Proposed Research Program on Polycyclic Aromatic Hydrocarbons

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NTP Board of Scientific Counselors Meeting
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Outline

- Introduction to PAHs
  - Toxicity
  - Defining the problem
- *Charge Questions 1-2*
- Background on Concept Development
  - Mixtures
  - Contacts
- Proposed Research Approach
  - Elements
  - Significance and outcomes
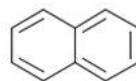- *Charge Questions 3-8*

What are PAHs?

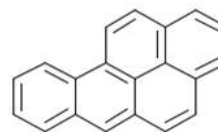**Polycyclic Aromatic Compounds (PAC) -**

Are composed of two or more fused rings containing at least one aromatic ring and include:

- Unsubstituted
- Alkylated

  Polycyclic Aromatic Hydrocarbons (PAH)

- N-, S-, and O-substituted
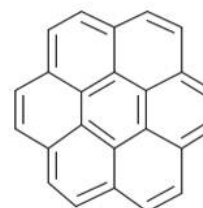
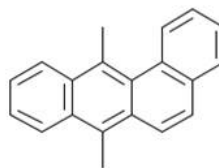- Heterocyclics with N-, S-, and O-containing rings

Napthalene

Benzo(a)pyrene
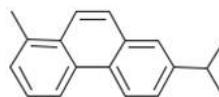
Retene

Choronene

7,12-Dimethylbenzanthracene

Dibenzothiophene

Acenaphthenequinone

6-Chrysenamine
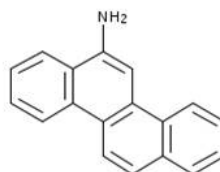
2-nitro-6h-dibenzo[b,d]pyran-6-one

PAHs in the environment

- PAHs occur naturally in petroleum and coal or are created and released into the environment through natural events (e.g., volcanic eruptions, forest fires) and human activity (e.g., combustion processes)

- Over 1500 distinct PAHs have been referenced in the literature, though millions of structural configurations are possible

- Exist in complex and dynamic mixtures
  - Profiles of PAHs differ by source (e.g., pyrogenic versus petrogenic)

Properties of PAHs

- Exhibit a wide range of biological properties due to the structural diversity across the class

- Lipophilic and readily absorbed (2-3 ring more so than 5-6 ring)

- Widely distributed with some accumulation in adipose tissue and breast milk

- Rapidly metabolized (primarily by cytochrome P450 enzymes in the liver)

- Eliminated as conjugated metabolites in bile and urine
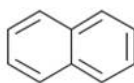
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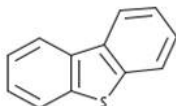
Dibenzothiophene
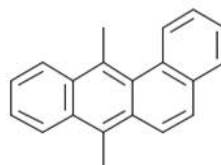
7,12-Dimethylbenzanthracene
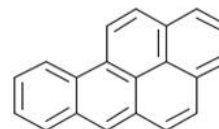
Benzo(a)pyrene

PAH metabolism

Benzo[a]pyrene

Benzo[a]pyrene → (CYP) → Epoxides 4,5- 7,8- 9,10-

Benzo[a]pyrene → Phenols 1- 7- 3- 9- 6-

Epoxides → GSH conjugates

Phenols → (PS) → Quinones 1,6- 3,6- 6,12-

Quinones → GSH conjugates

Quinones → (QR) → Hydroquinone derivatives

Epoxides → (EH) → Dihydrodiols 4,5- 7,8- 9,10-

Dihydrodiols → Glucuronides and sulfate esters

Phenols → (CYP) → Phenol dihydrodiols 9-OH-4,5-diol 6-OH-7,8-diol 1-(-3)OH-9,10-diol

Phenol dihydrodiols → Glucuronides and sulfate esters

Dihydrodiols → (CYP PS) → Dihydrodiol epoxides 7,8-diol-9,10-epoxide 9,10-diol-7,8-epoxide

Dihydrodiol epoxides → GSH conjugates

Dihydrodiol epoxides → Tetrols

Adapted from IARC, 2010

Carcinogenicity of PAHs

- History
  - 1775 Percival Pott identifies soot as the cause of scrotal cancer in chimney sweeps

  - 1875-1892 High incidences of skin cancer reported among workers in paraffin refining, as well as shale oil and coal tar industries

  - 1915 Katsusabu Yamagiwa produced squamous cell carcinoma from skin painting studies with coal tar

  - 1930's E.L. Kennaway *et al.* isolated individual carcinogenic PAHs from coal tar

- Major cancer types associated with PAHs include <u>lung</u>, bladder, and skin

- Multiple other types of cancer suspected to be associated with PAH exposure (e.g. renal cell, stomach, pancreatic, prostatic)

Mechanisms of carcinogenicity

- Activation by CYPs to reactive metabolites that form stable, covalent DNA adducts
  - Monooxygenation
    - Bay and fjord region activation to diol epoxides
    - Cyclopenta-ring oxidation
  - One electron oxidation to form radical cations
- Formation of ortho-quinones and generation of reactive oxygen species
- Meso-region biomethylation and benzylic oxidation
- Aryl hydrocarbon receptor (AhR) binding
- Inhibition of Gap-Junctional Intercellular Communication
- Suppression of the immune system

Bay region

Fjord region
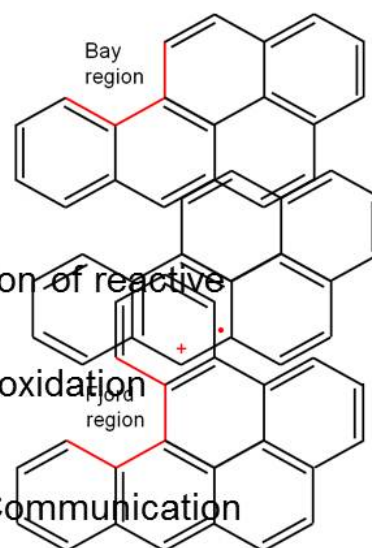
Mechanisms of carcinogenicity

Carcinogenesis
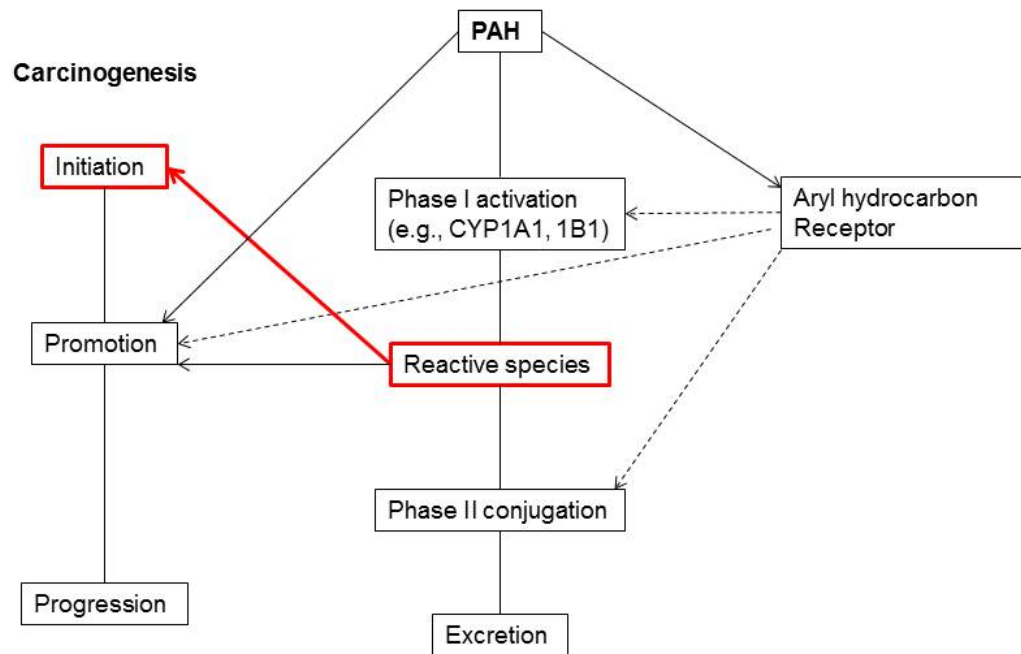
Adapted from Sjögren et al. (1996)

Immunotoxicity of PAHs

- Suppression of the humoral immune response
- Mechanisms includes AhR-dependent and independent pathways, but are not fully characterized
- There is some concordance between the PAH structure-activity relationship for cancer and immune suppression

Serum Anti-SRBC IgM Antibody Titers in Female B6C3F1/N Mice Treated with DBZA for 28 Days
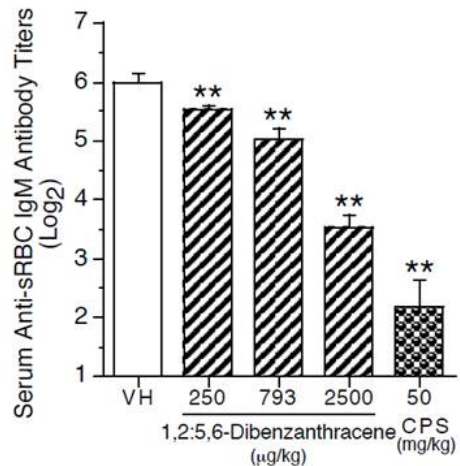
Reproductive and developmental toxicity of PAHs

- Reproductive Toxicity
  - Male reproductive toxicity
    - Decreased sperm
  - Female reproductive toxicity
    - Ovarian follicle loss
  - Estrogenic activity
    - Positive in uterotrophic assay

- Developmental Toxicity
  - Impaired development of male and female reproductive systems
  - Reduction in fetal birth weight and length, impaired intrauterine growth
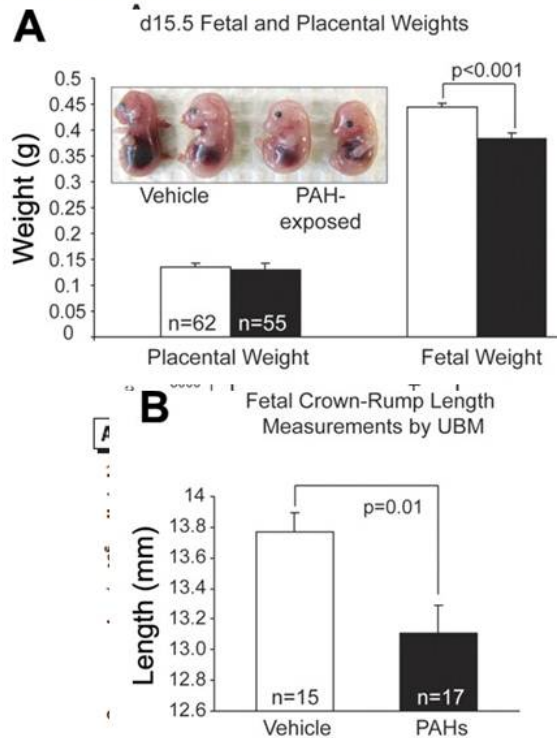
A d15.5 Fetal and Placental Weights

B Fetal Crown-Rump Length Measurements by UBM

From Sagvaek et al 2012 Am J Physiol Endocrinol Metab 297:E557-E930

From Chang et al 2011 DOI 10.1289/1574 Clinical Investigation

Neurotoxicity of PAHs

Results from Morris water maze

- ## Neurotoxicity
  - ### Animal studies
    - Impaired learning and memory
    - Motor effects
    - Increased aggressive behavior
    - Increased anxiety-related behaviors
  - ### Human studies
    - Anxiety/depression
    - Decreased IQ scores
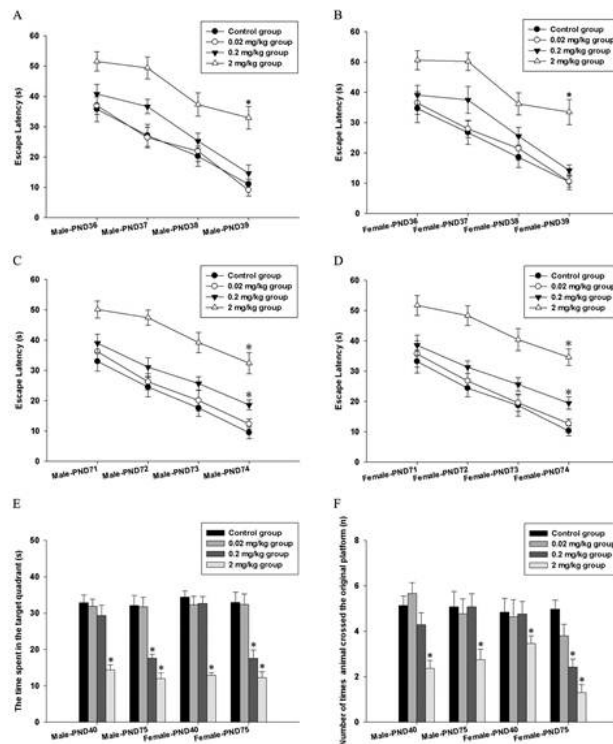
From Chen *et al*. 2012. Toxicol. Sci.125:248-261

Human exposure

- PAHs are ubiquitous environmental contaminants

- Typical exposure scenarios include:
    - Consumption of PAH-contaminated food (e.g., seafood)
    - Non-dietary ingestion (e.g., house dust)
    - Inhalation of polluted air (e.g., cigarette smoke, diesel exhaust)
    - Dermal contact through occupation (e.g., road-paving)

Problem

Estimating human health risk from exposure to environmental PAH mixtures

Whole Mixtures
Requires toxicity data on whole mixtures
- Data on mixture of interest
- Data on "sufficiently similar" reference mixture

Component-based
Requires toxicity data for individual chemicals within the mixture
- Dose addition
  - Relative Potency Factor
- Response addition

Risk assessment of PAH mixtures

- Relative Potency Factor approach (dose additivity)

Reference compound
(benzo(a)pyrene)

D A

C B

Convert to BaP
equivalents

D A

C B

Add to get total
mixture dose

A

Response

BaP dose
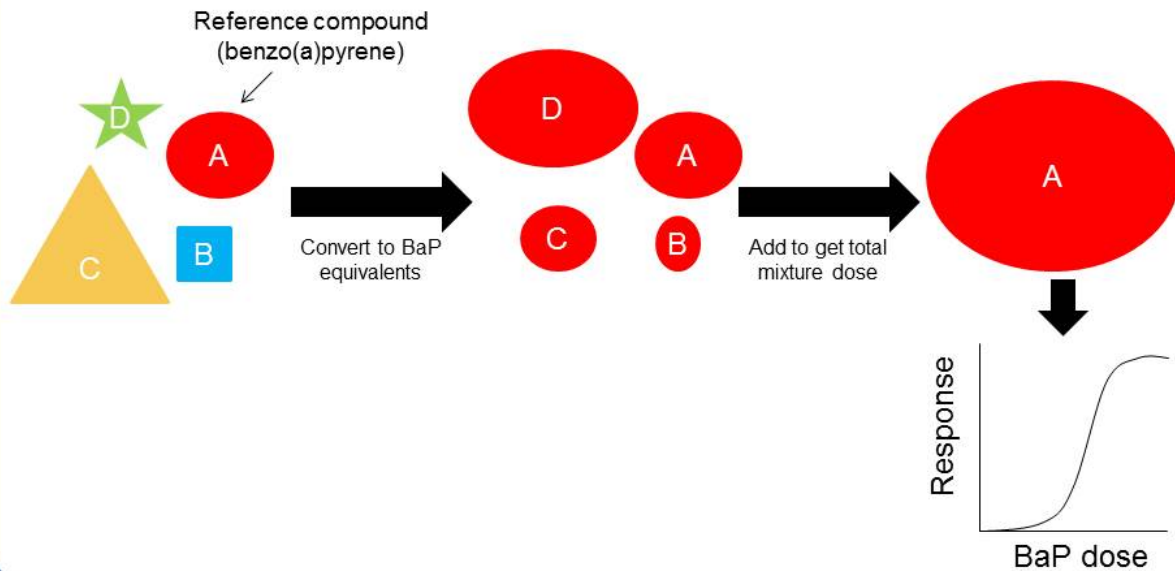
Risk assessment of PAH mixtures

- Sufficient similarity of whole mixtures
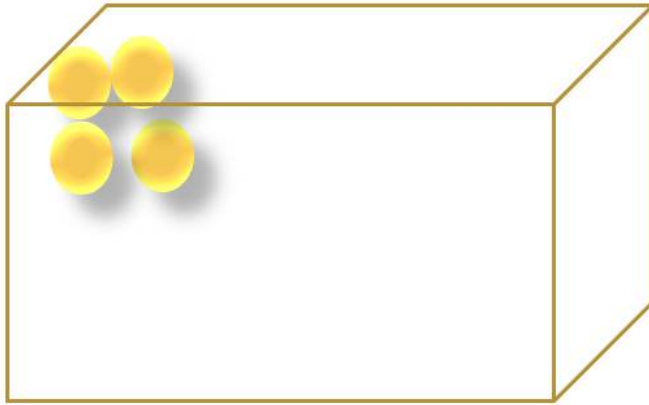
Risk assessment of PAH mixtures

- **1993** - US EPA Provisional Guidance for Quantitative Risk Assessment of Polycyclic Aromatic Hydrocarbons

- **2002** - US EPA hosted a Peer Consultation Workshop on Approaches to Polycyclic Aromatic Hydrocarbon (PAH) Health Assessment

- **2010** - FDA and NOAA used a relative potency factor approach in assessing the risk associated with contaminated seafood following the Deepwater Horizon Oil Spill

- **2010** - US EPA IRIS draft document "Development of a Relative Potency Factor (RPF) Approach for Polycyclic Aromatic Hydrocarbon (PAH) Mixtures" reviewed by a Scientific Advisory Board

Regulatory aspects

- 16 (unsubstituted) PAHs are commonly monitored because they are identified as priority pollutants under the Clean Water Act

Benzo(a)pyrene	Benzo(a)anthracene	Chrysene	Indeno(1,2,3-cd)pyrene
Acenaphthene	Benzo(b)fluoranthene	Dibenzo(a,h)anthracene	Napthalene
Acenaphthylene	Benzo(k)fluoranthene	Fluoranthene	Phenanthrene
Anthracene	Benzo(ghi)perylene	Fluorene	Pyrene

- 1993 PAH Guidance document assigned RPFs for 7 PAHs, which have been widely used by EPA and other regulatory agencies (including FDA)

- The draft 2010 document expands the number of PAHs with assigned RPFs
  - 74 PAHs with toxicological data available in the literature
  - 27 PAHs assigned RPFs (3 assigned RPF = 0)

Scientific Advisory Board recommendations

- In parallel to continued use of the RPF approach, EPA should begin developing a whole mixtures approach to achieve two goals: (1) potentially validate the RPF approach, and (2) explore it as a possible replacement for the RPF approach in the near future

  The Agency should seek support from the National Toxicology Program to **test a portfolio of 12-15 different complex mixtures in animal studies**. These mixtures should represent a diverse array of mixtures but also represent the most important mixture classes of concern to EPA (based on the level of health concerns and/or extent of exposure) such as coal tar, manufactured gas plant (MGP) residues, coke oven emissions, diesel and gasoline exhaust, coal plant emissions, etc.

- **Challenges in testing 12-15 complex mixtures in NTP bioassays:**

  – Multiple issues in prioritization of PAH-containing mixtures

  – Logistical challenges in acquiring and analyzing complex mixtures

  – ~30-60 kg sample required for each 2-year cancer bioassay

  – Very resource intensive

  – Once data are generated, significant work required to develop sufficient similarity methods

Problem

EPA approach

Estimating human health risk from exposure to environmental PAH mixtures

Future

Sufficient Similarity of Whole Mixtures

Unknowns:
- Little toxicity data on whole mixtures
- No accepted methods for determining sufficient similarity
- What proportion of whole mixture toxicity is due to PAHs vs. other contaminants

Component-based (Relative Potency Factor)

Assumptions:
- No interactions among PAHs
- PAHs elicit cancer via same MOA
- Route to route extrapolation is valid

Unknowns:
- No assessment of RPF methods
- Inadequate individual chemical data
- Other toxicities (immunotoxicity)
- Toxicities of other PAHs (alkyl, oxy)

Major knowledge gaps

- Exposure
  - Do the 16 commonly monitored PAHs capture the class?
- Hazard characterization
  - The vast majority of PAHs have not been characterized
  - Genotoxicity/carcinogenicity have been the focus of characterization, when it is clear that PAHs display additional toxicities
- Risk assessment
  - There is a great deal of uncertainty in the application of the RPF approach to PAH risk assessment
  - There is no path forward for developing a sufficient similarity approach to assess the risk associated with complex PAH mixtures

Charge questions

1. Comment on the clarity and validity of the rationale for the proposed research program as articulated in the NTP research concept document. Has the scope of the problem been adequately defined? Are the relevant knowledge gaps and key issues identified and clearly articulated?

2. Does the concept document demonstrate that the NTP has sufficiently considered the advantages and disadvantages of using mixtures science to investigate PAH toxicity versus conducting cancer bioassays on selected PAHs or PAH mixtures?

Background on Concept Development

Trans-NIEHS Mixtures Working Group

<u>Division of the National</u>
<u>Toxicology Program</u>
Division Director's Office
Nigel Walker

Office of Health Assessment
and Translation
Kembra Howdeshell

Office of Nominations and
Selections
Scott Masten

Biomolecular Screening
Branch
Scott Auerbach
Liz Maull

Toxicology Branch
Chad Blystone
Mike DeVito
June Dunnick
Paul Foster
Cynthia Rider

<u>Division of Extramural</u>
<u>Research and Training</u>
Susceptibility & Population
Health Branch
Caroline Dilworth
Kim Gray
Claudia Thompson

Cellular, Organs & Systems
Pathobiology Branch
Jerry Heindel
Annette Kirshner
Mike Humble
Lisa Chadwick

Center for Risk & Integrated
Sciences
David Balshaw
Danielle Carlin
Bill Suk

Grants Management Branch
Barbara Gittleman

Scientific Review Branch
Janice Allen
Sally Tilotta

<u>Division of Intramural</u>
<u>Research</u>
Biostatistics Branch
Gregg Dinse
Grace Kissling
Keith Shockley
Dave Umbach

Epidemiology Branch
Todd Jusko
Freya Kamel

Toxicology & Pharmacology
Laboratory
David Miller

NIEHS
National Institute of
Environmental Health Sciences

NIEHS Mixtures Workshop – Sept 26-27, 2011

- Organized by the 3 divisions at NIEHS
  - NTP, Intramural, Extramural
- Identify and focus on key issues that present challenges in mixtures research
  - Use to inform the development of an intramural and extramural mixtures research strategy
- Broad discipline representation of invited participants
  - Representatives from statistics, biology/toxicology, epidemiology, exposure science, and risk assessment
- Format
  - Stage-setting invited speakers.
  - Discipline-based and interdisciplinary based breakout sessions
- Report
  - Available on website

http://tools.niehs.nih.gov/conferences/dert/mixtures/
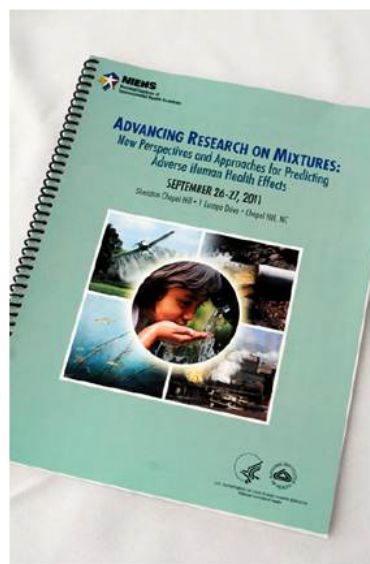
Key issues

- *In vitro* and *in vivo* approaches needed to address mixtures questions

- Cross-disciplinary effort required

- Systems-based approaches for studying mixtures are recommended

- Sufficient similarity as a key approach that requires development

- Need for development/refinement of both bottom-up (component-based) and top-down (whole mixtures) approaches for predicting toxicity of mixtures

- Federated databases should be developed to manage mixtures data, including exposure, *in vitro*, animal, and human data

- Tools and methods for prioritization of chemicals/mixtures are needed

Other mixture-related activities

- Elsevier International Toxicology of Mixtures Conference
  - Mixtures Research at the NIEHS and NTP: An Evolving Program - Nigel Walker (NTP/NIEHS)

- NAS Emerging Science for Environmental Health Decisions Workshop "Mixtures and Cumulative Risk Assessment: New Approaches Using the Latest Science and Thinking about Pathways"
  - Case Study: Potential of Genomic Data on PAHs to Inform Cumulative Assessment – Lyle Burgoon (US EPA/NCEA)

Goal 4 of the NIEHS Strategic Plan

Understand how combined environmental exposures affect disease pathogenesis.

a) Assess the joint action of multiple environmental insults, including chemicals, nonchemical stressors, and nutritional components, on toxicity and disease, and identify interactions resulting from combined exposures.

b) Study the role of the human microbiome and its influence on environmental health, and explore the role of the microbiome in responses to environmental exposures.

c) Study the interactions of infectious agents with environmental exposures.

d) Understand how nonchemical stressors, including socioeconomic, behavioral factors, etc., interact with other environmental exposures to impact human health outcomes, and identify preventive measures that could be taken.

NIEHS
National Institute of Environmental Health Sciences
2012-2017
STRATEGIC PLAN
Advancing Science, Improving Health:
A Plan for Environmental Health Research

Society of Toxicology 2012 Annual Meeting

- Workshop Session - Sufficient Similarity of Whole Representative Mixtures or a Relative Potency Factor Approach: Polycyclic Aromatic Hydrocarbons as a Case Study

- Chairpersons: Cynthia Rider and Julia Gohlke (University of Alabama at Birmingham)

    – Lessons from the Deepwater Horizon Blowout: Developing Approaches to Estimate Risk from Complex Exposures – **Julia Gohlke**

    – Comparing Whole Mixture and Component Mixture Risk Assessment Methods – **Glenn Rice** (US EPA/NCEA)

    – Multiple Mechanisms of PAH Toxicity Revealed through Screening with Zebrafish Embryos – **John Incardona** (NOAA)

    – Utilizing Quantitative Structure-Activity Relationships (QSARs) to Predict Toxic Endpoints for Polycyclic Aromatic Hydrocarbon Risk Assessment – **Erica Bruce** (Baylor University)

    – The Relationship between Aromatic Ring Class Content of High-Boiling Petroleum Substances – **Russell White** (American Petroleum Institute)
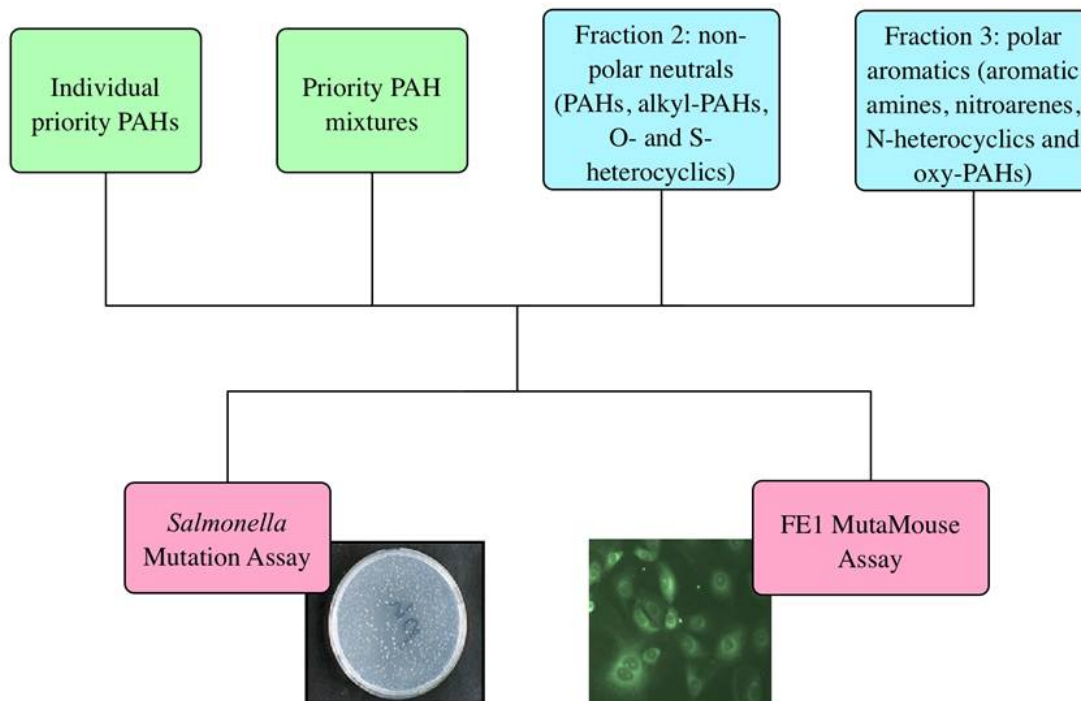
Extramural

- Deep Water Horizon Research Consortia
  - LSU, Tulane, UF, UT-Galveston
  - Focus: Improving characterization of exposure via contaminated seafood and exploring health effects in human populations
- Oregon State University
  - Kim Anderson
    - Exposure characterization
  - Robert Tanguay
    - Zebrafish assays with individual PAHs and complex environmental mixtures

Proposed research approach

Major knowledge gaps

- Exposure
  - Do the 16 commonly monitored PAHs capture the class
- Hazard characterization
  - The vast majority of PAHs have not been characterized
  - Genotoxicity/carcinogenicity have been the focus of characterization, when it is clear that PAHs display additional toxicities
- Risk assessment
  - There is a great deal of uncertainty in the application of the RPF approach to PAH risk assessment
  - There is no path forward for developing a sufficient similarity approach to assess the risk associated with complex PAH mixtures
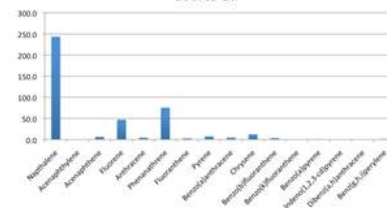
Exposure characterization

- Key issues

    – There is a deficiency in the characterization of complex PAH mixtures used in toxicity assessments

    – The majority of the limited carcinogenicity studies available have been conducted with dermal exposure, when real-world exposures often occur through oral or inhalation routes

    – Data are needed to link the external exposure (e.g., diet, air) to internal dose

Hazard characterization

- Key issues
  - A limited number of PAHs (mostly unsubstituted) have been characterized
  - A lack of characterization of PAH-driven effects on non-carcinogenic endpoints (e.g., immunotoxicity, reproductive and developmental toxicity)
  - Inadequate understanding of mechanisms and pathways of toxicity thereby restricting the development of predictive models

Risk characterization

- Key issues associated with the Relative Potency Factor Approach
  - Limited individual chemical data
  - Uncertainty in application of the RPF method to PAHs
  - Focus exclusively on carcinogenesis
- Key issues associated with the Whole Mixtures Approach
  - Lack of characterization of complex mixtures used in toxicity studies
  - Uncertainty regarding the proportion of toxicity elicited by whole complex PAH-containing mixtures explained by the PAH fraction
  - Uncertainty in the predictability of whole mixture toxicity based on a subset of individual PAHs

Specific aims

1. Assess chemical, toxicokinetic (TK), and absorption, distribution, metabolism, and elimination (ADME) properties of select, individual PAHs and mixtures to gain insight into exposure and dosimetry

2. Characterize the toxicity of a broad range of individual PAHs, defined PAH mixtures, and complex environmental mixtures containing PAHs using a short-term panel that incorporates *in vitro*, alternative animal, and *in vivo* models and captures a diverse array of endpoints/effects

3. Compare predicted mixture toxicity results using component-based models that incorporate calculated relative potency factors or whole mixture approaches (e.g., complex mixture fractionation approaches, models based on sufficient similarity of whole mixtures) to observed toxicity

Proposed approach

1. Targeted selection of test articles

2. A flexible, iterative format

3. Incorporation of a broad spectrum of endpoints (including *in vitro*, alternative animal model, and mammalian *in vivo* assays) that will capture a range of relevant toxicities in a short-term testing panel

4. Cross-disciplinary and institutional collaboration

Selection of test articles

- Considerations for selecting individual PAHs
  - Environmental exposure potential
  - Structural diversity
  - Representation from the following categories:
    - PAHs with wide-ranging RPFs assigned with high confidence
    - PAHs that are not commonly monitored and have potent RPFs assigned with low to moderate confidence
    - Substituted PAHs from different classes (e.g., alkylated and oxygenated)

Selection of test articles

- Both designed mixtures and complex environmental mixtures will be assessed
  - Designed mixtures will be created based on individual chemical data in order to assess the RPF approach
  - Select, complex mixtures will be included in the testing program based on consideration of exposure potential, impact to the overall research program, and public health concern
    - Extracts collected from passive sampling devices in oil-contaminated waters
    - Coal tar extracts tested in the Health Canada PAH program
    - PAH-containing standard reference materials (e.g., NIST mussel tissue)

Short-term toxicity testing panel

- Decision point: few test articles in 2-year bioassays or many test articles in a short-term testing panel
  - Advantages of short-term panel
    - Represent the breadth of toxicities that have been associated with PAHs
    - Maximize the number of test articles that can be evaluated
    - Allows for comparisons across endpoints
    - Rapid generation of data for use in developing whole mixtures approaches
    - Provides data for prioritization of test articles for future testing
  - Disadvantages
   - Lack of characterization of chronic effects

Short-term toxicity testing panel

- Mammalian *in vivo* component
  - Subacute exposure in male and female rodents
    - 28-day oral gavage exposure regimen used in Health Canada PAH program
    - 28-day exposure is standard in assessing short-term toxicity (OECD 407)
    - Preferred to shorter exposures because of greater likelihood of pathological changes in target tissues
  - Route of administration
    - Oral gavage preferred as main route
    - Potential to use dermal and/or inhalation routes for comparison across routes
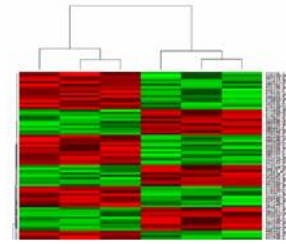
Mammalian *in vivo* component

- ADME/TK studies
  - Select studies will address uncertainties in route-to-route extrapolation
- Immunotoxicity cohort
  - Antigen-specific antibody forming cell assay with sheep red blood cell (sRBC) as antigen
    - Sensitive
    - Available literature on PAHs with this endpoint
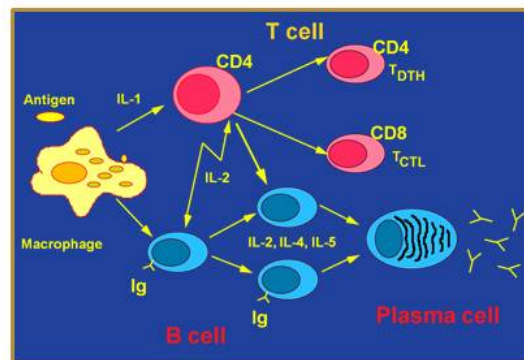

From Kimber White

Mammalian *in vivo* component

- General toxicity and genotoxicity cohort
  - Gene expression in lung, liver, and gonads
    - Elucidation of key pathways involved in toxicity at probable target sites
  - Pathology
    - Provides a phenotypic anchor for gene expression results
  - Hematology
    - Represents a sensitive endpoint for PAHs
  - Pig-a gene and micronuclei in red blood cells
    - Indicators of genotoxicity
   - Provide overlap with endpoints assessed by Health Canada

In vitro component

- Available HTS data on PAHs
- Aryl hydrocarbon receptor transactivation assay
- Genomic evaluation in 3 diverse cell lines (HepaRG, HL60, MCF7)

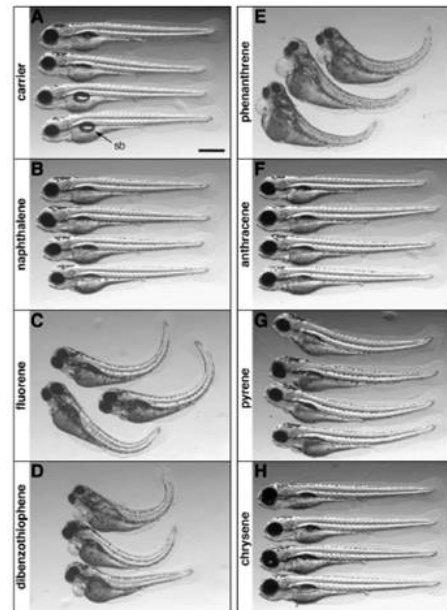

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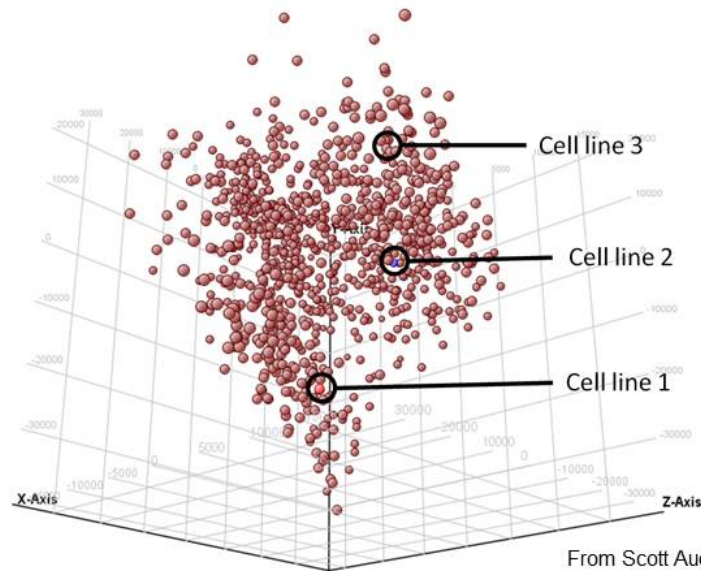
Alternative animal component

- Zebrafish developmental assay

  - Well-established alternative animal model

  - Developmental and neurotoxicological endpoints available

  - Historical data available with PAHs

  - AhR, CYP localization and manipulation possible
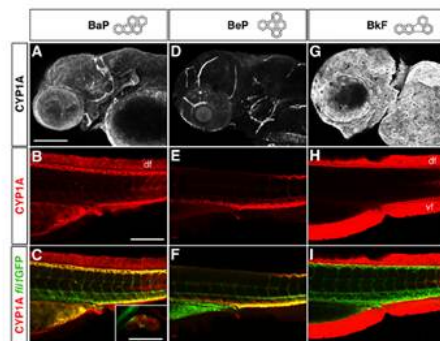
From Incardona et al. 2004. Toxicol. Appl. Pharm. 257:242-249

From Incardona et al. 2004. Toxicol. Appl. Pharm. 196:191-205

Proposed approach – iterative design

Round 1:

- PAHs with RPFs
- Alkylated PAHs
- Oxygenated PAHs
- Complex environmental mixture = simulated seafood extract (e.g., from passive sampling devices)

Round 2:

- Defined mixture: equipotent mix of individual chemicals with good dose-response data from round 1
- Complex environmental mixture = coal tar
- Targeted individual PAHs

Round 3:

- To be determined based on results from previous rounds

Evaluation of Round 1

- Assess testing battery
- Hazard characterization of individual PAHs and complex mixture
- Generate RPFs for each endpoint
- Compare non-cancer RPFs to cancer EPA RPFs
- Use RPFs to predict toxicity of a defined mixture to be tested in round 2
- Determine feasibility/challenges of testing environmental mixture

Evaluation of Round 2

- Assess predicted versus observed toxicity of defined mixture to evaluate RPF approach
- Compare results from complex mixtures 1 and 2 (evaluate chemical and biological range of complex mixtures).

Proposed approach – iterative design

Round 1:

- PAHs with RPFs
- Alkylated PAHs
- Oxygenated PAHs
- Complex environmental mixture = simulated seafood extract (e.g., from passive sampling devices)

Evaluation of testing battery

- Compare results from testing panel to available literature and Health Canada results (for overlapping endpoints) to assess biological plausibility and reliability
- Compare across endpoints to evaluate which endpoints are most informative and sensitive

Adjust testing panel as needed for next round of testing

Evaluation of Round 1

- Assess testing battery
- Hazard characterization of individual PAHs and complex mixture
- Generate RPFs for each endpoint
- Compare non-cancer RPFs to cancer EPA RPFs
- Use RPFs to predict toxicity of a defined mixture to be tested in round 2
- Determine feasibility/challenges of testing environmental mixture

Proposed approach – iterative design

Round 1:

- PAHs with RPFs
- Alkylated PAHs
- Oxygenated PAHs
- Complex environmental mixture = simulated seafood extract (e.g., from passive sampling devices)

Hazard characterization

- Generate dose-response data for test articles at each endpoint
- *Identify test articles that require further assessment or pose a particular human health hazard*

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Relative Potency Factor calculation

- Multiple approaches available for calculating RPFs, for example

$$RPF = \frac{ED_{50\,BaP}}{ED_{50\,PAH}}$$

- Cross-disciplinary collaboration to find best statistical models for calculating relative potencies
- Data will be made publicly available to support methods development and evaluation

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Compare cancer to non-cancer RPFs

- Assess correlation between cancer RPFs and non-cancer RPFs to increase understanding of mechanisms of action for different effects

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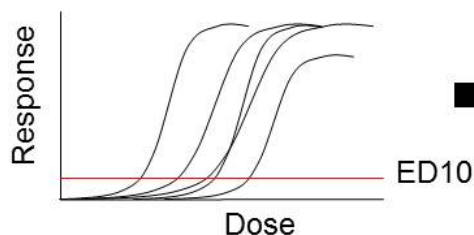# Proposed approach – iterative design

Round 1:

- PAHs with RPFs
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- Oxygenated PAHs
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Assessment of environmental mixture

- Challenges:
  - Acquiring homogeneous sample
  - Chemical analysis
    - Non-PAH contaminants
    - Acceptable unidentified fraction
  - Testing *in vitro* and in zebrafish model
- Develop methods to address challenges

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Round 2:

- Defined mixture: equipotent mix of individual chemicals with good dose-response data from round 1
- Complex environmental mixture = coal tar
- Targeted individual PAHs

Evaluation of Round 1

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Cross-disciplinary collaboration strategy

- A Division of Extramural Research and Training (DERT) representative will participate in study design meetings and act as a liaison to the division

- Extramural exposure scientists will be consulted in selection of complex mixtures for testing

- Options for making materials resulting from the testing program (e.g., study samples) available to extramural partners interested in performing additional analyses will be investigated

- Complementary extramural PAH research will be explored

- Continued discussion of lessons learned and suggested paths forward will be conducted with Health Canada, EPA, FDA and others

Significance

- The proposed work will address a ubiquitous, diverse class of compounds with members that display a broad range of toxicities

- Exposure to PAHs is widespread and occurs from various sources via multiple routes

- Decreasing uncertainty associated with cumulative risk assessment of PAHs will inform public health decisions

Expected outcomes

- PAHs as a class
  - Further characterize the toxicity of PAHs
    - Expand the endpoints evaluated for well-studied PAHs
    - Assess the toxicity of "unknown" PAHs (e.g., alkylated, oxygenated)
  - Strengthen the basis for assessing risk from PAH exposure by providing dose-response data for a variety of individual PAHs and PAH mixtures for multiple endpoints, as well as data to decrease uncertainty associated with route-to-route extrapolation
  - Provide recommendations on sensitive, reliable short-term assays for future PAH evaluation
  - Provide chemical analysis of PAH-containing complex environmental mixtures
  - Contribute to understanding the role of PAHs in the toxicity of complex environmental mixtures

Expected outcomes

- Opportunity to address mixtures knowledge gaps
    - Building bridges between *in vitro* and *in vivo* approaches
        - Incorporation of both will allow for direct comparison and evaluation
        - Iterative design will facilitate development of studies needed to strengthen extrapolations
    - Cross-disciplinary effort
        - Toxicologists, exposure scientists, epidemiologists, risk assessors, and biostatisticians
    - Systems-based approaches
        - Gene expression, zebrafish, and AhR transactivation assays will help to elucidate the complex pathways involved in PAH toxicity
        - This information will be exploited to develop future mixture hypotheses
    - Need for both component-based and whole mixtures approaches
        - Begin to develop a path forward for comparing predictive mixture modeling approaches

Charge questions

3. There are challenges inherent to achieving the aims of any proposed research program. Are the relevant challenges identified and clearly articulated? Where approaches to overcome challenges are proposed, are they appropriate?

4. Comment on the strategy and approach for the identification and selection of test articles (individual chemicals and mixtures). Are there additional factors not outlined in the concept that the NTP should consider?

5. Regarding the models and endpoints proposed for the short-term testing panel, comment on whether there are certain models and/or endpoints that should not be considered for inclusion. Are the relevant important toxicities captured? If not, suggest alternatives models or endpoints for the NTP to consider.

Charge questions

6. The NTP has proposed an iterative/evolving testing approach incorporating *in vitro*, alternative animal model, and short-term mammalian studies. Is the approach logical and appropriate? Identify any limitations that are not sufficiently explored in the concept document.

7. Based on your evaluation of rationale, scope and strategy, rate the public health impact of this research program as low, moderate, or high. Are there parts of the research program that are higher priority than others? Are there parts of the research program that have a higher likelihood of success at meeting pre-defined specific aims?

8. Provide any other comments you feel NTP staff should consider in developing this research program.