RESEARCH PROTOCOL and STATISTICAL ANALYSIS PLAN

September 13, 2021

and

INFORMED CONSENT FORM

October 10, 2018

Benzo[*a*]pyrene Ultralow Dose-Response Study

Principal Investigator: David E. Williams, PhD

NCT03318978

RESEARCH PROTOCOL

September 13, 2021

1. Protocol Title: Benzo[*a*]pyrene Ultralow Dose-Response Study

PERSONNEL

2. Principal Investigator — David E. Williams, PhD
3. Student Researcher(s) — N/A
4. Sub-investigator(s) — Sandra Uesugi, RN; Lisbeth Siddens
5. Study Staff — N/A
6. Investigator Qualifications:

   Williams, PhD in Biochemistry, is a Toxicologist with over 40 years of experience working with Polycyclic Aromatic Hydrocarbons (PAHs) and has directed similar human studies involving micro-dosing of PAHs at OSU.

   Uesugi, RN, the Clinical Research Nurse Coordinator (nurse coordinator) has extensive experience in clinical research coordination, laboratory research, science communication for the lay public, and project management. In addition to being a registered nurse, Ms. Uesugi has completed a certified phlebotomy training course, intravenous therapy training, and a clinical research coordinator (CRC) training course. Uesugi has also completed the OSU Radiation Safety training and has previous laboratory experience working with 14C labeled compounds.

   Siddens has performed carcinogen and radioisotope handling over the course of 32 years, including 15 years with the study PI. Siddens is trained and approved to handle extreme carcinogens and participates in OSU's medical surveillance program. As the person in charge of the LPI extreme carcinogen facility it is her responsibility to dilute extreme carcinogens such as benzo[a]pyrene to concentrations considered safe for handling per the OSU EHS guidelines. Siddens also maintains chemical and ionizing isotope inventories. Siddens is trained in proper shipping and handling of biological and hazardous materials.. For this protocol, Siddens will perform handling of extreme carcinogens, dilute carcinogen solutions as well as BaP capsule preparation. Siddens was trained to carry out the capsule preparation protocol by Dr. Erin Madeen, a former graduate student in the Williams lab and person responsible for capsule preparation on previous IRBs. Madeen was a pharmacy technician before entering graduate school. The training first entailed observing Madeen prepare capsules for several cycles. Under Madeen's guidance Siddens prepared a set of capsules. Capsules were evaluated for the proper level of 14C-BaP with liquid scintillation counting. This was repeated over several weeks to ensure consistency over time.

7. Training and Oversight:

   Williams is responsible for the conduct and oversight of the study, for ensuring that privacy/confidentiality of subjects is maintained and that all individuals working on the study are properly trained to perform their role on the study.

   Nurse coordinator Uesugi will obtain informed consent from study subjects. She will also oversee recruitment and enrollment, closely monitor recruitment to prevent over enrollment, and ensure all regulatory requirements are followed.

Extended absence of the PI is not anticipated because the PI will not use sabbatical leave during the study, is appointed at 1.0 FTE (9-month) and a tenured faculty member. A certified phlebotomist may occasionally be employed to collect blood samples as a back-up to Uesugi.

8. Conflict of Interest

   No study team member has a conflict of interest with this study.

FUNDING

9. Sources of Support for this project (unfunded, pending, or awarded)

   - Indicate internal and/or external funding source: External (pending, proposed start date 12/01/2017)
   - Grant/contract number: PHS grant, National Institutes of Health, R01 ES028600
   - Name of PI on Grant: David E. Williams, PhD
   - Grant title: Benzo[*a*]pyrene Micro-dosing of Humans: A New Tool for Exposure, Risk Assessment and Prevention
   - Any external source(s) of material, equipment, drugs, supplements, or devices: None

DESCRIPTION OF RESEARCH

10: Description of Research

Benzo[*a*]pyrene (BaP) is the most intensely studied polycyclic aromatic hydrocarbon (PAH), environmental pollutants formed naturally from forest fires, volcanoes, etc. Anthropogenic sources include coal, tobacco smoke, creosotes, coal tar-based pavement sealants, petroleum products including diesel and gasoline, wood and mixtures from production of coke, aluminum and graphite, among others[1]. BaP is a class 1, known human carcinogen (Internal Agency for Research on Cancer)[2] currently 8th on the ATSDR[3] (Agency for Toxic Substances and Disease Registry, a division of the Centers for Disease Control and Prevention) list of agents of concern at high-priority pollutant sites. BaP is strongly associated with lung cancer (number one cause of cancer mortality). Previously, EPA IRIS estimated the oral exposure slope factor for lifetime cancer risk at 7.3 mg/(kg-day) (Linear Extrapolation Model, no threshold) but recently adjusted this risk factor to (1 mg/kg-day)[4,5]. This risk assessment is based on high dose rodent studies. Few studies have been done examining PAHs in human plasma and none following administration of defined doses.

Our hypothesis is that plasma levels of [^{14}C]-BaP and metabolites will exhibit a dose-response consistent with predictions based on a previously developed Physiologically-Based Pharmacokinetic (PBPK) model for BaP[6]. This hypothesis will be tested by micro-dosing individuals with [^{14}C]-BaP at levels of 25, 50, 100 and 250 ng (2.7-27 nCi). To date, our accelerator mass spectrometry (AMS) studies with PAHs have employed a single dose[7,8]. Pharmacokinetic parameters for [^{14}C]-BaP and metabolites will be assessed by UHLPC-AMS in plasma and urine collected over 48 hours. Metabolite profiles and kinetics of elimination over this dose range are predicted to be consistent with the BaP PBPK model developed by our present and past collaborators at Pacific Northwest National Laboratory (PNNL)[6]. The results from this study will be disseminated in peer-reviewed toxicology/environmental health journals, at scientific meetings and provided to the U.S. EPA for incorporation into their risk assessment models. The experimental procedure employs accelerator

mass spectrometry (AMS) for analysis. AMS (10^3-10^9 more sensitive for ^{14}C than scintillation counting) is increasingly used to determine pharmacokinetics of drugs under development. A dose given to human volunteers of no more than 1/100 the expected therapeutic dose or less than 100 µg of [^{14}C]-labeled drug (usually 100-200 nCi) is referred to as "microdosing" (Phase 0 studies or eIND studies). This approach has been validated for exploratory clinical development by the Consortium for Resourcing and Evaluating AMS Microdosing (CREAM Trial) and the EU Microdosing AMS Partnership Programme (EUMAPP). This early use of human subjects in drug development is consistent with the goals of the FDA Critical Path Initiative and AMS in exploratory IND applications has been addressed by FDA[9]. AMS has been used in toxicology and carcinogenesis studies. AMS sensitivity allows the study of pharmacokinetics and DNA binding of environmental contaminants known to be animal carcinogens and suspected of being human carcinogens including the cooked meat mutagens PhIP and MeIQx, the mycotoxin and human hepatocarcinogen AFB_1, in addition to BaP[10]. It is noteworthy that our group has successfully completed AMS studies with AFB_1 and the PAH, dibenzo[*def,p*]chrysene (DBC)[7,8,11]. IARC estimates that a non-smoker, not exposed occupationally, will receive a daily dose of 270-700 ng of BaP; about 95% through the diet[2]. The European Union maximum limit for BaP in fish is 2,000 ng/Kg f.w. and the FDA Limit of Concern (LOC) is 35,000 ng/Kg. The dose of 25 ng would be the equivalent of eating 12.5 or 0.7 g of fish at the EU, and FDA limits, respectively. The World Health Organization has set an estimated safe daily lifetime (70 years for a 70 Kg individual, cancer endpoint) exposure to BaP of 42-350 ng[12]. With respect to the internal dose of [^{14}C] of 2.7-27 nCi, this represents 0.3-3% the dose given in a common diagnostic procedure (^{14}C-urea test for *Helicobacter*)[13] and 4 and 5 orders of magnitude lower than a recently published paper dosing people with 300 µCi of epicatechin[14]**.** Therefore, from the standpoint of both chemical and radioisotope dose to the volunteers, this protocol represents *de minimus* risk and that was the finding of the FDA (IND 117175) in a "study may proceed" determination for our current study (IRB protocol 5644) which uses a dose of 46 ng (5 nCi).

11. Background Justification

An estimated 95% of daily BaP exposure (270-700 ng, non-occupational; non-tobacco) is dietary[1]. Especially high in charcoal-broiled or smoked meats and cheeses, almost all foods contain appreciable amounts. Exposure to PAHs, including BaP, is associated with cancer of the lung, skin, stomach, ovary and testis in addition to non-cancer chronic disease such as asthma, cardiovascular disease and diabetes[2]. The fetus and infant are especially susceptible to PAH exposure. Even though PAHs are ubiquitous environmental pollutants of potential concern to human health, there is little or no information on the pharmacokinetics of PAHs in humans. With the utilization of AMS located at Lawrence Livermore National Laboratory (LLNL), interfaced with Ultra-High Pressure Liquid Chromatography (UHPLC), it is possible to measure [^{14}C]-isotopically-labeled BaP and metabolites over time in human plasma and urine following administration of micro-doses.

The oral exposure slope factor for cancer risk (70 years, 70 Kg adult) for BaP using a linear, non-threshold model (EPA-IRIS)[5,15] was ,until recently, 7.3 mg/kg-day^{-1} when EPA issued a new report on BaP and lowered the cancer risk slop factor to 1 mg/(kg-day).[5] This risk assessment is based on high dose (1-100 mg/kg-day) exposures in rodents- **5-6 orders of magnitude higher doses than human exposures**. This risk assessment may not adequately protect some populations. Studies, including our own, with PAH mixtures indicate the linear extrapolated dose may under-estimate actual human risk using the Relative Potency Factor (RPF) approach[4,15]. The RPF approach assumes linear pharmacokinetics for PAHs and their metabolites with dose but this has never been assessed in

humans. For comparison, our preliminary results show the plasma levels, as determined by AMS at C_{max} (highest concentration obtained) after dosing individuals with 46 ng [^{14}C]-BaP, were ~ 8 fM (femto(10^{-15} moles/L)), **or 10^7 lower than an *in vitro* study (50 nM) showing no adverse impact on hepatocytes.** The sensitivity of AMS allows for micro-dosing with [^{14}C]-BaP in humans with *de minimus* risk to determine [^{14}C]-BaP and individual metabolites in the low fM range (2-20 femtograms/mL plasma). Our premise is- **the best model for humans is humans.** Utilizing PBPK models, we can provide a much more accurate estimate of BaP levels in human tissues following exposure at environmental levels as well as individual metabolites representing reduced or enhanced risk.

12. Multi-center Study – N/A
    LLNL will only receive coded, de-identified samples for analysis. Pacific Northwest National Labs will only receive coded, de-identified data for analysis.

13. External Research or Recruitment Site(s) - N/A

14. Subject Population

- Subject characteristics: Male and female subjects, 21-65 years of age
- Subject ethnicity and race – Subject race and ethnicity are expected to reflect the local population demographics. No attempt will be made to exclude or enrich for any ethnic or racial group. Our study is small, so we will recruit from the local population with English language skills sufficient to comprehend study consent documents and the potential risk and benefits involved in study participation. We will not be preparing documents in an alternative language, so it is possible that the ethnicity and race of our study subjects may be somewhat different from the distribution in the local community.
- Total target enrollment number: Up to 50 people will be enrolled in this study. Our goal is for 7 subjects to complete the study. We expect a high percentage of screen failure. If a large number of study enrollees qualify to participate in the study, we will stop enrolling subjects prior to reaching our maximum enrollment figure.
- Description of any vulnerable population(s): We will not involve vulnerable populations including children (20 years of age or younger), pregnant women, prisoners, non-English speakers, non-literate subjects, and adults lacking capacity to consent.
- Inclusion and exclusion criteria:

  Inclusion criteria for women:

  - Age 21-65 (inclusive)
  - Must be post-menopausal or have had surgical sterilization to eliminate any possibility for fetal exposure
  - Willing to defer blood donation for one month before, throughout, and one month after completion of study activities
  - Willing to avoid consuming cruciferous vegetables, I3C or DIM supplements, smoked or cured meat or cheeses, or charcoal-grilled meats for 2 weeks prior to and during each study cycle (gas grilled foods acceptable)

  Inclusion criteria for men:

- Age 21-65 (inclusive)
- Willing to defer blood donation for one month before, throughout, and one month after completion of study activities
- Willing to avoid consuming cruciferous vegetables, I3C or DIM supplements, smoked or cured meat or cheeses, or charcoal-grilled meats for 2 weeks prior to and during each study cycle (gas grilled foods acceptable)

Exclusion criteria for both men and women:

- Smoker (tobacco or other substances) or use of smokeless tobacco in past 3 months or living with smoker
- Regular use of medications that affect gut motility or nutrient absorption (e.g. cholestyramine, sucralfate, orlistat, pro- or anti-motility agents)
- History of gastrointestinal surgery (e.g. bariatric surgery, cholecystectomy) or gastrointestinal disorder (Crohn's disease, celiac disease, IBS, or colitis)
- Current or history of kidney or liver disease
- Prior high-dose ^{14}C exposure from medical tests. (micro-dose ^{14}C exposure not exclusionary)
- Occupational PAH exposure (e.g. roofers, asphalt pavers, fire-fighters, etc.)

- Description of any vulnerable population(s): We will not involve vulnerable populations including children 20 years of age or younger, pregnant women, prisoners, non-English speakers, non-literate subjects, and adults lacking capacity to consent.

  No attempt will be made to exclude or enrich for any ethnic or racial group; however, participants who cannot clearly understand the risks and benefits of the research as communicated to them in verbal and written English will be excluded. We do not have the resources to translate documents into other languages or to provide translators over the course of the study, so the demographics represented in the study subjects may be different from the distribution found in the local community.

- Recruitment: We will recruit subjects through postings in *OSU Today,* Craigslist, local regional and local media, and flyer advertisements placed on the OSU campus and throughout the Corvallis area (See attached recruitment documents). We also anticipate recruiting from the LIFE registry maintained by the Center for Healthy Aging Research (CHAR) at OSU. Except students and employees directly supervised by Williams, we will allow OSU students or employees to participate in the study. With the exception of contacting individuals on the LIFE registry, only subjects requesting information about the study will be contacted, and no subjects will be prospectively contacted by researchers without contact first being initiated by the individual.

  In situations that potential subjects respond to an abbreviated version of the full text recruitment posting (e.g. OSU Today's 75-word limit for postings), the coordinator can provide the full text of the recruiting flier via phone or email if more information is requested. Prior to study enrollment, the coordinator may also respond to general inquiries about study logistics (location, number of visits, study activities, the basic study schedule and type of specimen collected).

15. Consent Process

Waiver of documentation- We seek a waiver of documentation of written informed consent for the telephone screening to be able to collect information over the telephone prior to an in-person visit. This is necessary so that we can screen subjects for entrance criteria by telephone, thus saving the subject the inconvenience of making an unnecessary visit to the Clinical Research Center (CRC) if

they do not qualify and will permit study team to make the best use of their time by screening only those subjects who meet the minimum entrance criteria.

The **Telephone Screening Script** describes the process of obtaining verbal consent by asking the question "Are you interested in learning more about the study?" at the onset of the phone screening and providing a check box for "yes" or "no". If the potential subject agrees, the "yes" box is checked and the interview continues. If the box "no" is checked, the interview is terminated and any information gathered is shredded immediately. The **Telephone Screening Script** also contains study information to be provided to subjects before screening information is collected. A paper copy for subjects who meet minimum entrance criteria is filed in the subject's research record to document the telephone screening verbal consent along with their signed consent form obtained at the screening visit.

Written consent – The nurse coordinator will verbally review the informed consent document at the screening visit prior to any study activities taking place. We will offer subjects an opportunity to voice any questions or concerns, to take time to consult others (family members, health care providers), or do any other research that would help them understand the study activities before they sign the consent form. We will give a copy of the signed document to the subject with study team contact information.

Discussions regarding consent with potential subjects and acquisition of consent will take place in a private location with measures taken to ensure privacy (closed doors, periods of time between appointments with other subjects).

This consent process will be followed for all subjects, including those who have previously participated in Protocol 5644. Those subjects who choose to participate in both studies will be counted towards enrollment in both Protocol 5644 and this study (Protocol 8233).

- **Obtaining consent online**- N/A.

- **Assessment of comprehension**- We will assess comprehension of consent by asking subjects to briefly, in their own words, describe the details of the study, required activities, and their understanding of the risks involved.
- **Children** – N/A
- **Non-English speakers**- N/A. We do not propose to recruit non-English speaking subjects.
- **Student records** – N/A
- **Signatures on the consent form:**
    - Subject: Subject signatures indicate that the study has been explained to them, all of their questions have been answered, and they agree to be in the study.
    - Researcher: Researcher signatures indicate that the study was explained to the subject, comprehension was assessed and found to be sufficient, and the subject provided consent to participate in the study.
    - Witness/Translator signature: N/A.
    - Parent, guardian, or legally authorized representative: N/A.
- **Disclosure of significant new findings**- If, at any time during the study, new findings indicate a

possible increased risk or societal benefit which may influence the subjects desire to continue in the study, we will relate those findings to the subject.

- **Adult subjects with diminished capacity to consent** – N/A

16. **Assent Process**- N/A. No children will be recruited.

17. **Eligibility Screening**

We will initially screen interested subjects by telephone including questions from the eligibility checklist to determine if subjects meet minimum entry criteria. (See Telephone Screening Script.) Request for waiver of documentation of written informed consent for the telephone screening interview is described in the Consent Process section.

Subjects who qualify by telephone screening will be scheduled for a screening visit, which includes an in-depth informed consent discussion, brief health assessment and physical exam. (See Health Assessment Form) No screening visit activities will take place before the consent process.

18. **Methods and Procedures**

All study activities will take place in the Clinical Research Center in the Linus Pauling Science Center, room 407.

Screening visit (60 minutes)

The nurse coordinator will review the informed consent document including study activities, schedule and diet restrictions, answer questions, and provide further information as requested. After written consent is obtained, the nurse coordinator will collect demographic information, health history, height, weight, blood pressure and heart rate (see Health Assessment Form and Demographic Form). Female subjects will be asked to provide a spot urine sample for a pregnancy test. The study physician will perform a physical exam. Fasting is not required for this visit.

Evaluation of eligibility

The study physician will review Health Assessment information to assess eligibility to continue with the study. Subjects who qualify for this study will be notified of their eligibility and invited to participate.

Diet Restrictions

Subjects will be asked to avoid consuming cruciferous vegetables, I3C or DIM supplements, smoked or cured meat or cheeses, or charcoal-grilled meats for 2 weeks prior to and during each study cycle. Gas-grilled foods are allowed. Subjects will be provided a list of cruciferous vegetables and condiments to avoid.

Subjects will be asked to confirm they have followed the dietary restrictions before study cycle activities begin. Subjects who indicate that they have not followed the diet restrictions for 2 weeks will be allowed to have up to two dietary lapses with subsequent washout extension before they will be automatically removed from study participation due to lack of compliance.

Study cycles (4 total - 25, 50, 100 and 250 ng):

Subjects will be asked to complete a 3-day food diary covering the 3 days prior to each study cycle. Administration of this diary in our current study (IRB protocol 5644) showed that, on average, volunteers decreased their dietary BaP exposure by 35-54 ng/day[16]. During the study volunteers are required to keep a similar food diary. The results will be used to estimate dietary intake of PAHs using

the latest data from the Joint FAO/WHO Expert Committee on Food Additives (JECFA, http://www.food.gov.uk/multimedia/pdfs/poly-aromatic-hydrocarbons.pdf, accessed 11-7-2016).

Subjects will fast overnight (no food or drink besides water) before Day 1 of each cycle. Subjects will be asked to provide a spot urine sample for baseline analysis and then to empty their bladders completely. Female subjects' urine will also be used for a urine pregnancy test and test results must be negative to proceed with study activities. The results of these pregnancy tests will not be revealed to the subject in compliance with the Oregon Health Authority, Laboratory Compliance Section. Female subjects' pregnancy status will be confirmed via urine test at the start of each of the 4 cycles.

The IV catheter will be placed in an appropriate vein in the forearm or antecubital region by the nurse coordinator. Subjects will swallow a capsule containing the [^{14}C]-BaP with 100 mL water. Prior to every micro-dosing with [^{14}C]-BaP, a time zero blood sample of 20 mL will be collected with 10 mL analyzed for background plasma levels of BaP (and 62 additional PAHs) with GC-MS/MS[17] and the remaining 10 mL analyzed for [^{14}C]-BaP and metabolites as with subsequent time points.

Blood will be sampled at 0, 0.25 0.5, 1.0, 1.5, 2, 3, 4, 8, 24, and 48 hours (11 draws total, 8-9 of which come from the catheter). The catheter will remain in place for blood draws through hour 4. After the 4 hour blood draw, subjects will have the choice of having the catheter removed and returning for the 8 hour blood draw by straight stick, or they can keep the catheter in place and remain on-site until after the 8 hour blood draw. The subjects will be monitored by the nurse coordinator between blood draws. The 8 (possibly), 24, and 48 hour blood draws will be done with straight stick phlebotomy. No more than three (3) skin punctures will be made in an attempt to draw blood at each visit. The first blood draw of each cycle will be 20 mLs and then subsequent blood draws will be 10 mL for a total amount of 120 mLs (8 tablespoons) per cycle.

Subjects will be instructed to collect all urine during the entire 48-hour study cycle in containers provided by the study team. Subjects will be provided a discrete soft-sided cooler bag to store collected urine samples. They will be instructed to store samples at room temperature between visits and to return any filled containers at their next visit. The longest subjects will need to store samples between visits is 24 hours between Visits 3 and 4.

Two hours after swallowing the capsule, subjects can order breakfast from a menu from Ava's Café or other local cafe, after which subjects may resume normal eating and drinking. Packaged snacks or juice will be provided prior to the standardized breakfast if the study team determines it is necessary.

This process will be followed for each dose (25, 50, 100 and 250 ng [^{14}C]-BaP) for a total of 4 cycles per subject. There will be at least three weeks' washout period between cycles. Previous multiple micro-dosing of the same individual has shown that after 3 weeks there is no detectable [^{14}C] in plasma or urine, validating the choice of 3 weeks as adequate for a "washout period"[7,8]. Extending the washout period will not adversely impact the data collected from the subjects but will minimize the subject's exposure to PAHs.

Subjects will be asked to defer blood donation for one month before, throughout, and for one month after completion of the study. At any point during the study, subjects will be referred to their physician if the study physician or nurse consider this advisable.

Processing of samples:

Blood samples will be centrifuged to obtain plasma and periperal blood mononuclear cells (PBMCs). The [^{14}C]-BaP and metabolites will be extracted directly from plasma by liquid/liquid extraction with

ethyl acetate. The urine will be pooled over the time periods pre-capsule (baseline), 0 – 12, 12-24 , and 24-48 hours. After volumetric quantitation of each pool of urine, 12 mL will be collected, 4 mL will be adjusted to pH 5 and half treated with β-glucuronidase/sulfatase prior to extraction with ethyl acetate. The plasma and urine extracts will be blown down under argon, sealed in amber vials and shipped to LLNL where they will be injected onto an Ultra-High Pressure Liquid Chromatograph (UHPLC) interfaced via a "moving wire" into the AMS[18]. We will be able to determine femto(10^{-15})g/mL levels of BaP and BaP metabolites (by co-elution with unlabeled standards available from the OSU Superfund Research Center PAH repository). The time-course of blood and urine levels of parent BaP and metabolites will be used to construct a pharmacokinetic model[6]. In addition, DNA isolated from PBMCs at 0 hr and 48 hr will be counted directly on the AMS to check for levels of covalent DNA binding.

19. Compensation

Subjects will receive $125 for each completed cycle. Subjects who discontinue the study early will be paid an amount pro-rated to the percentage of total blood samples completed. The amount will be determined by multiplying the number of study blood samples completed by $11.36.

20. Costs

There will be no cost to subjects for being in the study. A parking space outside of LPSC is reserved for study subjects during their visits.

21. Drugs or Biologics

a) Name: [7-^{14}C]-benzo[*a*]pyrene (BaP), specific activity 27 µCi/µmol
b) Approval status: not approved for therapeutic use
c) Chemical formula: $C_{20}H_{12}$
d) Dosage strength(s): 25, 50, 100 and 250 ng
e) Rationale for choosing the drug or substance dose: The U.S. EPA is considering a model of risk assessment for PAH mixtures that utilizes the Relative Potency Factor (RPF) approach, derived from animal studies[4]. BaP is the reference compound and has an RPF of 1. The accuracy of the RPF approach is based on the assumption that the interaction between all PAHs is additive (same Mechanism of Action or MOA) and that high-dose animal data can model human exposure at environmentally relevant levels (orders of magnitude lower than the animal studies). Until now, it has not been possible to test these caveats in humans. The administration of four microdoses of [^{14}C]-BaP will allow us to better determine PAH risk assessment and pharmacokinetics at environmentally relevant levels of exposure in humans.
f) Method/route of administration: Benzo[a]pyrene is not a drug and will be referred to as the "test article" product. Capsules intended for human use will be prepared in the Linus Pauling Science Center room 383, in a clean hood dedicated to radiolabeled chemicals. The test article product will be prepared as a capsule containing the test article product and lactose as an excipient. A work area in the fume hood will be sterilized with bleach and alcohol and all work surfaces will be covered with fresh, plastic backed laboratory bench paper. All pipettes are calibrated and only used with filter pipette tips and all Hamilton syringes are purchased new, checked for accuracy, and dedicated to this project. Solaray empty vegetarian capsules, size 0, composed of vegetable cellulose, will be opened and held in a microtube rack. The empty capsules will be filled with pharmaceutical grade lactose monohydrate NF (Spectrum Chemical Mfg. #61-1730890). The dosing solution will be applied to the lactose with a Hamilton syringe (2.7, 5.4, 10.8, or 27 nCi in 25 µL ethanol), allowed to evaporate briefly, then sealed with capsule caps. All capsules are manufactured within 7 days of ingestion and stored at -20°C in an airtight

container with desiccant packets prior to ingestion. An excess of 3 capsules minimum are prepared to verify the consistency of the dose. These quality control capsules are vortexed in 5 mL of pH 7 water for 5 minutes, at which point they are dissolved. Fifteen mL of scintillation cocktail is then added prior to scintillation counting. If radioactive content shows variation greater than 10 percent from the expected dose or from each other, the batch will be rejected and a new preparation will be made. Prior to being filled, empty Solaray® capsules are stored in an airtight bag in a gasket sealed plastic box, in a cabinet in a lab that does not utilize scintillation detectable radioactivity or human tissues. This box contains all microdosing supplies in a separate area than traditional laboratory supplies. After dosing capsules are prepared, they are stored in a new glass scintillation vial containing silica desiccant packets with a PTFE lined airtight cap. The vial is stored in a sealable plastic bag in a -20o C freezer until use, which must be within five (7) days of preparation. Each ziplock bag will have the appropriate label applied (see sample label). The batch size will be up to five (5) capsules so that up to two (2) volunteers enrolled per week plus three (3) capsules for quality control. Several batches will be prepared over the course of the study due to rate of volunteer recruitment, the time interval between dose cycles and different dose cohorts. Capsules that are rejected for human use or those that have expired will be destroyed and disposed of in the radiochemical waste at Oregon State University.

g) Mechanism of action: BaP is not a therapeutic, so the mechanism of action described involves the current understanding of BaP carcinogenesis. The most well accepted mechanism for BaP carcinogenesis involves metabolic activation[19]. Bioactivation to the ultimate carcinogen is initiated by epoxygenation at the 7,8-position. The epoxide is hydrolyzed by the action of the enzyme epoxide hydrolase and a second epoxygenation produces the ultimate mutagenic and carcinogenic metabolite, the BaP-7,8-dihydrodiol-9,10-epoxide. The (+)-*anti*-BaP-7,8-dihydrodiol-9,10-epoxide metabolite is the most mutagenic and carcinogenic form of BaP. The metabolite is formed by oxidative metabolism by cytochrome P450 enzymes (CYPs) enzymes, primarily in the liver (also to some degree in lung and GI). The epoxide is chemically unstable and has been shown to react with the N^2 position in guanine in DNA which can lead to mutations in genome sequence.

h) Known drug interactions: Not applicable at this microdose

i) Manufacturer/Sponsor: American Radiolabeled Inc. (ARC, custom synthesis)

j) Manufacturer/Sponsor location: St. Louis, Missouri

k) Name of supplier: Same as Manufacturer (custom synthesis)

l) IND Status: This protocol is an amendment to IND 117175.

m) Documentation or certification of quality or purity: The stock manufacturer provided documentation of radiochemical purity (99.2%) and a specific activity of 27 nCi/nmol. We will check the purity by collected HPLC fractions (U.V. detector) and the radioactivity by an on-line radioisotope detector. The specific activity will be confirmed by injection of the [^{14}C]BaP onto HPLC and quantifying the mass by U.V. using a standard curve of unlabeled BaP standard (NCI, Chemical Carcinogen Repository). The radioactivity in the BaP HPLC peak will be determined by on-line radioisotope detector. Both the chemical purity (U.V. peak co-eluting with unlabeled standard BaP) and the % of the total ^{14}C within the BaP peak must be 95% or greater. If the purity falls below 95%, the stock will be re-purified to the 95% standard before administration to study subjects. We do not expect a problem maintaining radiochemical purity as [^{14}C]-BaP is known to be stable under the storage conditions described below.

Quarterly purity analyses will be provided to the Oregon State University IRB and Radiation Safety

except in the case of a several month lapse in active study subjects or because the study is complete. In either case, the study team will notify Radiation Safety (Dan Harlan) and the OSU IRB. Prior to resuming study cycle visits, the study team will perform a purity test and quarterly analyses will resume henceforth. The study team will notify Radiation Safety and the IRB of the reactivation of quarterly tests and will provide test results to Radiation Safety for review of purity tests and calculations.

Sample Label:

7-^{14}C Benzo[a]pyrene Microdose Capsule
Dr. David E. Williams, Oregon State University
Linus Pauling Science Center
541-737-3277

RADIOACTIVE – 2.7 nCi/Capsule - 25 ng 7-^{14}C Benzo[a]pyrene
≥ 95 percent purity
Take 1 capsule by mouth
Prepared on DD/MM/YYYY

Caution: Limited by United States law to investigational use only

Summary of preclinical and early human studies (for studies with an IND): BaP is one of the most extensively studied PAH environmental contaminants. BaP is a skin carcinogen in the rodent 2-stage model involving dermal application and promotion by TPA[1,2]. In addition to dermal exposures, BaP has been documented as an animal carcinogen following oral or inhalation exposures. Target tissues include liver, lung, forestomach, esophagus, auditory canal and oral cavity. Occupational exposures in humans are associated with increased incidences of cancers of the lung, skin and bladder[2].

Animal studies in mouse and rat confirm the carcinogenic potential of BaP when administered by the oral route, a mimic of human exposure[2]. Dose-dependent appearance of tumors can be observed in the forestomach, lung, esophagus, breast, tongue and larynx in a somewhat reliable manner. A summary of animal studies in which BaP was administered by the oral route is provided in the table below, the dose information was converted to mg/kg for ease of comparison. The summary only includes studies which evaluated untreated control animals as a negative control.

Animal Studies with Orally Administered Benzo[a]pyrene.

Species	Dose (mg/kg)	Route; Regimen	Duration	Tumor Observations	Reference (all in Ref 2)
Mouse,A/J	0 550 3,350	Oral, Diet	260 days	Forestomach tumors 0/21 (0%) 5/25 (20%) 27/27 (100%)	Weyand et al., 1995 Chem Res Toxicol 8(7): 949-954

Mouse, B6C3F1	0 5 25 100	Oral, Diet	2 years	Forestomach tumors 1/48 (2%) 3/47 (6%) 36/46 (78%) 46/47 (98%)	Culp et al., 1998 Carcinogenesis 19(1): 117-124
Mouse,Swiss	0 50	Oral 2X/week, 4 weeks	27 weeks	Forestomach tumors 0/10 (0%) 10/10 (100%)	Badary et al., 1999 Eur J Canc Prev. 85 435-440
Mouse, Muta	0 75 125	Oral, 5 consec. days	41 weeks	Forestomach tumors 0/8 (0%) 10/10 (100%) 10/10 (100%)	Hakura et al., 1998 Regul Toxicol Pharmacol 27(3): 273-279
Rat, Sprague Dawley	0 6 39	Oral, 1x/9 days 5X/week	Lifespan	Combined tumors 3/64 (4.7%) 3/64 (4.7%) 10/64 (15.6%)	Brune et al., 1981 J Canc Res Clin Oncol 102(2): 153-157
Rat Crl:CD	0 63	Oral, 1x/week 8 weeks	49 weeks	Mammary Tumors 1/30 (3%) 11/30 (37%)	El-Bayoumy et al.,1995 Carcinogenesis 16(2):431-434

The mouse study of Culp et al., 1998 (see above Table) utilized the lowest **lifetime** dietary exposure of 5 mg/kg (a dose 100,000 times greater than the dose in our microdose studies) which did not produce a significant increase in tumor incidence. This is similar to the rat study of Brune et al., 1981 (see above Table) in which a dose of 6 mg/kg did not result in an increase in tumors when animals were exposed and monitored over their lifetime.

- Justification and safety information if FDA approved drugs will be administered for non-FDA approved indications or if doses or routes of administration or subject populations are changed: N/A.
- Justification and safety information if non-FDA approved drugs without an IND # will be administered: N/A.
- Plan for the storage, dispensing, handling, inventory control, and disposal of investigational and FDA-approved drugs and biologics:

  Storage and handling:

  The lab where the capsules are prepared (LPSC 383) is not a radiopharmacy. Because these capsules are created solely for the purposes of this study and are not to be considered a theraputic drug, they are not made to GMP standards but will be prepared with the highest quality, consistency and precision possible. Room 383 is a locked, secure laboratory, and no other activities occur in this space besides those related to BaP handling and capsule preparation. Siddens and Williams are the only persons with keys to access this space.

The [^{14}C]-BaP is shipped as a concentrated solution (0.10 mCi/ml; specific activity = 27 nCi ^{14}C/nmol BaP = 0.103 nCi/ng) in toluene. This toluene stock is stored in sealed containers as received from the supplier at -80°C in a secure carcinogen laboratory in LPSC 383. In order to ensure radiochemical purity (greater than or equal to 95% for the purified concentrated stock) and to prepare a diluted sample suitable for human consumption, the compound is collected as a single peak from an HPLC run. The identity of the compound is confirmed by co-elution with a non-radioactive standard.

A working stock solution will be prepared from the 0.10 mCi/mL solution at the highest concentration used in the dosing capsules, (250 ng BaP/25 µl =10 ng/µl; 27 nCi ^{14}C/25 µl=1.08 nCi/µl) using 95% food grade ethanol. For example to make 10 ml of a stock at this concentration, 108 µl of the original stock will be aliquoted into a clean vessel. Toluene is then carefully evaporated off and the ^{14}C-BaP aliquot resolubilized into 10 ml food grade ethanol. The radioactivity will be checked with a liquid scintillation counter and adjusted to the target concentration. This can then be aliquoted into clean, amber 2 ml vials and stored under argon at -80°C. All other dosing stocks will be made diluting this highest concentration with additional food grade ethanol to achieve all four doses (see table below). These working stocks are stored under argon at -80^{o}C when not in use. Each stock is checked for ≥ 95% radioisotope purity on a quarterly schedule throughout the study. Stocks will be checked on UPLC using UV detection for BaP and radioisotope detection for ^{14}C.

^{14}C Dose (ng)		Radioactivity (nCi)		Dilutions from ^{14}C-BaP 10 ng/µl stock to make 2 ml	
Per 1 µl	Per Capsule 25 µl	Per 1 µl	Per Capsule 25 µl	BaP 10 ng/µl stock	100% Food Grade Ethanol
1	25	0.108	2.7	0.2 ml	1.8 ml
2	50	0.216	5.4	0.4 ml	1.6 ml
4	100	0.432	10.8	0.8 ml	1.2 ml
10	250	1.08	27.0	2 ml	0 ml

Capsules intended for human use will be prepared in a clean hood dedicated solely to capsule preparation. ^{14}C-BaP stock solution is not diluted in the same area as capsule preparation. The hood where capsule preparation is performed will be surveyed for radioactivity by taking swipes and evaluating with liquid scintillation counting. The capsule preparation hood will be sterilized with bleach and alcohol, and all work surfaces will be covered with fresh, plastic backed laboratory bench paper. All pipettes are calibrated, dedicated to this procedure, and only used with sterile, filter pipette tips and all Hamilton syringes were purchased new, checked for accuracy, and dedicated to this project.

As an added safety measure, Uesugi will assist Siddens with capsule preparation for study subjects providing quality control and ensuring that a second trained individual is always present to assist, confirm and verify capsules are produced per specified SOPs.

Prior to being filled, empty capsules and lactose excipient are stored in an airtight bag in a gasket sealed plastic box, in a cabinet in a lab that does not utilize scintillation detectable radioactivity or human tissues.

Each capsule contains only the [^{14}C]-BaP test article product and lactose as an excipient. Solaray Size 0 empty vegetarian capsules, composed of vegetable cellulose, will be opened and held in a microtube rack. The empty capsules will be filled with pharmaceutical grade lactose monohydrate (Spectrum Chemical Mfg Group). The 25 µL aliquot of dosing solution of the chosen concentration (25, 50, 100 or 250 ng [^{14}C]-BaP) will be applied to the lactose in each of the 5 capsules using a calibrated pipet fitted with a sterile filter tip. The ethanol will be allowed to evaporate, then the capsules will be sealed with capsule caps.

Immediately after capsule preparation, 3 of the 5 capsules will be dissolved in 5 ml water and 20 ml scintillant and counted on a liquid scintillation counter. Results wil be emailed directly to Dan Harlan, the director of OSU Radiation Safety (RSO). Once RSO has determined that the three evaluated capsules are within 10% variance and 10% error, the results are emailed to Siddens. Siddens will then contact Williams and Uesugi that the cycle may proceed. If the capsules are not given RSO approval, the participant is rescheduled and a new set of capsule will be prepared. Two capsules will be stored in sealed vials in the dark in a zipper-lock plastic bag with silica desiccant at -20°C until subject consumption. One capsule per cycle will be given to subjects. The remaining capsule will be for back-up in case of any problems or disposed in radioactive dry waste if not needed. Any capsules not used within 1 week will be discarded.

Dispensing: Capsules will be transported to the Clinical Research Center (407 LPSC) in sealed glass vials within a zipper-lock polypropylene bag for secondary containment. The bag will be labled as described above and stored within a plastic box for transportation to the clinic.

One capsule containing 25, 50, 100 or 250 ng [^{14}C]-BaP will be administered to subjects with 100 mL of water by the nurse coordinator at the start of the study cycle.

Inventory control: Information including number of capsules, date of preparation, and operator are recorded immediately after capsules are prepared. Date of consumption and disposal of test capsules are recorded on day 1 of the cycle. If test capsules do not pass approval by the OSU radiation safety officer, the information is still recorded and a new set of capsules will be prepared.

Disposal: Capsules that are not needed, expired or rejected for human use will be disposed of in the radiochemical waste at Oregon State University.

- Explain whether the use of the test article involve a route of administration or dosage level, use in a subject population, or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with its use:

  The great majority of exposure (>95%) of the larger molecular weight PAHs, such as BaP, is through the diet in a variety of foods including breads and cereals, grains, vegetables and smoke-cured or barbequed meats[2]. Dietary intakes of total PAHs in the U.S. have been estimated at 160-1600 ng/day with BaP alone at 270-700 ng/day. A 2005 report from the FAO/WHO Joint Expert Committee on Food Additives and Contaminants listed a mean BaP daily dietary intake of 270 ng (70 Kg individual) with 700 ng as a high-level intake[1]. The European Union maximum limit for BaP in smoked meats is 5,000 ng/Kg fresh weight. The dose of 250 ng would be the equivalent of approximately 45 g of smoked meat at the EU allowable limit. Compiling all of the animal data, a **Virtually Safe (Lifetime) Dose (VSD) of 42-350 ng/person/day** has been established as the best estimate for a lifetime exposure to BaP producing no more than 1 cancer per million people[1-4]. Therefore, based on the chemical mass involved in microdosing (significantly lower than

background exposure and levels determined to be VSD), this study poses *de minimus* risk to subjects.

22. **Food.** Subjects will be provided breakfast on the first morning of each cycle. Up to $10 worth of food and/or beverage will be purchased at Ava's Café in LPSC or another local café.

23. **Medical Devices**- N/A.

24. **Radiation-** Radiation Safety approval form included.

    It should be noted that in our study, a cumulative total of 45.9 nCi for the 4 dose cycles of [^{14}C]-BaP is equivalent to about 170 minutes of natural background radiation. Patients have been given 1,000 nCi of ^{14}C in the urea breath test for the diagnosis of *Helicobacter pylori*[13] and a recent study utilized a dose to human volunteers of 300,000 nCi of [^{14}C]-epicatechin (a polyphenol from green tea)[14]. The chemical form of ^{14}C (BaP) to be used in the present study can not be broken down to CO_2, as in the urea breath test, and thus could not be incorporated into metabolic pathways in the body. In animal studies, PAHs are eliminated rapidly (hours to days) from the body and this has been confirmed by our PAH microdosing study with dibenzo[def,p]chrysene (Figure 1).[7,8]

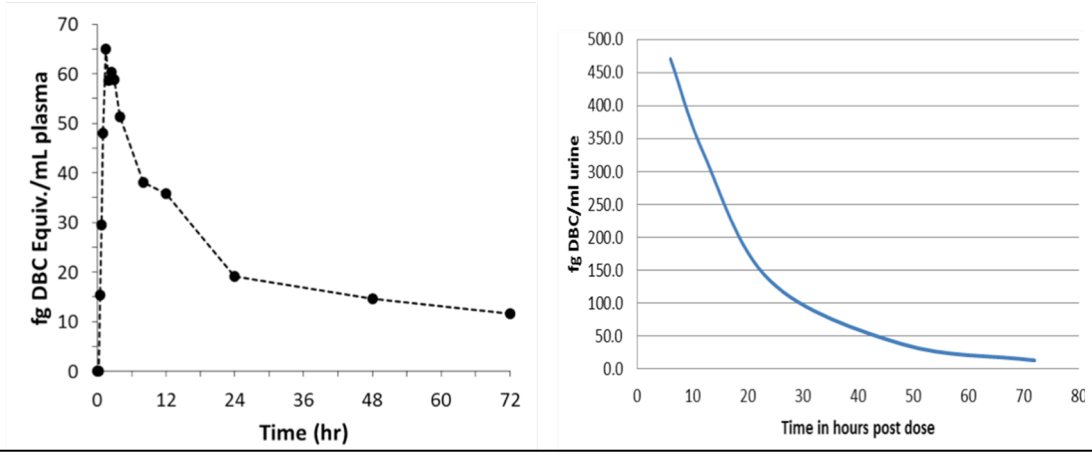

Figure 1. Pharmacokinetics of DBC in Humans Following a Dose of 5 nCi (29 ng) (IRB Protocol #3853)

25. **Biological Samples**

- A Biological Materials Form is included.
- Biological samples as described above in the Methods and Procedures section will be obtained from living subjects, prospectively, for this research.
- The total amount of blood collected from each subject is 480 mL (4 x 120 mL/cycle). The maximum amount of blood collected in an 8 week period is 240 mL.
- Samples will be identified by a code that will be protected as described in the Anonymity and Confidentiality section below. A portion of the de-identified coded study samples will be forwarded to LLNL for analysis with no personal identifying information contained within the shipment, so there is no risk of loss of confidentiality with the mailing of samples to LLNL nor the analysis of samples at LLNL. The remaining coded samples will be stored in LPSC 389 at OSU

indefinitely unless not allowed by subjects (see Anonymity and Confidentiality section). Coded samples will be retained for potential future research projects relating to the study objectives. The consent form includes a section for subjects to give permission to use their samples in future projects without being contacted for consent.

- Samples sent to LLNL will be processed at OSU before shipment and are no longer considered biohazardous materials after processing. The radioactivity level in collected blood and urine are well below what is considered a background level when we conduct routine laboratory swipe surveys (below the limit of detection by liquid scintillation counting) and thus, these samples are not considered radioactive material. OSU personnel processing the samples are not exposed to above background levels of radioisotopes and /or carcinogen. This material does not pose a biohazard, carcinogen hazard, or radioactivity health risk to postal carriers or LLNL employees.
- No medical records will be accessed.
- Only urine pregnancy tests will be conducted with female subjects at the screening visit and before each cycle. No results will be shared with subjects.
- Although one of the study aims is to determine if there is genetic variability between subjects that could account for variability in study outcomes, this is not "genetic testing" in the usual sense as only a few genes will be assessed for allelic variants and results will not be disclosed to subjects (as there is no established relationship between any of the allelic variants and disease). Coded samples will be stored in LPSC 389 at OSU indefinitely unless not allowed by subjects (see Anonyminity or Confidentiality section). Coded samples will be retained for potential future research projects relating to the study objectives.
- DNA will be stored and not shared. Subjects will not be contacted in the future as there will be no relevant clinical data to share. The subject can indicate on the consent form whether or not he/she agree to have a small number of genes (maximum 7) known to be involved in metabolism and excretion of BaP analyzed. The results will not be shared as there is no established disease risk; no family relationships will be tested or disclosed. For disclosure of clinically relevant information see bullet above.
- Plasma extracts and urine samples will not be returned from Lawrence Livermore National Laboratories but will be discarded by them upon completion of their analysis.

26. <u>Anonymity or Confidentiality</u>

- We will handle all research records as confidentially as possible and retain for three years post-study termination. We will protect all computer records by passwords and keep all paper study records in a locked cabinet in a locked room accessible only to the study team.
- Only the nurse coordinator will have access to the document linking identifiers to subject code numbers. Personal information collected during telephone screening, the consent documents, and compensation acknowledgement form will be the only documents containing subject name and personal information. We will collect direct identifiers (name, mailing address, email and phone number) for the purpose of contacting the subject througout the course of the study. We will store this information separately from coded data. Health assessment, subject demographic documents and other research information obtained during the duration of the study will also be identified only by ID code and kept in a location separate from any materials that may identify the subjects.

- At the completion of the data analysis of the study, we will keep only coded sample data. At this time, we will shred and destroy any information linking individual identifiers to subject codes.

- We will not provide materials containing ID codes and personal identifiers to anyone outside the research team. Also, we will not use individual identities in reports or publications resulting from this study. This study does not involve medical, educational or other personal records.

- We will report names of subjects enrolled from the CHAR Life Registry back to CHAR at the completion of the recruitment phase or earlier if requested.

- Data will be reported in publications in aggregate or coded such that subjects will not be indentifiable.

- Any information stored electronically will be on a computer system with a fully patched operating system and applications, and current antivirus software with current virus definitions and a plan for routine back-ups is in place.

- We will protect email correspondence by deleting after the subject has completed the study, is removed or withdraws from the study, or has been found to not qualify.

- We will only identify biological samples collected during the study by non-identifying code. We will process in-house or to LLNL for analyses with no personal information of the subjects provided.

- We will immediately destroy notes taken during telephone screening conversations with excluded subjects. We will keep records from subjects who are excluded from the study after being enrolled confidential and stored securely as described above.

- Coded blood and urine samples will be sent to LLNL for analysis of study endpoints. Any sample remaining after analysis will be destroyed at LLNL. Coded data will be sent to PNNL for analysis. Coded samples not sent to LLNL will be stored in LPSC 389 at OSU indefinitely unless subjects do not give permission to store samples and data for future studies.

- If a subject does not give permission to use of their data and samples for use in future studies, their samples will be discarded according to OSU Biosafety policies and their data will be excluded from data sets used for future studies.

- This research is covered by a Certificate of Confidentiality from the National Institutes of Health. This Certificate has been deemed issued.

27. <u>Risks</u>

Potential risks of participation in this study are as follows:

BaP: The International Agency for Research on Cancer (IARC) has determined that BaP is a Class 1 known human carcinogen[2]. Humans are exposed to PAHs, including BaP, from a number of sources, the highest being occupational and smoking, so we are excluding these populations. In the general population, the greatest BaP exposure is through diet (9.5-43.5 ng/day inhalation; 1 ng/day water;

160-1,600 ng/day diet)[1-5]. The dietary restrictions during this study are intended to offset the total 425 ng BaP consumed in 4 study cycles. This is equivalent to less than or equal to 4.2 ng/day over the 101 day (minimum) study period.We estimate that following our dietary restrictions will reduce subject daily dietary exposure to BaP by 35-54 ng/day. Therefore, subjects are not exposed to an increased risk of cancer.

Radiation: The total radiation dose of 45.9 nCi for the 4 dose cycles of [^{14}C]-benzo[a]pyrene is equivalent to about 170 minutes of natural background radiation. The risk from the ultra-low doses in this study is negligible.

Blood Sampling: Risks of blood sampling include pain, bruising, and in rare instances, infection. Some individuals can become lightheaded or nauseated. A qualified research nurse or certified phlebotomist will perform blood daws and carry out all necessary precautions to reduce the risk of injury to subjects.

Blood Loss: While the risk of negative impact due to blood loss is negligible, subjects will be asked to refrain from blood donation one month before the study until one month after completion of the final cycle.

Food allergens: Foods and beverages provided during this study may contain allergens. Ingredient lists and packaged foods with clearly labeled allergen information will be provided to subjects upon request. Subjects will be asked to notify the study nurse of any food allergies before ordering breakfast.

Confidentiality/anonymity: Loss of confidentiality or anonymity is a potential risk. See Confidentiality and Anonymity section.

Reporting of adverse events: Adverse events will be reported to the IRB and the FDA in accordance with each entity's policy.

Data and Safety Monitoring Plan: The *Data and Safety Monitoring Plan* document outlines risks and measures to minimize risk as listed above, reporting frequency and report content that will be provided to NIEHS, FDA and the OSU IRB. We do not have an independent Data and Safety Monitoring Board because this is a single-center study.

28. Benefits

This study is not designed to be of benefit to individual subjects. Potential benefits to society include essential information to aid regulatory agencies with respect to how environmental PAHs, such as BaP, at environmentally relevant levels of exposure are taken up by the G.I. (96% of carcinogenic PAH exposure in humans is dietary), metabolized and excreted from the body. That information can be used in modeling risk assessment rather than high-dose animal studies. This will provide a mechanism for improvement of public health

29. Assessment of Risk:Benefit ratio

The potential benefits in the form of knowledge gained for determination of exposure risk factors and promotion of human health outweigh the potential identifiable minimal risks.

References cited:

1. JEFCA (Joint FAO/WHO Expert Committee on Food Additives), 2005. Exposure to carcinogenic PAHs in the environment. Environm. Sci. Technol. 26, 1278-1284.

2. International Agency for Research on Cancer, 2010. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol. 92, Some Non-Heterocyclic Polycyclic Aromatic Hydrocarbons and Some Related Exposures, Lyon France.
3. ATSDR, 2013. Priority list of hazardous substances, https://www.atsdr.cdc.gov/spl/ (accessed 07/11/2017).
4. U.S. EPA (2010) Development of a relative potency factor (RPF) approach for polycyclic aromatic hydrocarbon (PAH) mixtures (External Review Draft), U.S. EPA, Washington, D.C., EPA/635/R-08/012A.
5. EPA (2017) Toxicological Review of Benzo[a]pyrene, Integrated Risk Information System, National Center for Environmental Assessment, Office of Research and Development, EPA, Washington, D.C., [CASRN 50-32-8]. EPA/635/R-17-003, www.epa.gov/iris.
6. Crowell, S.R., Amin, S.G., Anderson, K.A., Krishnegowda, G., Sharma, A.K., Soelberg, J.J, Williams, D.E. and Corley, R.A., 2011. Preliminary Physiologically Based Pharmacokinetic Models for Benzo[*a*]Pyrene and Dibenzo[*def,p*]Chrysene in Rodents. Toxicol. Appl. Pharmacol. 257, 365-376.
7. Madeen, E., Corley, R.A., Crowell, S., Turteltaub, K., Ognibene, T., Malfatti, M., McQuistan, T.J., Garrard, M., Sudakin, D. and Williams, D.E., 2015. Human in vivo pharmacokinetics of [(14)C] dibenzo[*def,p*]chrysene by accelerator mass spectrometry following microdosing. Chem. Res. Toxicol. 28, 126-134.
8. Madeen, E.P., Ognibene T.J., Corley, R.A., McQuistan, T.J., Henderson, M.C., Baird, W.M., Bench, G., Turteltaub, K.W. and Williams, D.E. (2016) Human microdosing with carcinogenic polycyclic aromatic hydrocarbons: in vivo pharmacokinetics of dibenzo[*def,p*]chrysene and metabolites by UPLC accelerator mass spectrometry. Chem. Res. Toxicol. 29, 1641-1650.
9. Food and Drug Administration, 2006. Guidance for industry, investigators, and reviewers-exploratory IND studies. U.S. Department of Health and Human Services, FDA, CDER, Rockville, MD.
10. Lightfoot, T.J., Coxhead, J.B., Cupid, B.C., Nicholson, S. and Garner, R.C., 2000. Analysis of DNA adducts by accelerator mass spectrometry in human breast tissue after administration of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine and benzo[a]pyrene. Mutat. Res. 472, 119-127.
11. Jubert, C., Mata, J., Bench, G., Dashwood, R., Pereira, C., Tracewell, W., Turteltaub, K., Williams, D. and Bailey, G., 2009. Effects of chlorophyll and chlorophyllin on low-dose aflatoxin B_1 pharmacokinetics in human volunteers. Cancer Prev. Res. 2, 1015-1022.
12. WHO, Benzo[a]pyrene (WHO Food Additives Series 28) in http://www.inchem.org/documents/ehc/ehc/ehc202.htm#SubSectionNumber:7.1.6 (accessed June 17, 2016), United Nations Environment Programme, International Labour Organization, World Health Organization, International Programme on Chemical Safety, Environmental Health Criteria 202, Selected Non-Heterocyclic, Polycyclic Aromatic Hydrocarbons, ISBN 92 4 157202 7,(NLM Classification: QD 341.H9),ISSN 0250-863X.
13. Atherton, J.C. and Spiller, R.C. (1994) The urea breath test for *Helicobacter pylori*. *Gut* 35:723-725.
14. Ottaviani, J.I., Borges, G., Momma, T.Y., Spencer, J.P.E., Keen, C.L., Crozier, A. and Schroeter, H. (2016) The metabolome of [2-^{14}C] (-)-epicatechin in humans: implications for the assessment of efficacy, safety, and mechanisms of action of polyphenolic bioactives. Nature Sci. Reports 6:29034.
15. U.S. EPA Toxicological review of benzo[a]pyrene (CASRN 50-32-8), www.epa.gov/iris.

16. Hummel, J.M., Madeen, E.P., Siddens, L.K., Uesugi, S., Krueger, S.K. and Williams, D.E. (2016) Estimated dietary exposure to benzo[a]pyrene (BaP) during clinical dietary restriction. Abstract, 2016 annual meeting of Diet and Optimal Health, Linus Pauling Institute, Corvallis, OR.
17. Forsberg, N.D., Wilson, G.R. and Anderson, K.A. (2011) Determination of parent and substituted polycyclic aromatic hydrocarbons in high-fat salmon using a modified QuEchERS, extraction, dispersive SPE and GC-MS. J. Ag. Fd. Chem. 59:8108-16.

18. Thomas, A.T., Ognibene, T., Daley, P., Turteltaub, K., Radousky, H. and Bench, G., 2011. Ultrahigh efficiency moving wire combustion interface for online coupling of high-performance liquid chromatography (HPLC). Analy. Chem. 83, 9413-9417.
19. Shimada, T., Gillam, E.M.J., Oda, Y., Tsumura, F., Sutter, T.R., Guengerich, F.P. and Inoue, K.. 1999. Metabolism of benzo[a]pyrene to *trans*-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene by recombinant human cytochrome P450 1B1 and purified liver epoxide hydrolase. Chem. Res. Toxicol. 12, 623-629.

Statistical Analysis Plan

Pharmcokinetic parameters were evaluated for linearity using a best fit modeling approach. First, pharmacokinetic parameters were evaluated for significant change as a functin of dose using a standard linear regression model including a fit y-intercept. Slopes were compared using a *t*-test and an alpha value of 0.05. If the parameter changed as a function of dose, we further evaluated the parameter with a linear regression model, with *k* as the slope through the origin and a Michaelis-Menten model, which assumes saturation at V_{max} and an affinity constant K, to individual parameters (p) as a function of extermal [^{14}C]-BaP dose. The Baysesian information criterion (BIC) was used to judge the best-fit model and provide evidence of linear or saturable pharmacokinetics.

CONSENT FORM

Project Title:	**Benzo[a]pyrene Ultralow Dose-Response Study**
Principal Investigator:	David E. Williams, PhD
Co-Investigators:	Douglas Aukerman, MD Sandra Uesugi, RN Lisbeth Siddens Jamie Pennington
Sponsor:	National Institutes of Health, National Institute of Environmental Health Sciences

---

1. WHAT IS THE PURPOSE OF THIS FORM?

This form contains information you will need to help you decide whether to be in this research study or not. Please read the form carefully and ask the study team member(s) questions about anything that is not clear.

2. WHY IS THIS STUDY BEING DONE?

The purpose of this research study is to better understand how our bodies absorb and eliminate a common pollutant called benzo[*a*]pyrene or BaP. BaP is one compound in the family of molecules called polycyclic aromatic hydrocarbons (PAHs), which come from burning material like cigarettes and coal. PAHs can cause health problems such as cancer and asthma. The most common source of PAHs for non-smokers is through foods.

The results of this study may help scientists understand how our bodies handle low levels of PAHs and may also help regulatory agencies better understand environmental pollution.

3. WHY AM I BEING INVITED TO TAKE PART IN THIS STUDY?

You are being invited to take part in this study because you are a healthy non-smoking adult aged 21-65. If you are female, you must be post-menopausal or surgically sterile.

4. WHAT WILL HAPPEN IF I TAKE PART IN THIS RESEARCH STUDY?

This research study involves a screening visit and four study cycles. We will ask you to take a different dose of BaP during each cycle (25 ng, 50 ng, 100 ng, and 250 ng). All the visits will take place in the Clinical Research Center (CRC) - Room 407 in the Linus Pauling Science Center at OSU.

Screening visit (60 minutes)

We will review the study activities, schedule and diet restrictions, and answer any of your questions before obtaining your written consent. We will collect demographic information, health history, height, weight, blood pressure and heart rate. If you are female, you will be asked

to provide a spot urine sample for a pregnancy test. The study physician will perform a physical exam and review your health history. Fasting is not required for this visit.

Four study cycles (48 hours, 4 visits per cycle):

For each study cycle, we will ask you to participate in these study activities:

Food Diary: We will ask you to record all food and beverages that you consume during the 3-days before each study cycle.

Diet restrictions: We will ask you to follow these restrictions for 2 weeks before and through the end of each cycle (16 days):

1. Avoid eating any smoked or cured meats and cheeses
2. Avoid eating any charcoal grilled meats (gas-grilled meat is ok)
3. Avoid eating cruciferous vegetables and condiments (see list)
4. Avoid taking any supplements that contain indole-3-carbinol (I3C) or 3,3'-diindolylmethane (DIM)

Overnight Fast: We will ask you to not eat or drink anything besides water for at least 8 hours before the first morning of each study cycle. We will provide breakfast on the first morning of each cycle, 2 hours after you swallow the BaP capsule. You will be able to order your own breakfast food and beverage from a menu, and ingredient information can be provided if you have any food allergies or dietary restrictions.

Study visits:

Visit 1 - (0-4-hour time points, 4.5 hours): On the first morning of each cycle, we will ask you to provide a urine sample and to empty your bladder. If you are female, we will also do a urine pregnancy test at the start of each of the 4 cycles. The study nurse will measure your weight and then place an IV catheter in a vein in your inner elbow.

We will draw a baseline blood sample and then provide a BaP capsule for you to swallow with water (time 0 hours). The study nurse will draw a blood sample at 0.25 0.5, 1.0, 1.5, 2, 3, 4 hours from the IV catheter. You will have the choice to keep the IV catheter in place until the 8 hour blood draw or have it removed after 4 hours. You will need to remain near the CRC if you choose to keep the IV catheter until the 8-hour time point.

Visit 2 – (8 hour time point, 15 minutes): If you chose to have the IV catheter removed after 4 hours, you can leave the building. We will ask you to return to the CRC at the 8-hour time point for a straight needle stick blood draw.

Visit 3 – (24-hour time point, 15 minutes): We will ask you to return to the CRC at the 24-hour time point for a straight needle stick blood draw.

Visit 4 – (48-hour time point, 15 minutes): We will ask you to return to the CRC at the 48-hour time point for a straight needle stick blood draw.

We will collect a total of 120 mL (8 Tbsp.) of blood in each cycle.

Urine collection: We will ask you to collect all of your urine for 48 hours in containers that we provide. We will also provide a discrete soft-sided cooler bag for transportation. You can store any collected urine samples at room temperature in the bag until you return for your next visit. We will ask you to return any filled containers at the next visit until the end of the cycle. The longest you will need to store any samples is 24 hours between Visits 3 and 4.

Washout period: We will wait at least 3 weeks between study cycles to allow your body to completely eliminate each BaP dose.

Storage and future use of data or samples: We may store your blood and urine for possible future studies. A portion of your blood and urine samples will be sent for immediate analysis. We may store the blood and urine samples kept at OSU indefinitely. Because it is not possible for us to know what studies may be a part of our future work, we ask that you give permission now for us to use your sample without being contacted about each future study. Future use of your samples will be limited to studies about health effects of pollution. We will not pay you for the use of your sample or any products, patents, or licenses that result from these samples. If you agree now to future use of your samples, but later decide that you would like to have them removed from research tests before the end of the study, please contact Dr. David E. Williams, Department of Environmental and Molecular Toxicology, Oregon State University, 473 Linus Pauling Science Center, Corvallis, OR 97331, 541-737-3277.We will be destroying all identifying information at the end of the study. Once the identifying information is destroyed, we will not be able to remove your information from the larger dataset.

_____You may store my information and/or samples for use in future studies.
Initials

_____You may **not** store my information and/or samples for use in future studies.
Initials

During this study some of your blood will be used to look at a small select number (1-7) of genes. A gene is the code (DNA) present in each cell in your body and controls the behavior of that cell. The genes we will look at are known to control the way BaP is handled in the body. This will help us understand if the genes under study increase or decrease the uptake, metabolism or excretion of BaP.

_____You may store and or use my information and/or samples for gene analysis.
Initials

_____You may **not** store and or use my information and/or samples for gene analysis.
Initials

Future contact: We may contact you in the future for another similar study. You can ask us to stop contacting you at any time.

Study Results: We will share any published results of the study with you if you request.

5. WHAT ARE THE POSSIBLE RISKS AND DISCOMFORTS OF THIS STUDY?

BaP: The International Agency for Research on Cancer (IARC) has determined that BaP is a Class 1 known human carcinogen. The amount of BaP you will take in this study is extremely small. It is less than you may eat already every day in your diet, especially in cooked meat. For example, one grilled hamburger could contain as much or more PAHs than the amount you will take in this study. The diet restrictions are designed to help reduce additional exposure to BaP and other PAHs during the study cycles, and your cancer risk is not increased by participating in this study.

Radiation: In order to track the BaP in your blood and urine samples, it contains a carbon-14 label. Carbon-14 emits very low levels of radiation. The total amount of radiation that you will receive in the 4 cycles is equivalent to 170 minutes of natural background radiation. At this level, the risks associated with radiation exposure are negligible and no higher than your everyday exposure.

Blood Sampling: The risks of having blood drawn from your arm include some pain when the needle goes in and a small risk of bruising, inflammation or infection at that site. Please alert the study nurse if you notice any symptoms during or after each study cycle.

Some people get lightheaded, nauseous, or faint. You are less likely to have these problems if you drink 1-2 glasses of water before your study visits.

The American Red Cross recommends that you do not donate more than 1 pint (32 tablespoons) of blood within a 2-month period. We request that you do not donate blood for at least one month after completing the final study cycle.

Food allergens: We will provide breakfast on the first day of each cycle and will provide clearly labeled packaged foods or ingredient lists if requested. Please notify the study nurse if you have any food allergies or diet restrictions.

Confidentiality and Privacy: There is a risk that we could accidentally disclose information that identifies you.

6. WHAT HAPPENS IF I AM INJURED?

Oregon State University has no program to pay for research-related injuries. If you think that you have been injured as a result of being in this study, please contact the study team immediately.

7. WHAT ARE THE BENEFITS OF THIS STUDY?

This study is not designed to benefit you directly. This study may help scientists and environmental regulatory agencies better understand the health effects of PAHs. Your participation will contribute to our scientific body of knowledge for risk assessment of an important group of environmental contaminants.

8. WILL I BE PAID FOR BEING IN THIS STUDY?

You receive $125 for each study cycle. The total amount you will receive for completing 4 cycles is $500. If you withdraw from the study, your payment will be prorated to the proportion of blood samples provided. For example, if you complete 9 out of 11 blood samples in a cycle you will receive $102.27 ($11.36/sample).

9. WILL IT COST ME ANYTHING TO BE IN THIS STUDY?

There will be no cost to participate in this study. Free on-site parking will be provided. You will be responsible for transportation to and from study visits and any costs associated with visits to your personal physician if follow-up is recommended.

10. WHO IS PAYING FOR THIS STUDY?

The National Institute of Environmental Health Sciences, National Institutes of Health is paying for this study.

11. WHO WILL SEE THE INFORMATION I GIVE?

The information you provide during this research study will be kept confidential to the extent permitted by law. Research records will be stored securely. Regulatory agencies including the Food and Drug Administration, the National Institute of Environmental Health Sciences and Oregon State University employees may access or inspect records pertaining to this research as part of routine oversight or university business. Some of these records could contain information that personally identifies you.

Some of your coded blood samples will be sent to outside laboratories for analysis. Outside laboratories will only have samples identified by code and will not have access to the key connecting your name to the code.

If we contacted you through the Center for Healthy Aging Research (CHAR) LIFE Registry, we tell CHAR if you chose to participate in this research study and will update them with your contact information.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

Most people outside of the study team will not see research information that includes your name. This includes people who try to get your information using a court order. We could give out this information if you gave us permission.

12. WHAT OTHER CHOICES DO I HAVE IF I DO NOT TAKE PART IN THIS STUDY?

Participation in this study is voluntary. If you decide to participate, you are free to withdraw at any time without penalty. If you choose to withdraw from this project before it ends, the

researchers may keep information collected about you and this information may be included in study reports.

We may take you off the study early if you do not follow study instructions, if the investigator stops the study, or if you develop serious side effects.

13. WHO DO I CONTACT IF I HAVE QUESTIONS?

If you have any questions about this research project, please contact: Dr. David E. Williams, Department of Environmental and Molecular Toxicology, Oregon State University, 473 Linus Pauling Science Center, Corvallis, OR 97331, 541-737-3277. You may also contact the study nurse Sandra Uesugi, RN at 541-737-3594 or Sandra.uesugi@oregonstate.edu.

If you have questions about your rights or welfare as a participant, please contact the Oregon State University Human Research Protection Program (HRPP) office, at (541) 737-8008 or by email at IRB@oregonstate.edu.

14. WHAT DOES MY SIGNATURE ON THIS CONSENT FORM MEAN?

Your signature indicates that you acknowledge that this study has been explained to you, that your questions have been answered, and that you agree to take part in this study. You will receive a copy of this form.

Do not sign after the expiration date: 10/09/2019

Participant's Name (printed): _____

_____ _____

(Signature of Participant) (Date)

_____ _____

(Signature of Person Obtaining Consent) (Date)