P are 100% and the metabolism of B(a)P are 30%)[18].

Table 2 Levels of Benzo(a)pyrene Found in Foodstuffs[4]

Food product	Levels (ug/kg)	Reference
Sausage and fish	1.7-10.5	Dobes et al. [19]
Fish and mutton	0.3-2.1	Bailey and Dungal [20]
Fish	1.7-53	Voitelovich et al. [21]
Fish	7	Gorelova [22]
Sausage and fish	0.1-1.4	Gorelova and Dikun [23]
Fish	4.2-60	Petrun and Rubenchik [24]
Fish (salmon)	2.6-3.0	Manelli [25]
Wurstel	0.4-1.0	Manelli [25]
Bacon	1.6-4.0	Manelli [25]
Salami	2.0-2.8	Manelli [25]
Sardines	1.8	Manelli [25]
Provola (cheese)	4.1-6.2	Manelli [25]
Haddock, salmon	0.3, 1.0	Lijinsky and Shubik [26]
Herring, sturgeon	1.0, 0.8	Howard et al. [27,28]
Ham	3.2	Howard et al. [27,28]
Ham, belly fat	1.0-14	Toth [29]
Fish	0.05-5.7	Dikun et al. [30]
Katsuobushi (bonito), Sabushi (mackerel), Urumegushi (sardines)	2.0-37	Masuda and Kuratsune [31]
Katsuobushi (bonito)	8.7-27.2	Shiratori [32]
Nori (seaweed food)	7.3-31.3	Shiratori [32]
Meats	9.0-55	Toth [33]

(continued)

Fish	11.5	Wierzchowski and Gajelwska [34]
Fish, mutton (commercially smoked)	1.0	Thorsteinsson [35]
Mutton (home smoked)	23.0	Thorsteinsson [35]
Hot sausage	0.8	Malanoski et al. [36]
Ham	1.0	Malanoski et al. [36]
Turkey fat	2.1	Malanoski et al. [36]
Whitefish	4.3	Malanoski et al. [36]
Whiting	6.9	Malanoski et al. [36]
Chubs	1.3	Malanoski et al. [36]
Cod	4.5	Malanoski et al. [36]
Meats (bologna, frankfurters, salami, pepperoni, sausages, ham, bacon, beef, pork)	0.2-2.0	Panalaks [37]
Fish (herring, canned, oysters)	0.5-15	Panalaks [37]
Cheese, Gouda	0.5	Panalaks [37]
Bacon	1.2-3.6	Rhee and Bratzler [38]
Cured meats, sausage, fish, cheese	< 5	Lucisano et al. [39]
Sausages (mutton) bologna	0-0.15	Fretheim [40]
Fish, oysters	0-9	Swallow [41]
Meat	< 0.5	Swallow [41]
Beef	18.8-24.1	Doremire et al. [42]
Pork	25.8-31.6	Doremire et al. [42]
Lamb	8.8-12.3	Doremire et al. [42]

(Table 2 Adapted From Ref. 4, Bjoreseth,A.)

Table 3 has estimates of daily human exposure to total PAH as well as B(a)P from air, water and food based on much earlier data from New Zealand. The present work will employ multimedia exposure data obtained by oue research group to understand the exposure of residents of the United States exposure during the late 1980s.

Table 3 Estimated Daily[43]

Human Exposure to PAH from Air, Water and Food

Source	Benzo(a)pyrene (ug)	Total PAH (ug)
Air	0.0095-0.0435	0.207
Water	0.0011	0.027
Food	0.16-1.6	1.6-16.0

(Table 3 Adapted From Ref. 43, Chen, S.)

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CHAPTER II

PRESTUDY DESIGN AND FIELD STUDY

The present study, titled Total Human Exposure to Environmental Substances (THEES), involves the measurement of human exposure to B(a)P from ingestion of food and water, inhalation of indoor and outdoor air in Phillipsburg, New Jersey. This city was identified as a location with unusually high atmospheric B(a)P levels possibly caused by space heating, motor vehicles and a metal foundry.[1]

The NJDEP funded a joint project between New Jersey Institute of Technology and the University of Medicine and Dentistry of New Jersey (UMDNJ) to investigate total human exposure to B(a)P in Phillipsburg in all environmental media. The exposure assessment guidelines were developed by the United States Environmental Protection Agency (USEPA) thus regulating some of the important types of pollution source, measurement and pathway information to perform a multi-media analysis.[2-4]

UMDNJ was responsible for all samples collection and project design for the three phases of the project. The THEES activities list is presented in Table 4.

2.1 Study Protocol

The study participants were selected from various locations within Phillipsburg through their responses to a mail solicitation. The location of respondent homes are:

homes 1 to 3 located in the valley near the metal foundry; homes 4 to 6 are located near the foundry but up a slight grade across the railroad tracks; homes 7 and 8 are located near outdoor sampling site #2; homes 9 and 10 are located across Route 22, approximately 2 kilometers to the north of the foundry.

Table 4 THEES Activies

THEES	Phase I	Phase II	Phase III
Period	Jan.29 -Feb.12,87	Jan.8 -Jan.22, 88	Sep.16 -Sep.30, 88
Soil sampled	Yes	No	No
Drinking Water sampled	Yes	No	No
Number of outdoor sites sampled	3	3	2
Number of home sampled for air	11	11	11
Personal air sampled	No	Yes	Yes
Food samples	Each meal in separate weekly box	One box per day for all meals	One box per day for all meals
Percent of adult meal requested as sample	1/3	1/4	1/4
Blood sampled	Once at end of study	Once at end of study	Once at end of study
Urine sampled	No	Yes	Yes
NO_2 Measured	Yes	No	No
Ventilation measured	No	Yes	Yes
Cooking stove monitored	No	No	Yes

The 10 THEES volunteers were not meant to be a statistically selected subgroup or representative of the people living in this area. The selection of a limited number of participants was based upon the need to examine the daily contributions of indoor and local outdoor air pollution and other media contributing to human exposure in detail over the course of a two-week period.

An initial questionaire solicited each household to indicate home contruction, heating and cooking devices in the home, ventilation, time spent in the home, and other various life style patterns. Each home had some features which were common to all, and others which were unique to individual homes. Some characteristics of the households are listed in Table 5.

Table 5 Household Characteristics

(1) all homes are over fifty years old.

(2) nine homes are of wood construction.

(3) no home has an attached garage.

(4) no home has a fireplace.

(5) all homes are less than 5 meters from street.

(6) three of the homes had at least one smoker in residence.

(7) six of the homes had children living at home.

(8) five of the homes used gas cooking ranges and ovens.

(9) five of the homes used electric ranges and ovens.

(10) five of the homes had microwave ovens.

(11) three of the homes had toaster ovens.

2.2 Sampling Methodology

The food samples were collected from the in-home meals eaten by a single individual from each household. Each collected meal is about one fourth of an adult portion in Phase II and III. All food samples were stored in labelled aluminum containers and kept frozen at $-20^{o}C$ except when being processed or analyzed. The samples from each household were separated by one box per day for all meals. The individual sets of food samples were prepared for analysis following processing by Mr. Peter Creighton of UMDNJ Robert Wood JOhnson Medical School. The procedure involved:

* Weighing
* Disintegrating in commerical design food processor
* Returning to the original container
* Putting one half of each disintegrated sample from one participant for one week into a commercial design blender and thoroughly homogenizing. A measured amount of distilled water was added to the blender, if necessary to help pulverization and homogenization.
* The weekly homogenized composite was put into a folded aluminum container, labelled and frozen.
* The weekly composite samples were transported to NJIT for analyses and stored in a freezer until analysis. Manifest records were kept to record transfers to and reports by NJIT.

Food samples were analyzed for B(a)P using a technique modified by Ms. Suyi Luo who was responsible for the THEES Phase I analyses. Our subsequent modifications focused on treatment of Florisil employed in analyses. The food sampling centered on the eating habits of an identified individual in each household, and included breakfast, lunch, dinner, and snacks. The only restriction to the protocol was that we could only collect food eaten at home and a complete accounting of meals taken in and out of the home was documented by a questionnaire to allow for adjustment of in-home food measurements. Table 6 lists some representative daily meal diaries in a single household.

Although food samples were obtained from family meals each day, due to practical problems in the collection of food, some participants could only provide as little as three meals (of a possible 31) a week. The range of B(a)P per kilogram of weight of food was between 0.012 to 1.023 ug/kg (ppb) depending on the nature of the particular foods and how they were cooked. The results from THEES II and III are listed in the Appendix, and are discussed further in Chapter IV.

Table 6 Daily Meals in a Single Family

Sample 111-02

Breakfast	cold cereal, raisins, banana
Lunch	fried eggs, margarine, catsup, pumpernickel bread, milk, tea, pear, ginger snaps.
Dinner	fried beef patty,potato,carrot, spinach, margarine, lettuce, celery, tomato, mayonnaise, orange, cookie, coffee, milk.
Snack	milk, toast, margarine, Taffy candy.

Sample 111-03

Breakfast	dry cereal, raisins, banana, cinnamon, milk, coffee.
Lunch	pizza (cheese), pear,milk, brownie.
Dinner	lasagna, salad: Spinach, lettuce, onion, cauliflower, mayonnaise, orange, brownie, milk coffee.
Snack	toast, margarine, milk.

Sample 111-08

Breakfast	rhubarb,raisins, cottage cheese,banana, bran, flakes, cinnamon, toast, margarine, coffee, milk.
Lunch	grilled cheese sandwich, green pepper, rump, nectarine, roast potato.
Dinner	spaghetti, meat sauce, salad: spinach, tomato lettuce, onion, toasted soy bean, olive oil, Italian dressing, orange, gingersnaps, milk, coffee
Snack	toast, margarine, milk.

Sample 111-12

Breakfast	whole wheat toast,margarine,jam,milk, coffee.
Lunch	pita bread, baked beans, margarine, apple, gingersnaps, milk, tea.
Dinner	beef patty with smoky cheese, potato, carrot, margarine, salad: lettuce, celery, onion, orange, milk, coffee.
Snack	margarine, milk.

Sample 111-13

Breakfast	dry cereal, bran flakes, raisins, cinnamon, banana.
Lunch	macaroni and cheese, green pepper, apple, tea brownie, milk.
Dinner	turkey, mushroom,onion,broccoli, corn, salad: spinach lettuce, olive oil, orange, cookie bread, milk, coffee.
Snack	toast, margarine, milk.

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CHAPTER III

METHODS OF ANALYSIS

3.1 Historical Background

During the 1950s, concern over human exposures to carcinogenic PAH in food, in industrial and ambient atmospheres, in cigarette smoke, and in other media provided motivation for the development of analytical methods for these compounds.[1]

Column chromatographic separation coupled with absorption and fluorescence spectrophotometry were widely used for PAH analysis during that decade. At the same time, the technique of TLC was rapidly developing due to the growing interest in environmental analysis.

After 1958, when TLC equipment and materials became popular, the theory and applications of TLC expanded enormously. These parallel developments merged in the decade of the 1960s. During the 1960s, most of the emphasis was on the development of analytical methods for PAH in airborne pollutants and in food products.

For the analysis of PAH in food products, one of the first TLC methods reported was that of Genest and Smith[2] which employed silica gel G/2,2,4-trimethylpentane(isooctane) benzene (97:3) and multiple development to reduce background interferences in the determination of B(a)P. Howard and co-workers[3] developed a method for the determination of PAH in foods and modified[4] the method of Genest and Smith for B(a)P

to obtain a lower limit of detection of 0.5 ppb. The method, developed by Howard and co-worker for B(a)P in smoked foods, was adopted as an official procedure by the AOAC in 1972[5] and accepted by the IUPAC as a recommended method in 1975.[6] It involved food sample saponification by ethanolic potassium hydroxide and extraction by aliphatic solvent, followed by column chromatography on Florisil. They also employed acetylated cellulose/ethanol-toluene-water (17:4:4=v/v/v) to isolate and thin-layer chromatography with UV and fluorescence spectrometry to quantitate B(a)P from smoked food. The average recovery of B(a)P were 70-90 % at the 10 ppb level for the overall analysis.[7,8]

In studies by other researchers, determinations of B(a)P in smoked foods with similar procedures to the official one were adopted. Xia and co-worker[9] use the same saponification step, subsequently, extracted the solution with petroleum ether and washed the extract with H_2O. They concentrated the extract and subjected it to column chromatography on silica gel and neutral alumina. The benzene eluate was collected, concentrated and applied to paper chromatography developed by an ethanol/dicloromethane mixture. B(a)P was measured and over 90% recoveries were obtained by this method.

Saito and co-worker[10] employed a simpler procedure to determine B(a)P in food without saponification. B(a)P was extracted with a hexane/ether mixture (4:1) (presumably because it is found on the food surface), then purified through

an active alumina column and detected fluorimetrically. Recoveries of B(a)P from 14 kinds of food spiked at levels of 2 and 20 ppb were 50.0-80.6% and 79.5-93.8% respectively.

3.2 Principle

To measure the concentration of B(a)P in foods, the analytical methods used by our study involves digestion (saponification), extraction, column chromatography on Florisil and evaporation. After this procedure, the concentrate is dissolved in cyclohexane and aliquots separated by TLC on acetylated cellulose. B(a)P is quantitated by fluorescence scanning of the TLC plate.

3.3 Reagents and Chemicals

Methanol (HPLC grade)
Ethanol (anhydrous spectrophotometric grade)
2,2,4-Trimethylpentane (isooctane), HPLC, GC, Spectrophotometry)
Benzene (organic residue)
Cyclohexane (HPLC grade)
Dichloromethane (organic residue)
Sodium sulfate (ACS reagent grade, anhydrous, granular)
Potassium Hydroxide (KOH),(ACS reagent grade)
Florisil, 60-100 mesh, (Fisher Scientific Cat.No.F100-3)
Precoated TLC plates (20% acetylated cellulose, 250 microns, 20 cm x 20 cm, Analtech Co.)

3.4 Benzo(a)pyrene Standard Solutions

Standard solutions of benzo(a)pyrene [B(a)P] (Aldrich Chemical Co.) were prepared by dissolving weighed amounts in cyclohexane and stored in a freezer.

1. BaP stock solution:

   BaP 1000 mg/l in cyclohexane.

2. Working standards

   BaP 1 ng/l in cyclohexane

   BaP 5 ng/l in cyclohexane

   BaP 10 ng/l in cyclohexane

   BaP 20 ng/l in cyclohexane

   BaP 40 ng/l in cyclohexane

   BaP 70 ng/l in cyclohexane

   BaP 100 ng/l in cyclohexane

These working standards are made by diluting the stock solution in cyclohexane. All these standard solutions are stored in 10 ml amber vials, protected in a freezer at -20°C.

3.5 Apparatus

Apparatus used are usual laboratory equipment and the following items:

condenser,

500 ml round bottom flask,

separatory funnel (2L),

glass column (19 x 400 mm) fitted with a coarse fritted disk,

rotoevaporator,

vacuum oven,

TLC multi-spotter syringes (100 ug),

Analytical Instrument Specialties (AIS) TLC multi-spotter,

Rectangular glass chromatography tank (N-tank),

TLC/Spectrofluorometer apparatus (Perkin-Elmer, MPF-44B).

3.6 Procedure

3.6.1. Florisil Pretreatment and Test

150 g of Florisil (Fisher Company Cat. no. F100-3) is added to a 500 ml glass-stopped Erlenmeyer flask, then 250 ml of methanol is added. The flask is stoppered and shaken for three to five minutes with the stopper removed periodically to release pressure. The flask is left in the fume hood for 4 hours. The slurry is then transferred to a 600 mm diameter Buchner funnel fitted with filter paper of coarse porosity. The florisil slurry is allowed to sit in the funnel while methanol drains by gravity. This is followed by the application of vacuum to remove residual methanol by aspiration. The adsorbent in the funnel is then washed with an additional 170 ml of methanol which is allowed to drain as much as possible and then further removed by vacuum. The filtered Florisil is transferred to an aluminum foil receptacle and placed into a vacuum oven at room temperature (about 20°C) and left overnight (ca 18 hours). The dried Florisil is stored in an amber bottle and put in a dessicator.

Prior to use, 5% w/w of organic-free water is thoroughly mixed with the Florisil. Pretreatment and testing of Florisil is crucial to insuring quantitative recovery. To a 10 gram portion of the treated Florisil is added 5 wt % of organic-free water. The florisil is put into a glass column (19 mm by 400 mm) fitted with a course fritted disc to retain the solid. The column is gently tapped to compact the solid. On top of the florisil is placed 15 grams of granular anhydrous Na_2SO_4 and the column is tapped gently again. The column is washed with 50 ml of isooctane and the washing discarded. Pipetting of 1 ml of 100 ng/ml benzo(a)pyrene standard solution onto the top of column is followed by washing with four 20 ml portions of isooctane, with the column being allowed to drain completely before the next eluant is applied. The washes are collected and retained in a 250-ml round bottom flask. The column is then eluted with four 25-ml portions of benzene with each being allowed to drain completely before the next 25-ml benzene eluant is applied. The benzene eluates are collected in a second round bottom flask. Both flasks of eluates are separately evaporated under vacuum in a rotoevaporator at different water bath temperatures (90 ^{o}C for isooctane and 60 ^{o}C for benzene) until the volume is reduced to about 5 ml. Ten ml of cyclohexane is poured into each solution and evaporated until the volume is reduced to 1-2 ml. The residue is transferred to an 8-ml amber vial, and the round bottom flask is washed three times with 2 ml cyclohexane and the washings

are added to the residue in the vial. The volume in the 8-ml amber vial is reduced to exactly 1.0 ml by blowing under a gentle stream of nitrogen or air.

The concentrates of both the isooctane and benzene eluates are analyzed for B(a)P by TLC/Fluorescence. No B(a)P should be present in the isooctane elution and over 80% of the added B(a)P must be found in the benzene eluate.

3.6.2 Source Sample Preparation and Storage

The food samples were obtained from 10 volunteers in the Phillipsburg N.J. area, who, for a two-week period donated a one-fourth portion (estimated by each household) of every meal they ate. The participants placed the food portions into an aluminum foil container, which was collected daily and frozen. Each container was throughly blended into a homogeneous composite. These daily composites were then thoroughly blended into weekly composites. This resulted in a total of twenty weekly composite samples from the two week period for 10 participants. The participants were identified only by personal identification number (PID) to maintain anonymity. All samples were kept frozen until analyzed.

3.6.3 Extraction

All extractions and the purification procedure are carried out under minimal light in order to avoid possible photodecomposition of B(a)P.

100 grams of food sample are placed in a 500 ml round bottom flask then 200 ml of ethanol, 12.5 grams of KOH and a magnetic stir bar are added. The sample is digested and

saponified by refluxing gently at 90-95 ^{o}C for 2 hours with magnetic stirring to prevent the food from sticking to the bottom of the flask. The aqueous ethanol solution from the sample is collected in a 2-liter separatory funnel. The digested residual material is washed with three 50 ml portions of isooctane and these washings are used to extract the aqueous ethanolic solution in the separatory funnel. The aqueous ethanol solution is then further extracted with two 60 ml portions of isooctane. All five of the isooctane extracts are combined and washed several times with 60 ml of warm (60°C) water until the organic phase becomes clear. Any emulsion problems at this step can be clarified by adding 20-30 ml of saturated aqueous NaCl solution,if necessary. All of the isooctane extract is transferred to a 500 ml flask and two 20 ml portions of isooctane are used to rinse the separatory funnel and then added to the flask. 20 grams of anhydrous Na_2SO_4 is added to the isooctane extract to remove residual water and the extract is then filtered.

3.6.4 Column Chromatography on Florisil

The use of column chromatography can be advantageous for sample clean-up prior to TLC. First, 30 g of treated and tested Florisil (5% water w/w, from Part 3.6.1) is added to the glass column (19 mm X 400 mm) fitted with a coarse fritted glass disk and the column is gently tapped. 5 g of anhydrous Na_2SO_4 is added on the top of the Florisil and the column is again gently tapped. The column is then washed with 100 ml of isooctane and the washings discarded.

All of the isooctane extract from the sample is passed through the column, the 500-ml flask is subjected to rinse by one 20-ml portion of isooctane, and the washings are added to the column. B(a)P is retained on the column, after the isooctane solution is employed and allowed to elute completely from the column. Three 60 ml portions of benzene are applied to separate B(a)P from the column and the elutates are collected by a 500 ml round bottom flask.

3.6.5 Evaporation

Evaporation of samples to near dryness in the sample preparation procedure needs to be done very carefully, since care must be taken to concentrate the sample without losing or degrading B(a)P. The benzene eluate is reduced to 5 ml by rotoevaportion. 10 ml of cyclohexane is added and the volum reduced to ca. 2 ml for solvent exchange. The sample is transferred to an 8 ml amber vial along with three 2 ml cyclohexane washings of the round-bottom flask. The combined solution is blown down to 1 ml under nitrogen. We use a simple procedure for nitrogen blow-down which involves attaching a vial containing the sample to a ring stand in a fume hood. The vial is placed in a beaker and a disposable pipet attached via rubber tubing to a nitrogen tank is inserted in the vial. The solution will evaporate under nitrogen gas bubbled slowly into the vial. A similar setup also can be used with air instead of nitrogen by using an air filter between the pipet and tubing.

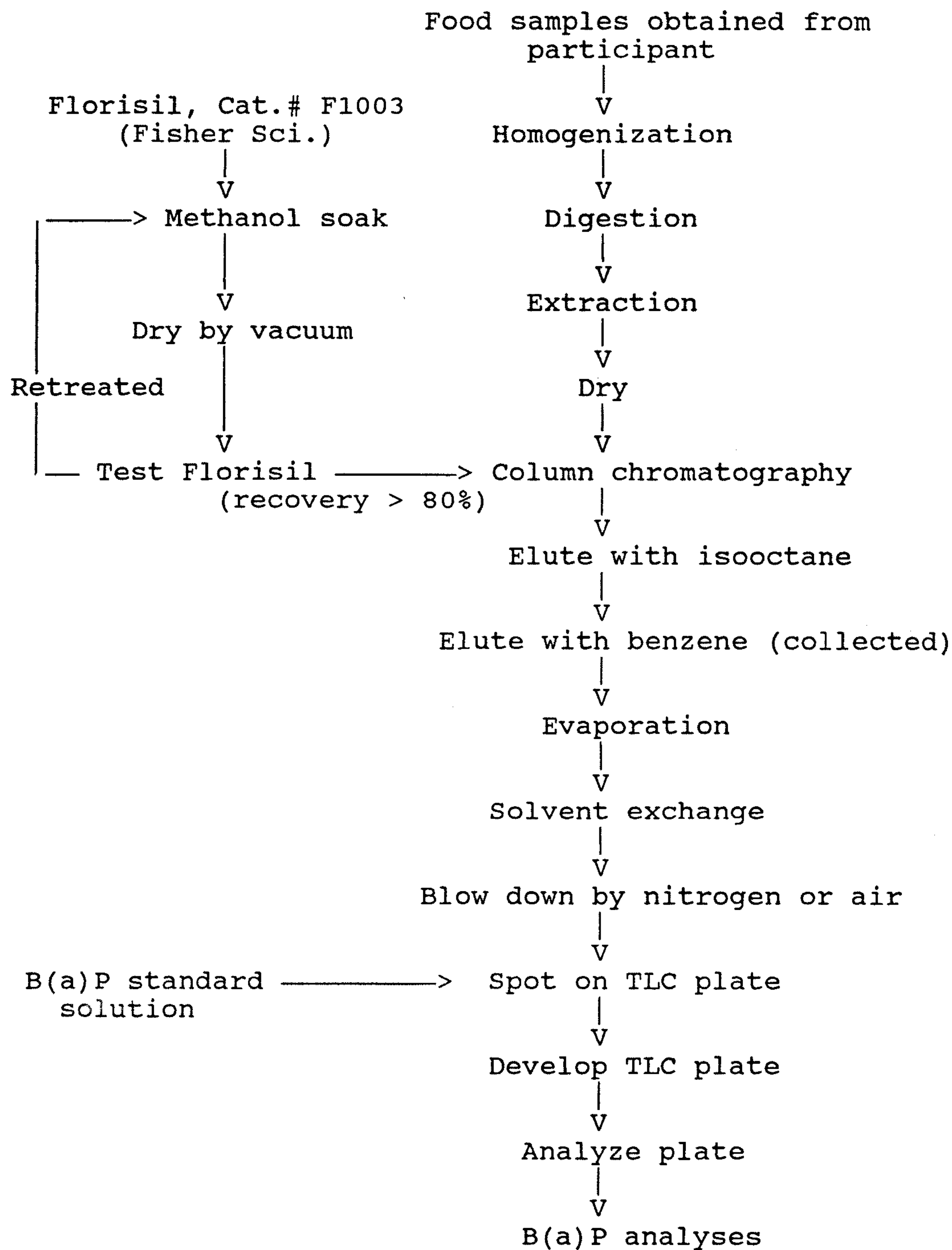
Following evaporation of samples, the residual solution volume is compared to that in an identical vial filled with an accurate 1.0 ml volume of cyclohexane. Caution must be used in drying the sample, since excessive air could alter or otherwise decompose labile B(a)P on the surface of sample solution.

3.6.6 Thin-Layer Chromatography

Accurate and consistent aplication of samples and standards to the plate in small, equally sized zones is critical for successful densitometry (Brain and Turner).[11] A 0.1 ml (100 microliter) aliquot of each sample is applied by syringe as a spot 2 cm above the bottom edge of the cellulose-acetate TLC plate using the Analytical Instrument Specialties (AIS) automatic spotter. Each plate has 18 channels and each of the samples is spotted into two channels for replication purposes. A series of B(a)P standards, 100 microliter aliquots, (e.g. 1 ng/ml; 5 ng/ml; 10 ng/ml; 20 ng/ml; 40 ng/ml) are applied in duplicate for calibration. After sample spotting, the plate remains under the fume hood for five minutes to dry and is developed with 150 ml ethanol-dichloromethane (2:1) in a rectangular glass chromatography tank (N-tank). The tank is closed with a glass lid and is covered with a box to avoid exposure to light during development which takes about 75 minutes. At the end of development, the plate is allowed to air dry in a dark place for about 5 minutes.

Using a plate-scanning spectrofluorometer (Perkin-Elmer,MPF-44B), B(a)P in food is identified by the retention time of the standard and quantitated by comparision of the sample peak area with a calibration curve composed by the series of B(a)P standard. A non-linear corrective equation of least squares parabola ($Y=A_o + A_1X + A_2X^2$) was applyed to calculate B(a)P concentration. The fluorescence conditions are 387 nm (excitation) and 428 nm (emission).

3.7 Schematic Representation of the Procedure



3.8 Recovery and Precision

3.8.1 Florisil Recovery Study

To certify the Florisil adsorbent for pretreatment of each batch is a critical aspect of this analytical method. Florisils, as received from different vendors, show different behaviors in their adsorption properties. Some activated Florisil samples tend to affect extensive tailing leading to poor recoveries. Thus, we attempt to make Florisil activation more consistent with methanol pretreatment followed by the 5% water loading employed in the present study and thus we obtain consistently high recoveries. Table 7 lists B(a)P recoveries from column chromography through different Florisil pretreatments. From this table, the best result is achieved with Florisil treated by methanol to remove impurities, left in vacuum oven at ambient temperature to dry and subsequently with 5% water added for deactivation. Table 8 shows the recoveries for THEES Phase II where the average recovery is 69.1 +/- 3.0%. Table 9 shows the recoveries for THEES Phase III where the average recovery is 84.5 +/- 10.7%.

Table 7

B(a)P Recoveries from the Column Chromatography by Using Different Florisil Pretreatment (B(a)P, 20-100 ng in solution was added to the top of the column)

Treatment Method	# Tests	% Recovery
A. # Pretreatment[a.] (Florisil as received)	2	6 +/- 6
B. Methanol Wash and Vacuum[a.]	3	43 +/- 37
C. Methanol Wash, put into Vacuum oven at 50°C 2% Water Deactivation	6	7 +/- 3
D. Methanol Wash, put into Vacuum oven at 20°C 5% Water Deactivation	26	80 +/- 15

a. Adapted From Luo S., Master Thesis, Ref. 12

Table 8

B(a)P Recoveries for Total Human Exposure to Environment Substance, Phase II (B(a)P , 100 ng in solution was added to the top of the column)

Batch	No. of Food Samples Analyzed with This Batch	% Recovery of Florisil Test
1	4	72.2
2	4	72.9
3	5	69.0
4	4	70.8
5	5	65.3
6	4	65.9
7	4	67.9
	Total 30	69.1 +/- 3.0

Table 9 B(a)P Recoveries for Total Human Exposure to Environment Substance, Phase III (B(a)P , 100 ng in solution was added to the top of the column)

Batch	No. of Food Samples Analyzed with This Batch	% Recovery of Florisil Test
1	4	64.7
2	4	68.0
3	5	63.3
4	4	77.3
5	5	84.1
6	5	91.8
7	6	87.5
8	5	80.5
9	5	78.3
10	5	98.8
11	4	81.2
12	4	81.9
13	4	92.7
14	4	93.2
15	6	89.7
16	5	98.6
17	6	88.5
18	6	90.7
19	2	95.2
	Total 89	Avg. 84.5 +/- 10.7

3.8.2 B(a)P Recovery Study

During the THEES study, B(a)P recoveries were tested to verify the accuracy of the analytical technique by sample spikes and duplicates.

In order to examine spike recoveries from real samples, 100 ng/l B(a)P solutions were injected into composited food samples, then subjected to the complete regular procedure. The recovery is determined by using

$$\% \text{ Recovery} = \frac{\text{B(a)P Found}}{\text{B(a)P Added + B(a)P Conc. in Food}} \times 100$$

As shown in Table 10, the average recovery is 82.4 +/- 6.4%. While the recovery of spiked sample is apparently different from the recovery of 100 ng B(a)P applied to the Florisil column, these three group results are within experimental error (69.1 +/- 3.0%, 84.5 +/- 10.7% and 82.4 +/- 6.4%).

The other test for B(a)P analysis from real food samples is the analysis of replicate. Tables 11 and 12 indicate the errors in replication of 16 food samples (6 for Phase II, 10 for Phase III). The percent errors range from 1-47%, and the average error is +/- 14.6. This reflects not only the deviation in Florisil pretreatment techniques, but also differences in investigators and their familiarity with the procedure.

Table 10 Recovery of B(a)P from Food Spiked with B(a)P Standard Solution

Sample	B(a)P Added (ng)	B(a)P Found (ng/100 g Food)	B(a)P in Food (ng/100 g Food)	% B(a)P Received
PID91 Week1	100	83.80	14.63	73.1
111-06	100	86.70	2.87	84.3
103 post	100	91.69	3.47	88.0
105 pre	100	87.59	4.13	84.1

Avg. 82.4 +/- 6.4

Table 11 Precision of B(a)P Assay in Food Using Duplicates from the Same Food Composite (THEES, Phase II)

Sample	Average B(a)P Level (ng B(a)P in 100 g Food)	Avg.	Avg. % Deviation from Analyses
PID01 Wk 1	1.26 1.93	1.60	20.94
PID01 Wk 2	3.30 4.15	3.87	14.86
PID02 Wk 1	3.90 3.86	3.88	0.52
PID11 Wk 1	9.55 3.44	6.50	47.00*
PID31 Wk 1	35.06 24.58	29.82	17.57
PID31 Wk 2	0.91 2.23	1.57	42.04*
		Avg.% deviation for all analyses	13.47

* These were among first samples analyzed in duplicate. We omit them as outlying data. If they are included the average % error is 23.8 and the average deviation is almost twice as large.

Table 12 Precision of B(a)P Assay in Food Using Duplicates from the Same Food Composite (THEES, Phase III)

Sample	Average B(a)P level (ng BaP in 100 g food)	Avg.	Avg. % Deviation from Analyses
PID11 Wk 2	13.18 14.85	14.02	5.94
PID42 Wk 1	2.22 3.09	2.67	16.29
PID72 Wk 1	3.83 3.85	3.84	0.26
PID72 Wk 2	8.77 19.83	14.30	38.67
PID91 Wk 2	78.32 89.10	83.71	6.44
103-pre	1.90 3.55	2.73	30.22
103-post	2.68 4.25	3.47	22.62
172-01	2.52 2.26	2.39	5.44
		Average % deviation for all analyses	15.73

3.8.3 Quality Assurance / Quality Control

A quality assurance program is an essential part of a secure analytical protocol. We use quality assurance to detect and correct B(a)P in our food samples and take every reasonable step needed to keep the measurement process reliable. Therefore, it is important that the limits of reliability be specified as part of the procotol.

The basic elements of quality assurance define the framework of all analytical procedures and must include:

(i) The sampling protocol and its relation to results should be made explicit. A detailed description of food sampling from Phillipsburg area, as well as procedures was prepared by Mr. Peter Creighton and included collecting, labeling, container preparation, storage, pretreatment and transportation to NJIT.

(ii) For the analysis of B(a)P in composite meals, HPLC grade solvents (isooctane, benzene, cyclohexane,etc) and well-washed glassware are used to prevent contamination. Syringes for spotting TLC plate are washed with hot methanol and cyclohexane.

(iii) Column chromography on Florisil is a critical step for this analysis. We employ Florisil pretreatment and testing to observe the recovery directly and to certify it.

(iv) Due to the variability of the individual food samples, these must be proved to be sufficiently homogeneous. Thus, the precision of analysis result was probed by replicate samples of food composites.

(v) Food spiked with B(a)P was also used for quality assurance in our analytical procedures.

(vi) Confidence in the measurement process can also be strengthened considerably by comparison of replicate results on blind samples which we did not know in advence were the same as those reported in the literature. Table 13 shows the B(a)P result of 22.5 ug/kg (ppb) in char-broiled beef. It is very consistent with a reported level of 21.5 ug/kg by Doremire et al.(1979)[13] In another comparison with published data, Table 14 lists three different categories of bacon: uncooked bacon; fried bacon, and oven cooked bacon. We found that fried bacon has the highest B(a)P contained in these three samples. These results are compared to the B(a)P level of 0.1 ug/kg by Joe et al. (1984) reported and others.[13]

3.8.4 Determination of Detection Limit

The qualitative definition of detection limit is the minimum concentration of analyte that can be detected at a known confidence level. When we define 3 times noise as a minimal value for blank measurements, the confidence level of detection will be 95% in most cases. The limit of detection for a single analysis of an aliquot of B(a)P is 0.05 ng. Since 100-gram food sample were extracted and finally concentrated to 1 ml, the analysis of a 100 microliter aliquot corresponds to a limit of detection (LD) of 0.005 ug/kg or ppb weight.

Table 13 B(a)P in CharBroiled Beef in a Blind Test of Replicate Samples

Sample #	B(a)P Level (ug/kg or ppb weight)
001 A	22.7
001 B	22.2
Avg.	22.5

* B(a)P content level found in char-broiled beef is 21.5 (ug/kg) from Doremire M.E., Harmon G.C. & Pratt D.E., 3,4-Benzopyrene in charcoal grilled meats, J. Food Sci., 44. 622-3. Adopted from Ref. 13.

Table 14 B(a)P Found in Bacons[a]

Sample	B(a)P Content (ug/kg or ppb weight)	Reference
Uncooked bacon	0.032	This work
Fried bacon	0.130	This work
Oven Bacon	0.043	This work
Bacon, fried	0.16	Lintas et al.(1979)[15]
Bacon	0.25	Lintas et al.(1979)[15]
Bacon	0.05	Crosby et al.(1981)[16]
Bacon	0.1	Joe et al.(1984)[17]

[a] Table adopt from Ref. 13.

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CHAPTER IV

RESULTS AND DISCUSSION

All the results of the analysis for THEES Phase I (investigated by Ms. S. Luo),[1] Phase II and Phase III are listed in the Appendix. These food samples were collected weekly for two one-week periods and daily over 14 days (THEES Phase III, Sampling no. 191, 111, 121, 172). The results revealed that actural meal consumption of B(a)P may vary by two orders of magnitude from home to home (e.g. compare home 02 and 03 in Appendix Table 1) or in one home from week to week (e.g. home 08, Appendix Table1; PID 31, 41, 42, 61 in Appendix Table 2; PID 31, 72, 91 in Appendix Table 3) or in a single person from day to day (e.g. PID 191-01 and 191-02 in Appendix Table 5).

4.1 B(a)P Exposure from Weekly Food

For most of the 15 to 21 meals per week acquired from each home, the concentration of B(a)P ranged 0.004 to 1.173 ppb. Table 15 list the arithmetric averages of B(a)P concentrations in weekly food composite samples for all three Phases of the THEES study, and the overall average B(a)P concentration from these studies is 0.161 ug/kg (ppb).

As we know, these three period results were not absolutely the same and thus the B(a)P weekly concentrations may vary considerably and still give us some consistent results.

Table 15 The Average of B(a)P Concentration in Weekly Food Composite Samples in the Three Phases of THEES

THEES (Phase #)	# of Samples Analyzed	B(a)P Concentration ug/kg, weight, weekly
I	20	0.168
II	20	0.105
III	18	0.210
	Avg.	0.161

4.2 B(a)P Exposure from Daily Food for THEES Phase III Study

For the THEES Phase III study, the net weight of food samples provided by each home were between 0.25 and 2.5 kg. Each collected meal represented approximately one fourth of an adult portion. The total weight of weekly composited meals eaten by each person may be estimated by

4 X (Net weight of food samples provided by each person)

We summarize the net weight of food samples provided, the estimated weight of actual food consumed in one week, the B(a)P concentration from analysis and ug B(a)P level per weekly ingestion in Table 16.

Table 16 Exposure to B(a)P via the Weekly Composite Meals

THEES Phase III

PID #	Week #	Net Wt. Food Samp. Provided kg	Total Wt. Food Samp. Consumed kg	B(a)P Anal. in Food Samp. ug/kg Wt.	ug BaP level / week
11	1	1.920	7.860	0.060	0.472
11	2	2.499	9.996	0.142	1.419
21	1	1.126	4.504	0.491	2.211
21	2	1.352	5.408	0.239	1.293
31	1	0.526	2.104	0.024	0.051
31	2	0.545	2.180	0.855	1.864
41	1	0.438	1.752	0.069	0.121
41	2	0.627	2.508	0.087	0.218
42	1	0.353	1.412	0.027	0.038
42	2	0.301	1.204	0.004	0.005
61	1	0.252	1.008	0.251	0.253
61	2	0.242	0.968	0.121	1.085
72	1	0.751	3.004	0.038	0.114
72	2	0.853	3.412	0.143	0.488
81	1	1.994	7.976	0.141	1.125
81	2	0.998	3.992	0.105	0.419
91	1	1.970	7.880	0.146	1.151
91	2	1.830	7.320	0.837	6.127
	Avg.	0.957	3.828	0.210	1.025

From Table 15, it appears that 3.8 kg is the average weight of food eaten by a single person in one week, and the average B(a)P concentration in THEES Phase III sample is 0.210 ug/kg food weight. The average B(a)P consumed in a week is therefore 1.025 ug B(a)P during the THEES Phase III weekly study, and which divided by 7, one will obtain the level of daily B(a)P ingestion amount. Therefore the average daily exposure to B(a)P level via food is 0.146 ug B(a)P/day (same as 146 ng B(a)P/day in scale) for the THEES Phase III weekly study.

The other estimation method is direct calculation from the average level of B(a)P concentration in daily food. For THEES Phase III, the B(a)P concentrations in daily food composite samples are listed in Appendix Tables 5-8. These samples may include 3 or 4 meals in each day, and also reveal variable levels of B(a)P in each analysis. The averages of B(a)P level in daily food composite meals from THEES Phase III are listed in Table 17. This table also shown the estimated daily ingestion food amounts of specific persons (PID number 11, 21, 72, 91) from Table 16 (the average B(a)P concentrationins in daily foods and the average weight of daily foods were measured from our analysis). The average B(a)P ingested levels in daily composite meals are calculated by

Avg. BaP Ingestion = Avg. BaP in Food X Avg. wt. of Food
(ug/day) (ug/kg-day) (kg)

Table 17 The Averages of B(a)P Concentration in Daily Food Composite Samples (THEES Phase III)

Sample #	Avg. B(a)P ug/kg wt. food, daily	Avg. wt. of daily food, kg	Avg. BaP ingestion ug/day
191	0.052	1.275	0.066
111	0.036	0.708	0.025
121	0.206	0.458	0.094
172	0.161	1.086	0.175
Avg.	0.114	0.882	0.090

If estimate the daily level of B(a)P from ingestion by using:

$$0.114 \frac{\text{ug BaP}}{\text{kg}} \times 0.882 \frac{\text{kg}}{\text{day}} = 0.101 \text{ ug BaP/day}$$

Then the daily level is 101 ng B(a)P per day. These results show large variations between people because the limited participating families have different eating and cooking habits. Hypothetically, the total daily B(a)P dose through food ingestion calculated from weekly estimates, 146 ng of B(a)P/day, is more consistent than that obtained from daily food composite samples.

4.3 Risk Assessment from Ingestion Exposure

For food dose exposure, we assume the level of B(a)P ingested is 100% absorption and the average body weight of male who lived in Phillipsburg area is 70 kg. The daily dose from ingestion is obtained by

$$146 \frac{\text{ng BaP}}{\text{day}} \times \frac{1 \text{ mg}}{106 \text{ ng}} \times \frac{1}{70 \text{ kg}} = 2.09\times10^{-6} \text{ (mg/kg/day)}$$

The ingestion cancer risk from a lifetime exposure to B(a)P is calculated as follow:

$$\text{Cancer Risk} = \underset{(\text{mg/kg/day})^{-1}}{q^*} \times \underset{(\text{mg/kg/day})}{\text{dose}} \qquad (2)$$

q^* carcinogenic potency factor;

it is the slope of dose response relationship for specific carcinogen.

q^* for BaP is 11.5 $(\text{mg/kg/day})^{-1}$

The cancer risk from ingestion is

$$11.5 \text{ (mg/kg/day)}^{-1} \times 2.09\times10^{-6} \text{ (mg/kg/day)}$$
$$= 24.04 \times 10^{-6}$$
$$= 2.4 \times 10^{-5}$$

It means that a person who is exposed to a B(a)P dose concentration from food daily at the level of 2.09×10^{-6} mg/kg for 70 years, has a chance to get cancer from food ingestion of 24 out of one million. Since we know 1×10^{-5} is the

minimim risk rate for an individual over a lifetime.[2] It is 2.4 times higher than this number, and thus pertinent regulation on foodstuffs, especially in smoked food, should be employed to safeguard the public health. The U.S. EPA should work with Food and Drug Administration (FDA) to consider the suitability of B(a)P in food as a level for maxium allowance.

4.4 Methodology Assessment

So far the developed method for a part of THEES study focusing on B(a)P in food, is a hybrid of the IUPAC and Chinese methods (see details in Chapter III). As previously described, this technique employs a hybrid of currently accepted methods to obtain a reliable one. The highly specific detection by spectrofluorometry of B(a)P allows some simplifications in the procedure and permits high sensitivity, reproducibility and selectivity. However, there is still a need for improvement in the procedure since benzene is used as the mobile phase on column chromatography. It is a well known carcinogenic chemical with a cancer risk even higher than B(a)P. It is necessary to improve this method in the future.

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CHAPTER V

CONCLUSIONS

5.1 Exposure to B(a)P in Food

The major focus of this study is to understand the exposure levels of residents of the United States to B(a)P during the late 1980s. We found the times when grills are used for cooking foods, and the eating habits play important roles to B(a)P exposure.

The result for average of B(a)P concentration from three phases of THEES study is 0.161 ug/kg (ppb). (see Table 15) Since the TLC/fluorescence detection limit is 0.005 ppb and this analytical technique has enough sensitivity to measure B(a)P in environmental samples,it is confident for the analysis of human exposure to B(a)P via the ingestion pathway.

Although this result was obtained from these phase studies, the concentrations of reliable and readily comparable results may still be considered highly limited. Thus, the conclusions resulting from this review must be regarded as merely tentative.

The other aspect of concern is the development of method that may be employed consistently. The quality control data obtained in this study indicate that this hybrid method is adequate, while the analysis shown 82.4% recovery on food spikes. Compare with the original IUPAC method, this method can reduce B(a)P losses from extraction steps after Florisil clean-up.

5.2 Assessment of B(a)P Exposure from Ingestion and Inhalation

In the Total Human Exposure to Environmental Substances (THEES) studies, the purpose of the investigation is to understand the multi-media exposure levels associated with B(a)P. The major focuses concern human exposure through inhalation and ingestion. We estimate that 0.15 ug B(a)P is the exposure level from daily food. It is slightly lower than the results in the earlier report from New Zealand (see Chapter I, Table 3). Because there are insufficient data on environmental exposures in real life, e.g. the weight of food intake for each person. we still must make assumptions in comparing our two weeks of ingestion data with 24 hour daily inhalation exposure (Chen's Thesis).[1] The average daily inhalation exposure to B(a)P is 0.028 ug. Thus, B(a)P in food ingestion provides B(a)P in amounts over 5 times higher than that contributed by inhalation. The occurrence of B(a)P in food should be viewed as a part of the much larger problem in the environment. It is also obvious that B(a)P in air, water and soil are being given much more emphasis with respect to their contamination of the food supply.

Since we now suspect that there may be health problems caused by B(a)P in food, further study should concern itself with ways to prevent B(a)P formation in food, and the biological monitoring of B(a)P exposure. Thereby, overall public health problems may be effectively controlled.

LITERATURE CITED

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2. Bjorseth,A., Handbook of Polycyclic Aromatic Hydrocarbons, Marcel Dekker, Inc. NY, 1983, Chapter 11.

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Table 1 The B(a)P Concentration in Weekly Food Composite Samples and the Total Number of Meals Composited in Each Home (THEES, Phase I)

Home[a] ID#	Week #	Number of Meals	Food B(a)P ug/kg, weight "as received" (ppb)
01	1	19	0.18
01	2	21	0.053
02	1	21	0.208
02	2	21	0.69
03	1	16	0.017
03	2	16	0.021
04	1	8	0.004
04	2	9	0.027
05	1	20	0.004
05	2	16	0.148
06	1	19	0.015
06	2	18	0.181
07	1	13	0.005
07	2	11	0.027
08	1	19	0.021
08	2	15	0.128
09	1	18	0.373
09	2	18	1.173
10	1	19	0.024
10	2	17	0.033

(Adopt From Ms. Luo S., Master Thesis.

a. The composites were identified only with a home number.

Table 2 The B(a)P Concentration in Weekly Food Composite Samples and the Total Number of Meals Composited in Each Home (THEES, Phase II)

PID #[a.]	Week #	Number of Meals	Food B(a)P ug/kg, weight "as received" (ppb)
001	1	11	0.016
001	2	13	0.039
002	1	3	0.039
002	2	8	0.022
011	1	21	0.065
011	2	22	0.023
031	1	11	0.298
031	2	14	0.016
041	1	7	0.014
041	2	12	0.279
042	1	9	0.094
042	2	12	0.678
051	1	18	0.013
051	2	16	0.039
061	1	31	0.276
061	2	23	0.025
082	1	10	0.012
082	2	22	0.023
101	1	22	0.031
101	2	15	0.103

[a] The first two digits in the Personal Identification (PID) Number refers to the house number. (houses 0-10; See Table 1) There were some subtractions and additions of houses between THEES Phase I and Phase II). The third digit identifies an individual in the household, part of whose meal is composited for B(a)P analysis (this approach differs slightly from the composite "household meal" in THEES Phase 1; See Table 1).

Table 3 The BaP Concentration in Weekly Food Composite Samples and The Total Number of Meals Composited in Each Home (THEES, Phase III)

PID number [a]	Week	# Meals	Food BaP ug/kg, (ppb)
011	1	6	0.060
011	2	8	0.142
021	1	6	0.491
021	2	7	0.239
031	1	8	0.024
031	2	6	0.855
041	1	4	0.069
041	2	7	0.087
042	1	6	0.027
042	2	7	0.004
061	1	7	0.251
061	2	7	0.121
072	1	5	0.038
072	2	8	0.143
081	1	20	0.141
081	2	8	0.105
091	1	8	0.146
091	2	8	0.837

a. PID refer personal identification number; see Table 2.

Table 4 The B(a)P Concentration in

Special Weekly Food Composite Samples (THEES, Phase III)

PID Number[a]	Period[b]	Food BaP ug/kg, (ppb)
01	pre	0.049
01	post	0.055
02	pre	0.029
02	post	0.040
03	pre	0.019
03	post	0.035
04	pre	0.059
04	post	0.011
05	pre	0.041
05	post	0.057

[a] PID refer personal identification number;as on Table 2

[b] These sampling periods are pre aggregate and post aggregate.

Table 5 The B(a)P Concentration in

Daily Food Composite Samples (THEES, Phase III)

Sampling number[a]	ug BaP/kg food (ppb)
191-01	0.021
191-02	0.195
191-03	0.023
191-04	0.048
191-05	0.021
191-06	0.027
191-07	0.024
191-08	0.026
191-09	0.026
191-10	0.147
191-11	0.032
191-12	0.018
191-13	0.065
191-14	0.050

a. Sampling number 191 means home ID number 09; personal ID number 01. The last two digits identifies Phase III two weeks study (Day 1 to Day 14). The composite meals usually include breakfast, lunch, dinner as well as snack.

Table 6 The B(a)P Concentration in

Daily Food Composite Samples (THEES, Phase III)

Sampling number[a]	ug BaP/kg Food (ppb)
111-02	0.033
111-03	0.048
111-04	0.057
111-05	0.015
111-06	0.029
111-07	0.035
111-08	0.078
111-09	0.038
111-10	0.025
111-11	0.030
111-12	0.023
111-13	0.025
111-14	0.028

[a] This sampling number refers to home ID 01, personal ID 01. The last two digits are as on Table 5.

Table 7 The B(a)P Concentration

in Daily Food Composite Samples (THEES, Phase III)

Sampling Number	Food B(a)P ug/kg, (ppb)
121-01	0.032
121-02	0.017
121-03	0.330
121-04	0.018
121-05	0.026
121-06	0.016
121-07	0.979
121-08	0.051
121-09	0.223
121-10	0.023
121-11	0.022
121-12	0.039
121-13	0.086
121-14	1.023

[a] This sampling number refers to home ID 02 Personal ID 01. The last two digits see as Table 5.

Table 8 The B(a)P Concentration

in Daily Food Composite Samples (THEES, Phase III)

Sampling Number	Food B(a)P (ug/kg, ppb)
172-01	0.039
172-02,03[b]	0.053
172-04,05[b]	0.206
172-06	0.312
172-07	0.269
172-08	0.389
172-09,10[b]	0.414
172-11	0.061
172-12	0.474
172-13,14[b]	0.041

a. This sampling numbers refer to home ID 07, personal ID 02. The last two digits are as on Table 5.

b. These samples were collected for two days in a sample.