NTP
National Toxicology Program

REACT PFAS

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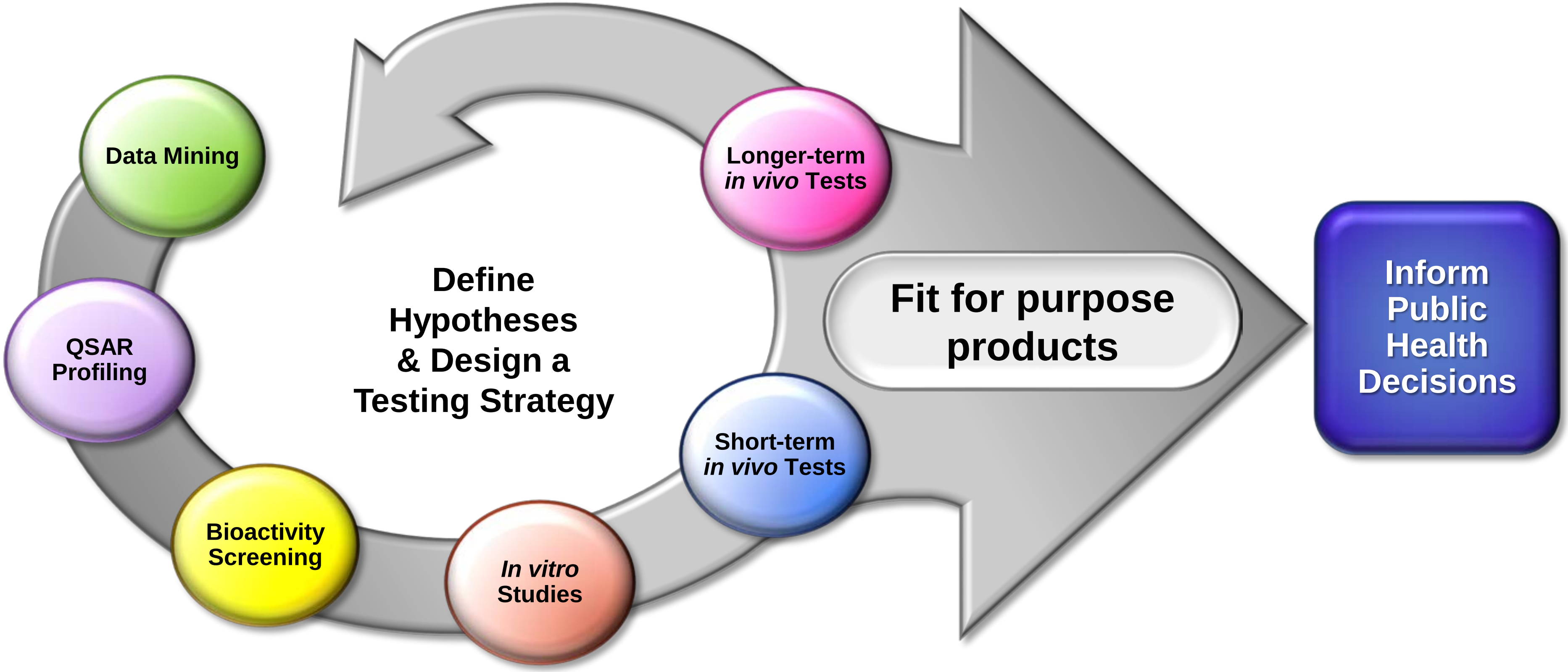
REACT PFAS

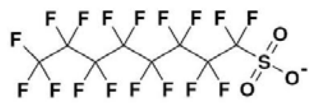
- Responsive Evaluation and Assessment of Chemical Toxicity (REACT)
  - REACT is a general approach that the NTP is developing to address environmental and public health challenges.
  - It is focused on fit for purpose solutions and involves literature mining, computational, in vitro and in vivo toxicological methods.
  - REACT PFAS is a REACT project focused on Per and Polyfluorinated Alkyl Substances (PFAS)

DNTP Translational Toxicology Pipeline Plan

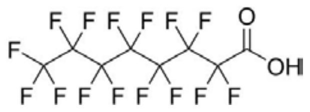


PFAS Background

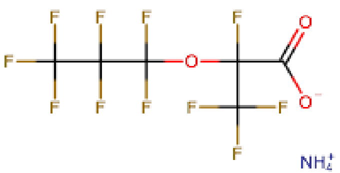
- Diverse group of compounds
- Used in carpeting, apparels, upholstery, food paper wrappings, and fire-fighting foams
- Persistent and bioaccumulative
- Long chain perfluorinated chemicals are well studied; their use is in decline
- Shorter and branched chain compounds increasing in production and use; less well studied



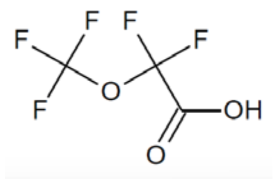
PFOS



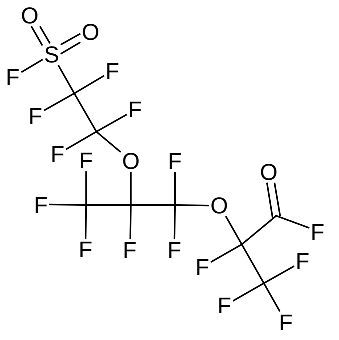
PFOA



GenX



PFMOAA



Nafion Monomer Precursor

PFOS and PFOA Alternatives of Interest

- Total number of PFAS >3000 chemicals
  - Includes products, impurities and degradants
  - EPA chemical dashboard has over 2700 listed
- Significant regulatory interest
  - USEPA: Several hundred of interest narrowing down to 75-150
  - FDA**:** Interested in PFAS used in packaging
  - DOD: Aqueous Fire Fighting Foams (AFFF)
  - ATSDR, CPSC, and state regulatory agencies
  - Federal Information Exchange on PFAS (Feb 2018)
    - National Science and Technology Council, Committee on Environment
    - EPA, DOD, NIH (co-chairs)

NTP Perfluorinated Compounds Evaluation

- Original nomination of perfluorinated compounds (PFCs) (2003) from EPA
- Studies focused on class assessment:
  - In vitro studies evaluated PFC classes for neurotoxicity, immunotoxicity, and mitochondria toxicity
  - Studies to evaluate toxicokinetics in NTP rat model: Hsd:Sprague Dawley SD
  - **Comparison of PFCs in vivo with focus on internal dose as marker of comparison using rat 28-day toxicity studies**
  - PFOA chronic and carcinogenicity rat study examines contribution of perinatal exposure to adverse outcomes

PFCs Guideline Toxicity Studies

- 28-Day toxicity studies: Comparing of 7 PFAS
  - Data available here: https://ntp.niehs.nih.gov/results/path/index.html
  - TOX Report 96: Sulfonates (2018) (PFBS, PFHxS, PFOS)
  - TOX Report 97: Carboxylates (2018) (PFHxA, PFOA, PFNA, PFDA)
    - Study included a PPARα agonist (WY-14643) as a comparator
- PFOA two-year study: Comparison of PFOA perinatal and non-perinatal effects
  - Data available in next 60 days: https://ntp.niehs.nih.gov/results/path/index.html
  - Draft NTP Technical Report (TR-598) to be posted early 2019 for peer review

PFAS Nominations

- January 2017, we received a list of 31 PFAS of interest from US EPA, National Center for Environmental Assessment (NCEA). At that same time, we began collaborations with colleagues at the USEPA National Exposure Research Laboratory on a separate list of PFAS. (Total of 38 PFAS and growing).
- July 2017, USEPA National Center for Computational Toxicology proposed a collaboration on 75 PFAS.
- We have over 100 PFAS under evaluation.

REACT PFAS Challenges

- Too many chemicals for traditional approaches
  - Over 100 chemicals under evaluation
- How do we develop a screening approach?
  - Prototypes PFOA and PFOS (?)
  - Wide variety of structures; PFOA and PFOS may not be good prototypes
    - EPA lists over 2700 PFAS on their dashboard.
    - In a subset of 268 PFAS, EPA has identified 53 structural classes.
    - 10 classes are in the EPA 75 chemical library.

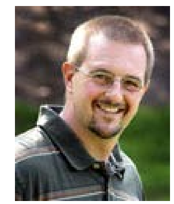
Problem Formulation and Approach

- What are the types of biological activity and toxicological information that NTP can develop in a *responsive timeframe* on these classes of chemicals?
  - How can this information be used to make public health decisions?
- What are the appropriate biological and computational tools to bring to this problem?
- How do we organize this information to provide useful products?
- How do we report this biological activity/toxicological information in a timely manner?

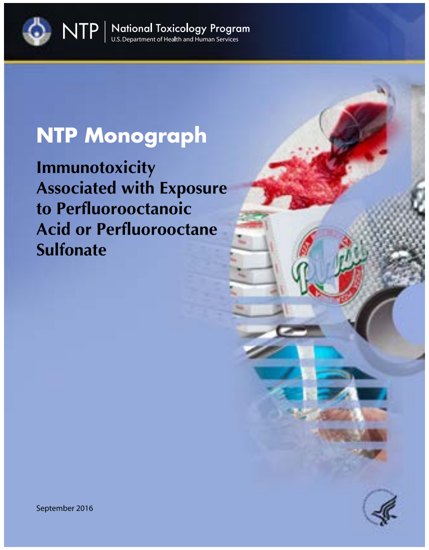
Staff Team Leads at NIEHS

- Literature Analyses – Andrew Rooney
- Chemistry – Suramya Waidyanatha
- In silico – Scott Auerbach
- In vitro – Sue Fenton
- In vivo – Chad Blystone
- Mixtures – Mike DeVito
- Reporting Plan – Mike DeVito





NTP Systematic Literature Review



NTP Monograph on Immunotoxicity Associated with Exposure to Perfluorooctanoic Acid or Perfluorooctane Sulfonate

- The NTP concludes that PFOA and PFOS are *presumed to be immune hazards to humans* based on a high level of evidence that PFOA and PFOS suppressed the antibody response in animal studies and a moderate level of evidence from studies in humans.
- https://ntp.niehs.nih.gov/go/749926
- We are developing a 2nd monograph on 7 additional PFAS at EPA's request.

REACT PFAS Efforts

- **EPA Library of 75 Chemicals**
  - Collaborative effort between NTP and EPA
  - Chemicals selected based on read across approaches
  - Forward looking library
- **NTP Exploratory Effort**
  - Hypothesis-based evaluation of in vitro models
  - Assays may use all or part of the PFAS library

- **Chemical Specific Studies**
  - Autoimmunity and PFAS in mice (Germolec)
  - GenX (Birnbaum/Fenton)
    - Toxicokinetics
    - Placental toxicity
    - Transporters
      - Blood brain barrier
      - Renal

How do we evaluate unknowns?

- QSAR Modeling
  - Leadscope QSAR models
  - Predict a variety of toxic responses
  - We use the predictions to inform us on in vitro model and assay selection
  - Evaluated the 268 chemical list from EPA
    - Hepatoxicity
    - Developmental toxicity
    - Neurotoxicity
    - Cardiotoxicity

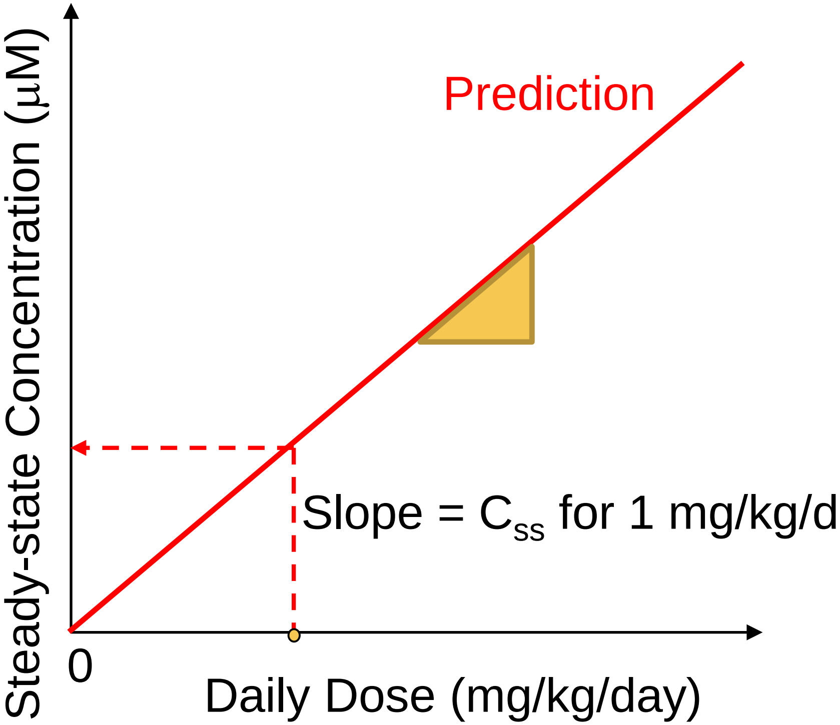
- High Throughput Transcriptomics
  - TempO-Seq and the S1500+ gene set
  - Allows for the evaluation of ~94% of known pathways

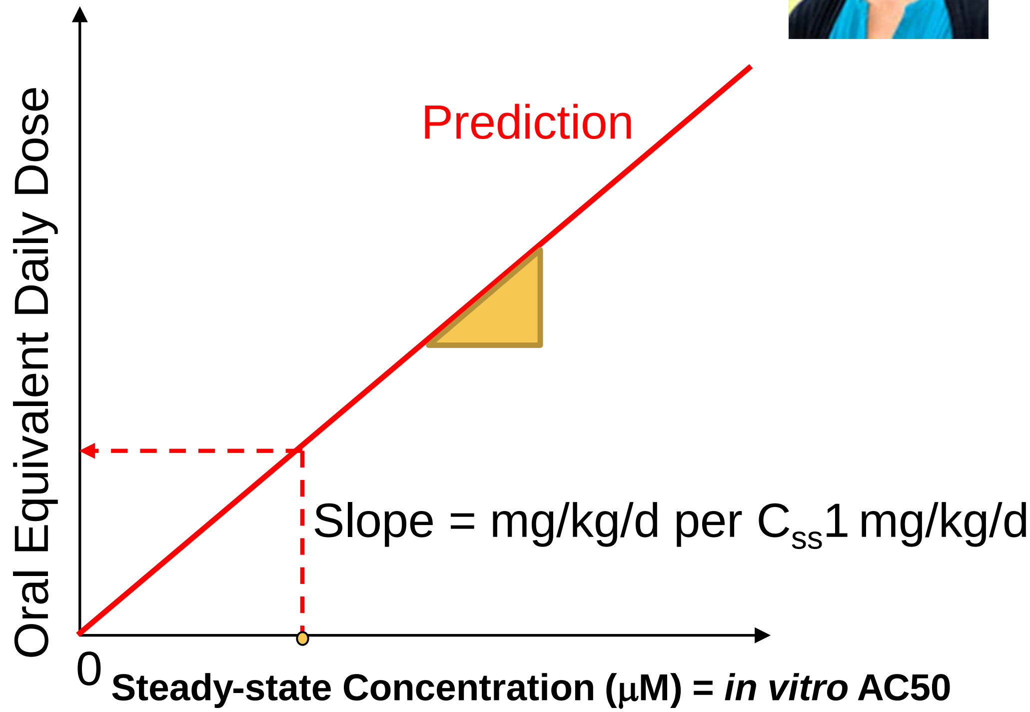
NTP-EPA PFAS Collaboration

Endpoint of Interest	NTP	EPA
Hepatotoxicity	3D HepaRG cell viability and transcriptomics	
Developmental toxicity		Zebrafish embryo assay
Developmental neurotoxicity		Multi-Electrode Array assay in neonatal cortical cells
Immunotoxicity	Cytokine alterations in human vascular endothelial cells (BioSeek)	
Hepatic Clearance	Metabolic clearance in 50 donor-pooled hepatocyte suspensions	
Plasma protein binding		Serum protein binding assay using human serum
Enterohepatic recirculation		Qualyst B-CLEAR hepatocyte transporter assay
In vitro disposition	In vitro disposition in 3D HepaRG cells	

In Vitro to In Vivo Extrapolation: IVIVE

Steady state in vitro-in vivo extrapolation assumption:
blood::tissue partitioning ≈ cells::medium partitioning





$$C_{ss} = \frac{\text{oral dose rate}}{(GFR * F_{ub}) + \left(Q_l * F_{ub} * \frac{Cl_{int}}{Q_l + F_{ub} * Cl_{int}}\right)}$$

- Swap the axes (this is the "reverse" part of reverse dosimetry)
- Can divide bioactive concentration by C_{ss} for for a 1 mg/kg/day dose to get oral equivalent dose

Wetmore *et al*. (2012)

Slide from John Wambaugh

Assays for NTP Exploratory Efforts

Endpoint of Interest	Assay
Hepatotoxicity	Mitochondrial toxicity in 2D HepaRG cells. (NTP Laboratory).
Immunotoxicity	Human and rat cytokine alterations in peripheral blood lymphocytes
Placental model	JEG cell viability and transcriptional changes(NTP Laboratory).
Milk protein production	Inhibition of milk protein production in MCF-7 cells (NTP Laboratory).
Renal Transport	Renal proximal tubule permeability assay in rats (NTPL) and humans (Contract)
Embryoid Bodies (Human Embyronic Stem Cells)	Transcriptional and immunohistochemical markers of differentiation (NTP Laboratory).

How do we organize the data?

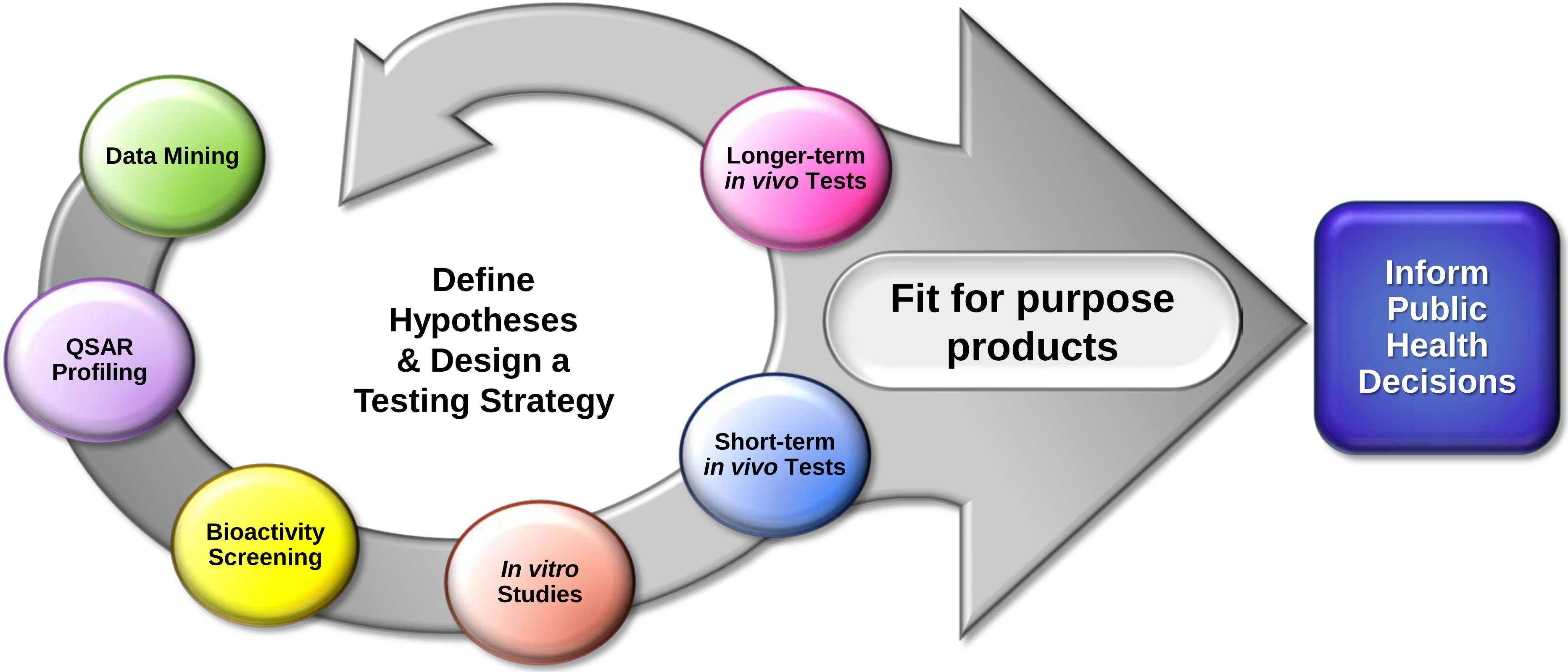
PFAS Assessment is based on Read Across

- Read Across
  - When the already available data of a data-rich substance (the source) is used for a data-poor substance (the target), which is considered similar enough to the source substance to use the same data as a basis for the safety assessment.
- PFAS will be clustered by structures and biological activity (includes toxicokinetic parameters).
- Use the PFAS from the NTP 28-day PFC class studies as anchor chemicals for read across.
- Likely need to run other PFAS as anchors.

Outputs

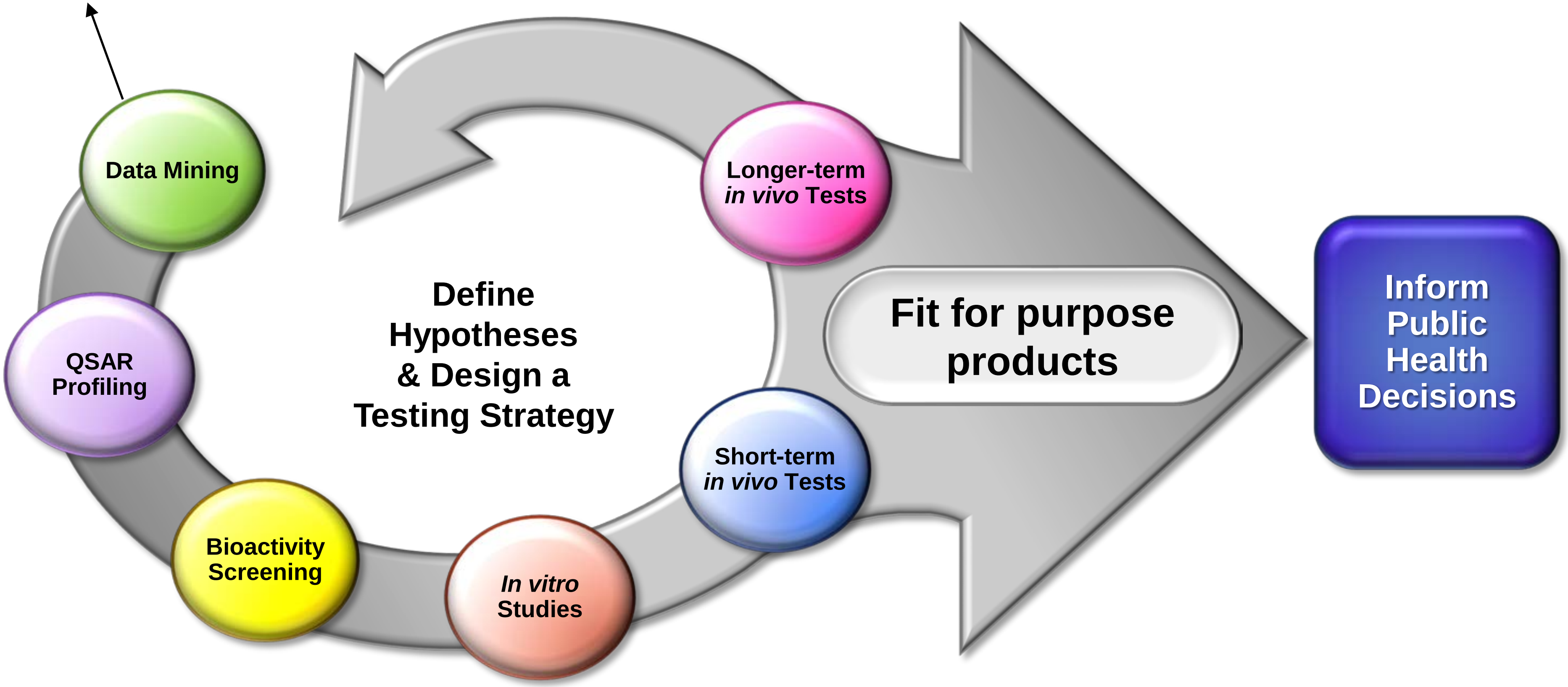
- In vitro assessment of >75 PFAS
- Grouping PFAS based on structure and biological activity
- Toxicokinetic data and IVIVE models for estimating oral equivalent exposures for PFAS
- Potential for follow-up in vivo studies as needed

DNTP Translational Toxicology Pipeline Plan



Systematic Literature Review of PFAS Immunotoxicity

DNTP Translational Toxicology Pipeline Plan



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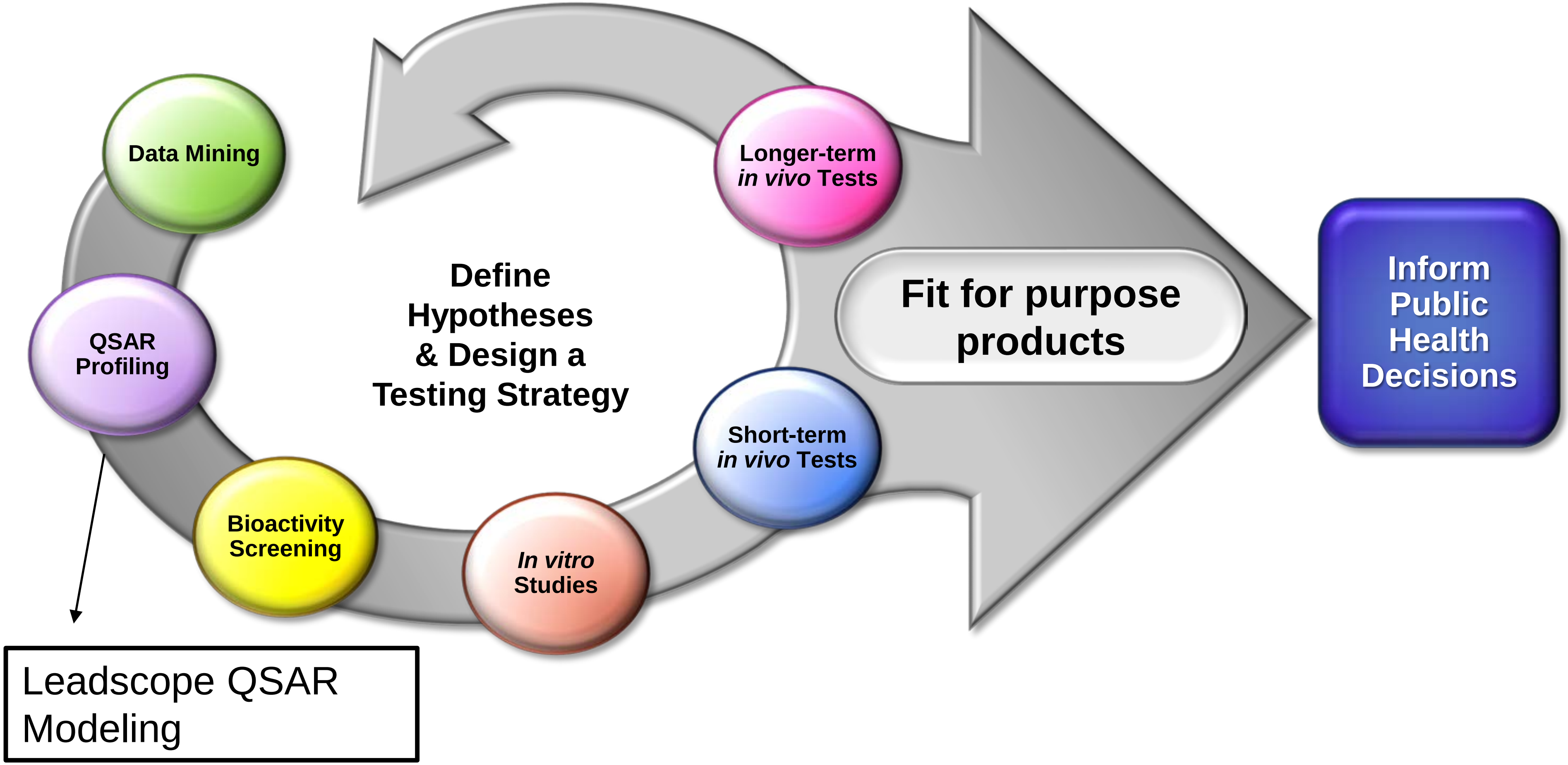

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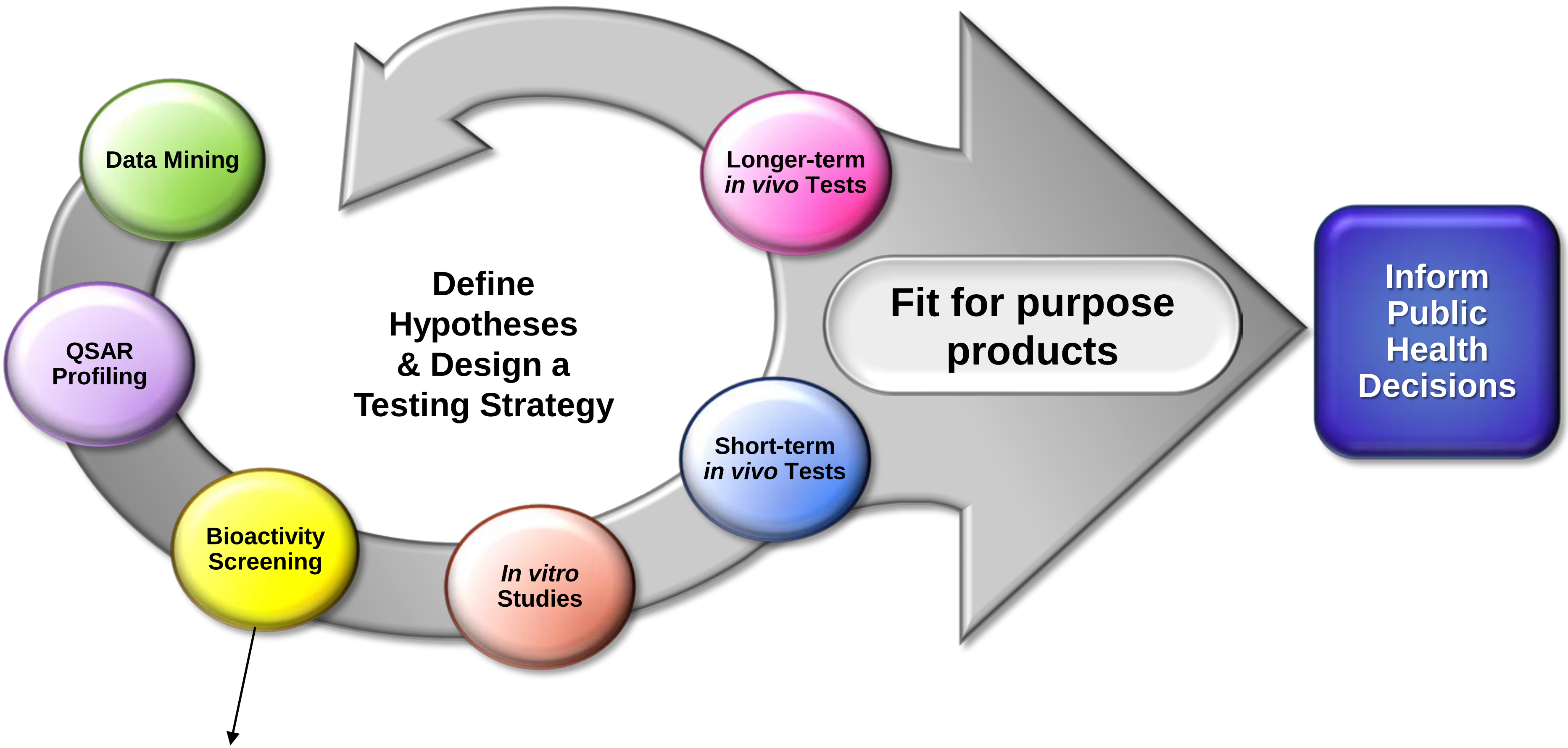
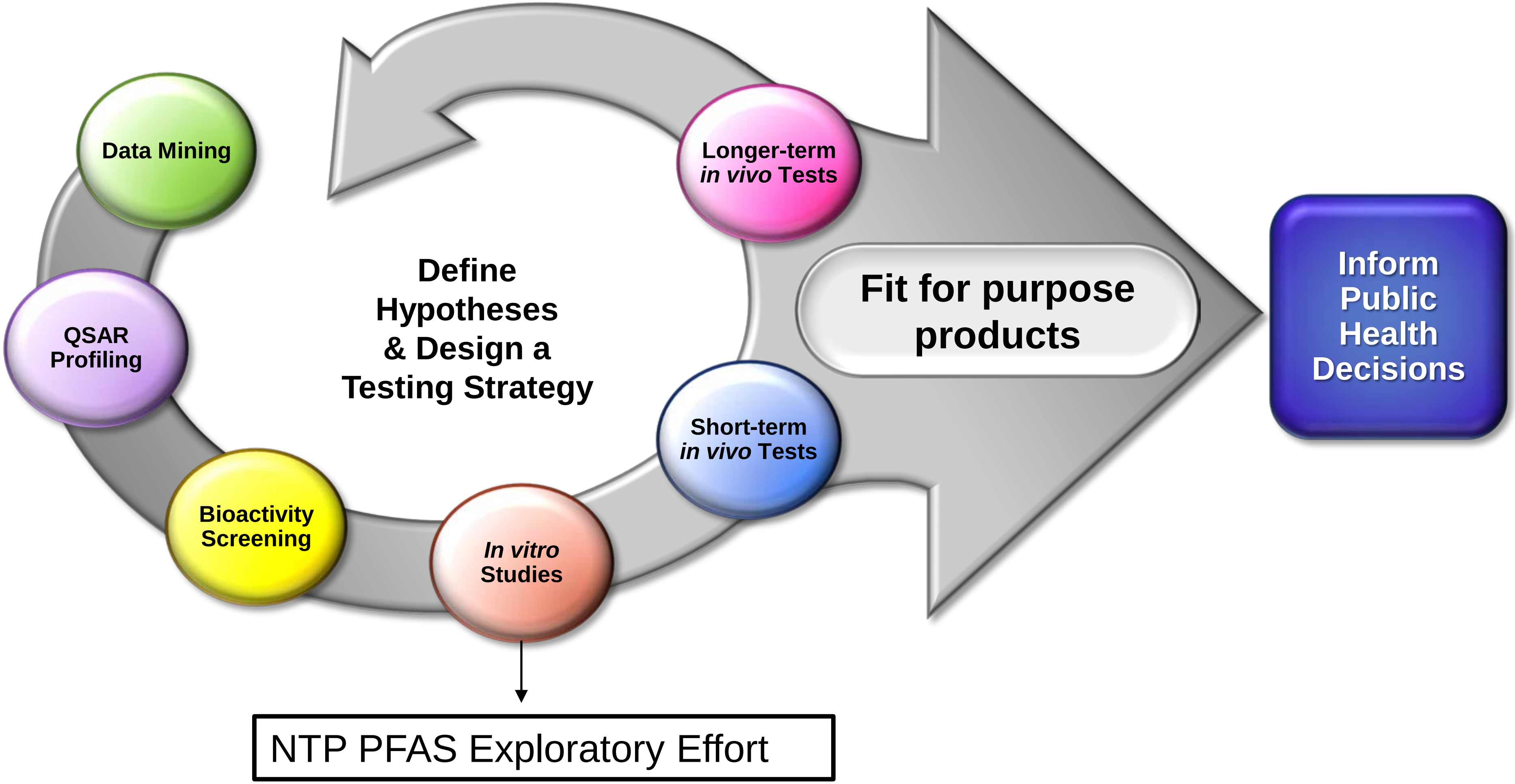


Tox21 Data
PFAS NTP-EPA Collaboration

DNTP Translational Toxicology Pipeline Plan



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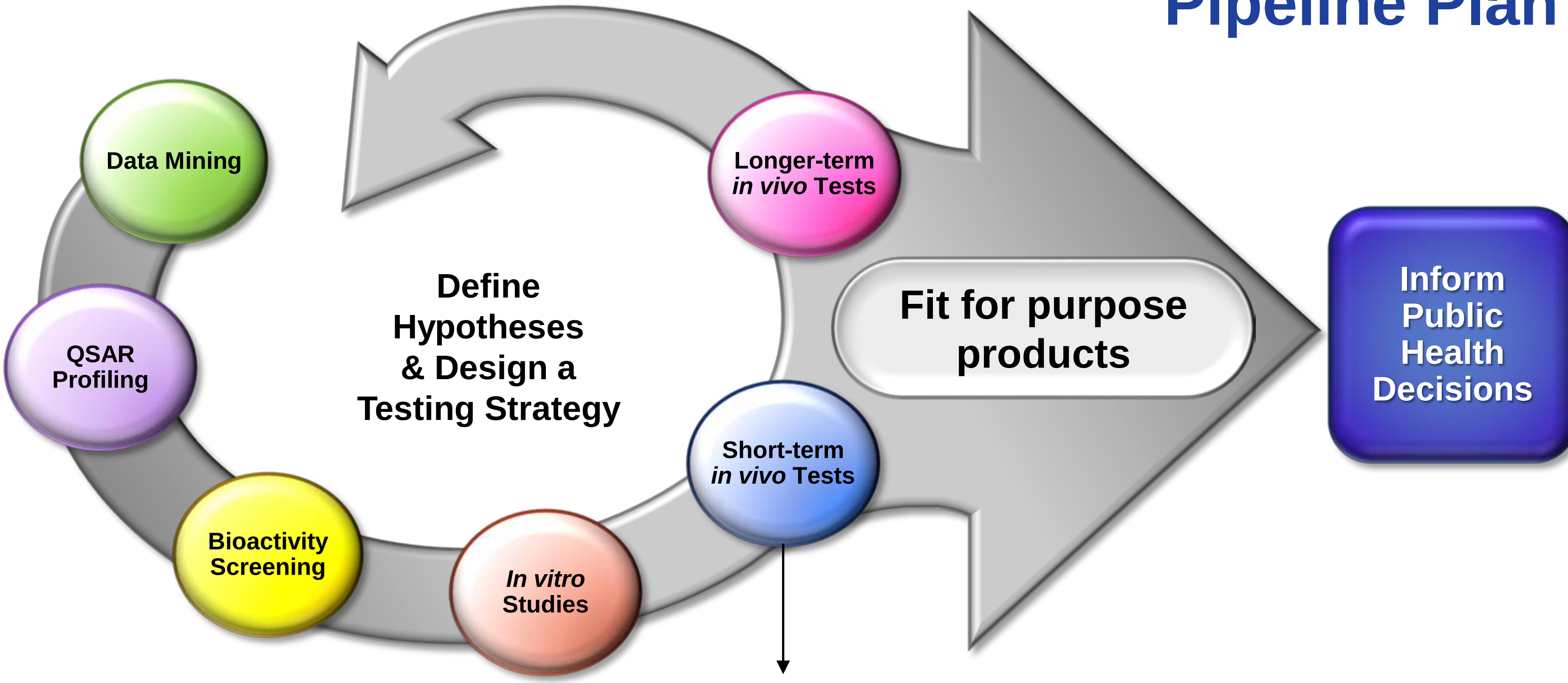
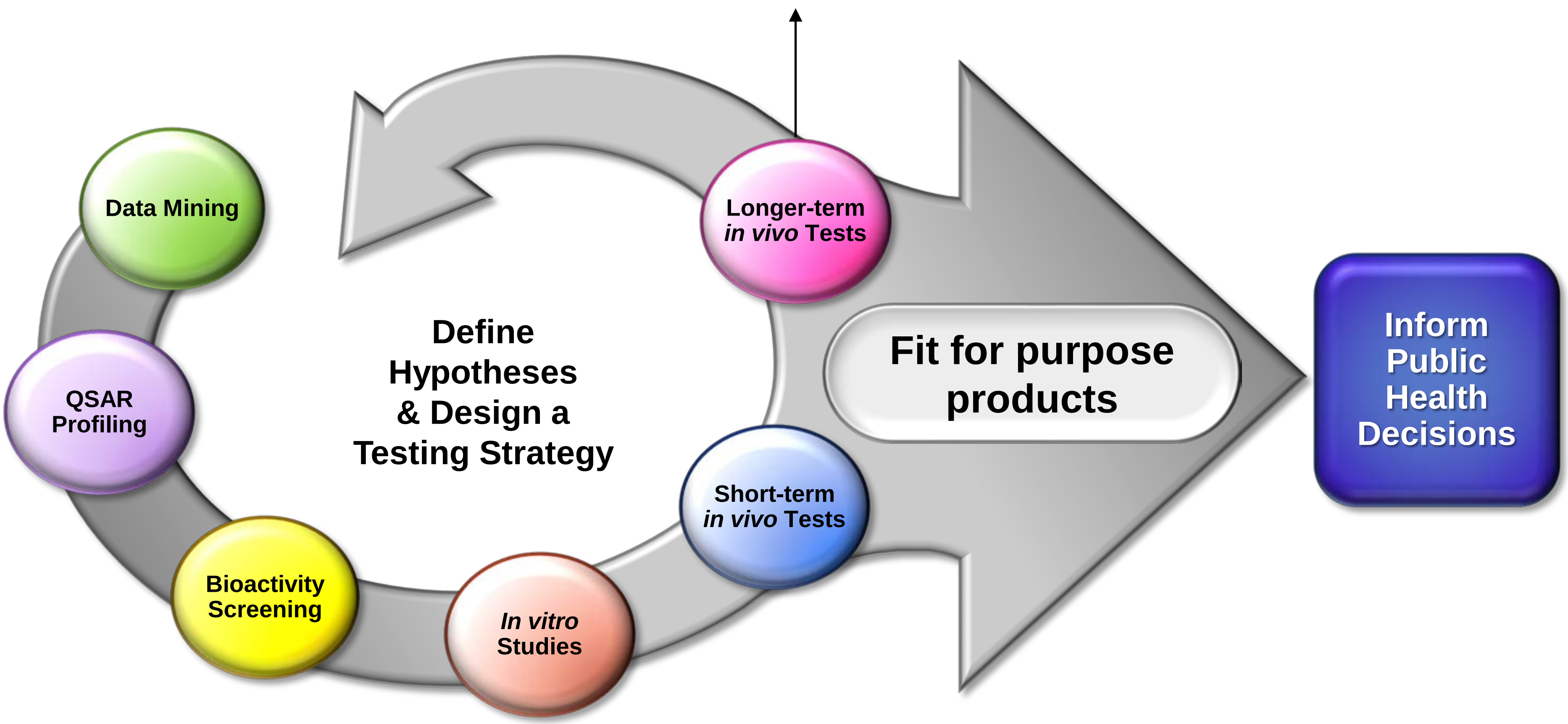


28-day Toxicity Studies
Immunotoxicity Studies
GenX Studies
Toxicokinetic Studies
5- and 28-day Transcriptomic Studies

DNTP Translational Toxicology Pipeline Plan

PFOA Carcinogenicity and Toxicity Studies



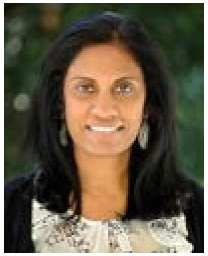
REACT PFAS Efforts to Date

- Developed a data analysis pipeline in the CEBS database for in vitro data from the NTP Laboratory
- Developed a transcriptomic analysis and reporting pipeline
- Evaluated subsets of the PFAS library in several of the exploratory efforts
- Developing analytical methods (have methods for ~15 PFAS)
- Obtained EPA library

REACT PFAS Disciplinary Leads

- **Hepatotoxicity – Steve Ferguson**
- **Immunotoxicity – Dori Germolec**
- **Endocrine toxicity – Sue Fenton**
- **In vitro toxicokinetics – Mike DeVito/Suramya Waidayanatha**
- **IVIVE – Nisha Sipes**
- **Embyroid bodies – Erik Tokar**
- **GenX studies – Linda Birnbaum/Sue Fenton**
- **QSAR modeling – Jui-Hua Hseih**





Questions?