NTP
National Toxicology Program

Integrating Literature Analysis into the NTP Research Pipeline

Windy Boyd

NTP Board of Scientific Counselors Meeting

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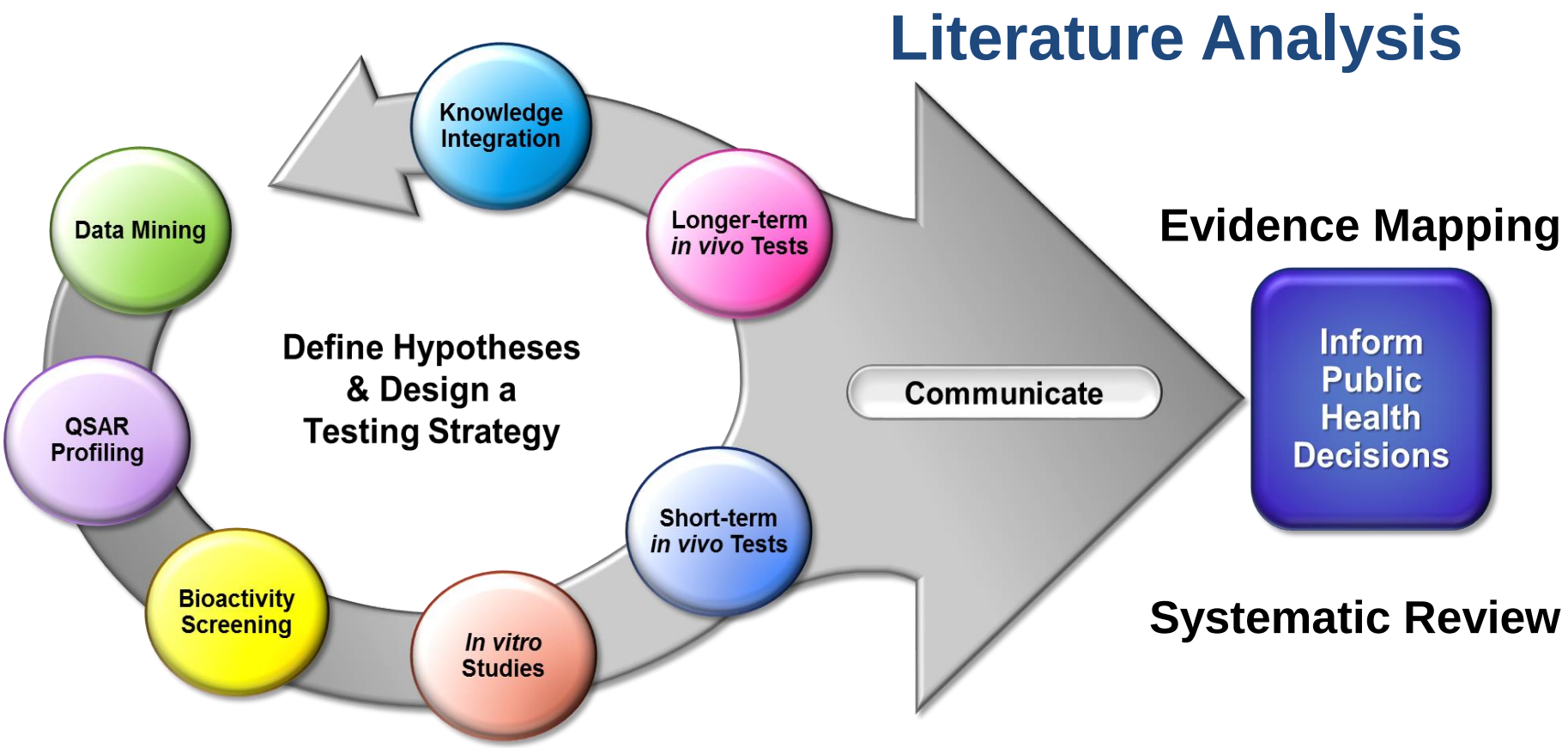
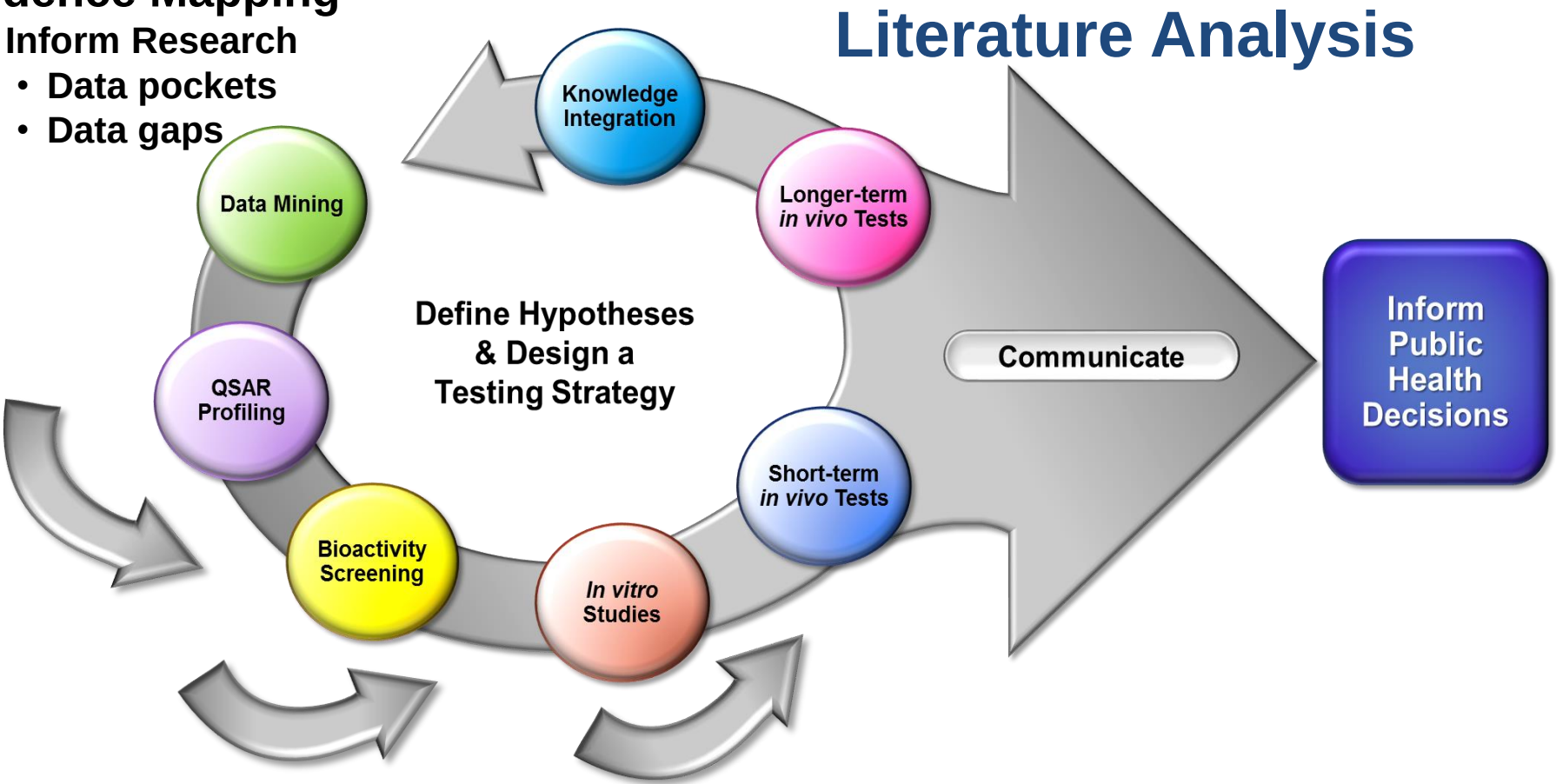
DNTP Translational Toxicology Pipeline Plan



DNTP Translational Toxicology Pipeline Plan

Evidence Mapping

- Inform Research
  - Data pockets
  - Data gaps



Background

- Parkinson's disease (PD) due to progressive neurodegeneration
  - Aggregation of α-synuclein in Lewy bodies
  - Loss of dopaminergic neurons in substantia nigra
  - Signs include tremor, rigidity, and shuffling gait
- Highly prevalent but etiology of most PD cases unknown
  - Genetics only account for ~10% of cases

Environmental factors

- Exposures to pesticides linked to Parkinson's in epidemiological studies
- Need for better understanding of which environmental factors may be contributing and how they act
- Neurodegeneration is not included in routine toxicological testing strategies
- Lack of methods to rapidly identify environmental exposures

Strategy to identify potential chemical contributors

- Project team
  - Combined scientific expertise in neurotoxicology, *in vitro* screening, toxicoinformatics, and literature analysis
- Goals
  - Identify previously evaluated chemicals, genes and pathways, and model systems
  - Develop a battery of *in vitro* and alternate model organism assays to screen chemicals for potential effects

NTP Parkinson's Disease Project

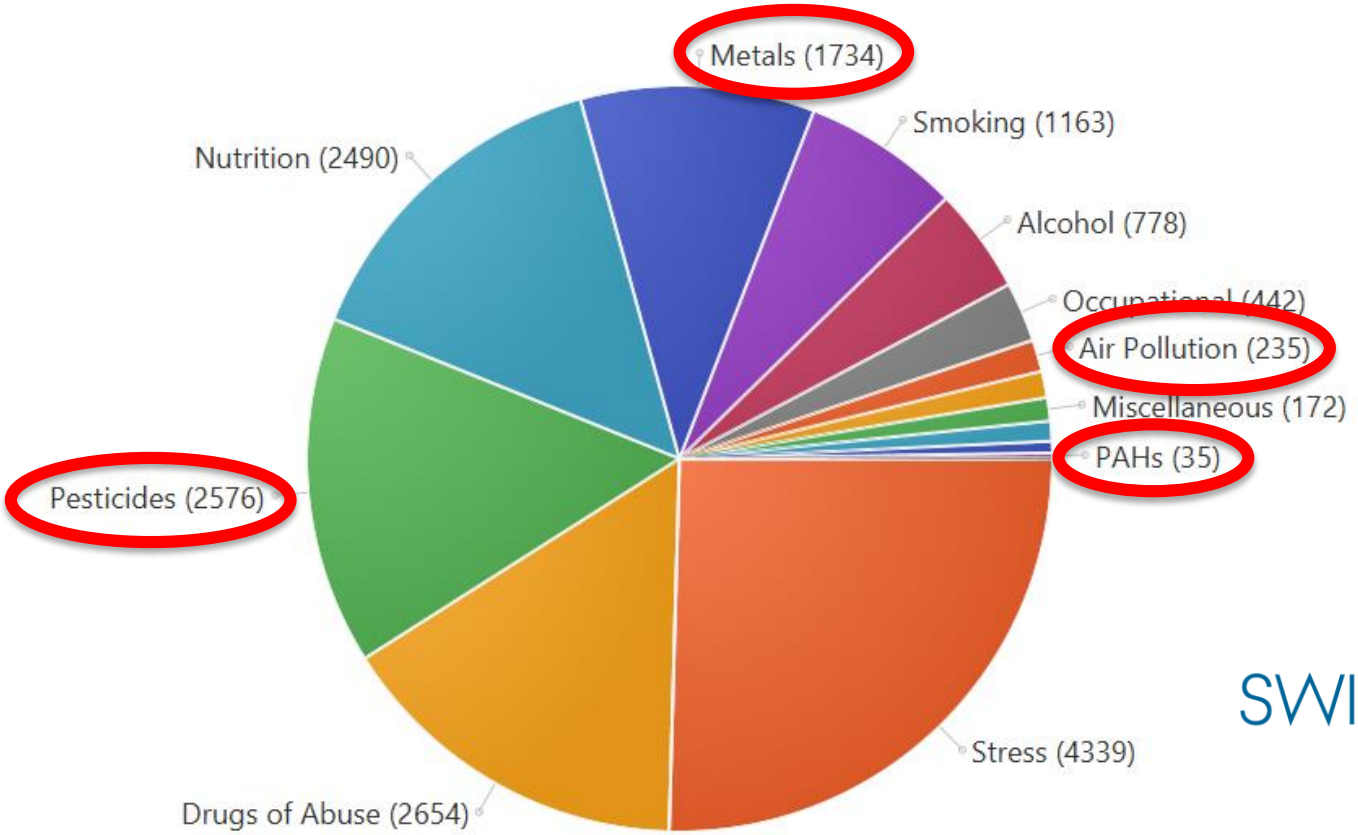
Strategy to identify potential chemical contributors

Literature analysis

- Questions: Which chemicals, genetic targets, and models have been reported in the scientific literature?
- PubMed search identified >90,000 records with mention of Parkinson's disease
- Screened studies for environmental chemical exposure and categorized by study characteristics

Exposures Associated with Parkinson's Disease

Automated Tagging of All Environmental Exposures



Ana Antonic
University of Melbourne

SWIFT REVIEW

Parkinson's Disease Evidence Map

		Human	*In vitro*	*In vivo*
Pesticides	Insecticides	33	504	375
	Herbicides	24	139	181
	Pesticides	11	3	1
	Fungicides	10	31	56
	Acaricides	1	1	
	Fumigants	1		
Metals	Metals	151	153	138
Nicotine	Nicotine	38	35	97
Other	Coolants	1		
	Disinfectants	1		
	Flame retardants		2	
	Fragrances			1
	Gases	1		
	Gasoline additives		1	
	Industrial	9	4	3

✓ Manual categorization of 1,840 studies revealed similar trend as automated tagging and allows researchers to explore published literature

Parkinson's Disease Evidence Map

Most-reported Environmental Chemicals

		Human	*In vivo*	*In vitro*
Manganese	Exposure	115	106	98
	Positive control		6	8
Paraquat	Exposure	22	135	97
	Positive control		37	36
Rotenone	Exposure	10	137	198
	Positive control		204	274
Nicotine	Exposure	21	36	17
	Treatment	16	61	18

Parkinson's Disease Evidence Map

Environmental Chemicals in >10 Studies

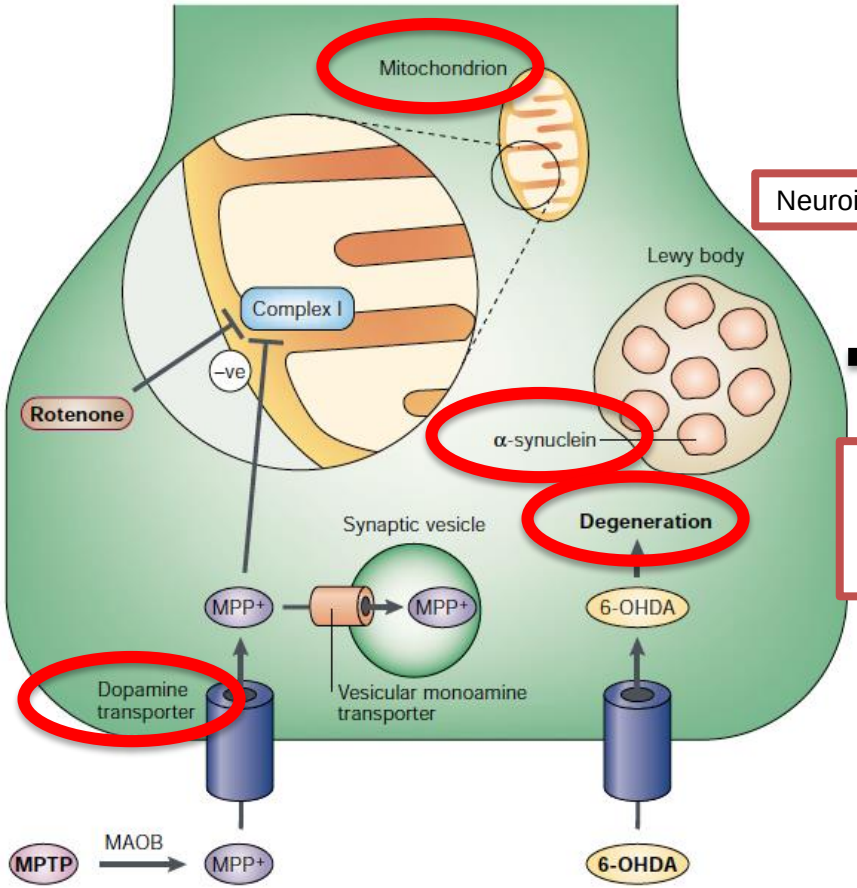
	Human	*In vivo*	*In vitro*
Maneb	8	43	22
Aluminum	17	14	14
Iron	17	8	19
Dieldrin	10	6	23
Mercury	19	1	6
Copper	10	6	12
Lead	20	2	4
Cadmium	5	2	10
PCBs	9	3	3
Zinc	8	5	2
Mancozeb	1	7	4

- Very few chemicals with multiple reports
- All metals and/or pesticides except PCBs
- Many chemicals with single study (not shown)

Candidate Chemical Library

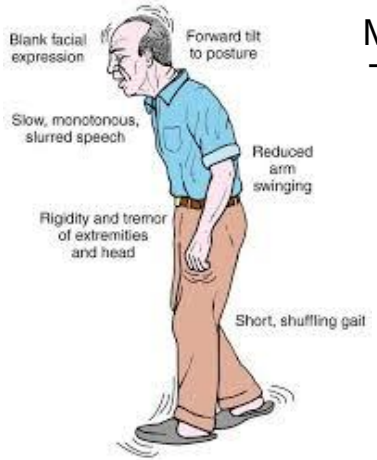
- **Predicted actives**
  - Positive controls
    - MPTP, rotenone, paraquat
  - Metals and metal compounds
    - Manganese tricarbonyl (MMT), maneb, methyl mercury, ziram
  - Organochlorines
    - DDT, heptachlor, dieldrin, lindane, endosulfan, TCE, hexachlorobenzene
  - Organophosphates
    - Chlorpyrifos, diazinon
  - Other pesticides
    - Permethrin, benomyl, tributyltin methacrylate, quintozene
- **Unknowns**
  - Triphenyl phosphate
  - Isopropylated phenyl phosphate
  - Captan
  - Glyphosate
  - Pyridaben
  - Acetaminophen
- **Predicted Negatives**
  - Saccharin sodium
  - L-ascorbic acid
  - D-glucitol
  - Acetyl salicylic acid

Informing Assay Selection for Targeted Testing



Neuroinflammation

Related changes in gene expression



Motor deficits

Mamta Behl
Tox Branch

Parkinson's Disease Evidence Map

in vitro Effects of Paraquat Exposure

Effect	Species					
	Human	Rat	Mouse	Rat x Mouse	Bovine	Grand Total
DA (TH+) neurons	2	8	9			16
Dopamine (DA and metabolite levels, DAT and receptor expression, TH immunoreactivity)	4	6	1	1		11
alpha synuclein, Tau phosphorylation, tubulin	11	4	2			16
Proteasome (Parkin, proteasomal activity)	10	3				13
Mitochondrial effects	22	13	2			37
Other (general expression changes, etc.)	40	28	13			78
Oxidative stress	40	39	13	2		89
Cell viability (LDH levels, apoptosis, total cell number)	65	60	19	2	1	137
Grand Total	81	74	30	2	1	178

*Some studies may have characterized multiple health effects or species and therefore may be represented multiple times. Row and column grand totals represent counts of distinct references.

Reported *in vitro* Models

Cell line	Category	Cell, tumor, subfraction type	Tissue origin	
SH-SY5Y	tumor	neuroblastoma	brain, bone marrow metastasis	49
SK-N-SH	tumor	neuroblastoma	nerve, bone marrow metastasis	7
primary mesencephalic cultures	primary	neurons	mesencephalon	22
		neurons, glia	mesencephalon	2
PC12	tumor	pheochromocytoma	adrenal gland	20
N27	transformed	neurons	mesencephalon	17
primary cerebral cortex cultures	primary	glia	cerebral cortex	1
		microglia	cerebral cortex	1
		neurons	cerebral cortex	7
		neurons, glia	cerebral cortex	1
		oligodendrocyte progenitors	cerebral cortex	1
primary cerebellar cultures	primary	granule neurons	cerebellum	3
		neurons	cerebellum	3
BV-2	transformed	microglia	brain	5
primary astrocytes	primary	astrocytes	brain	4
			cerebral cortex	1
brain cultures	ex vivo	mixed	brain	1

✓ majority of studies conducted in human and rat tumorigenic cell lines with fewer more relevant, complex models

Identifying Chemical-Gene Combinations

Toxicoinformatic Analysis


Ana Antonic


Nisha Sipes
BSB

- Selected genes associated with Parkinson's
  - Illumina's NextBio datamining software
  - Comparative Toxicogenomics Database (CTD)
- Grouped 233 genes into 15 disease-relevant pathways
- Linked genes to studies included in literature analysis
- Gene expression reported in 47% of relevant studies
  - 57% of 233 genes evaluated in those studies

Identifying Chemical-Gene Combinations

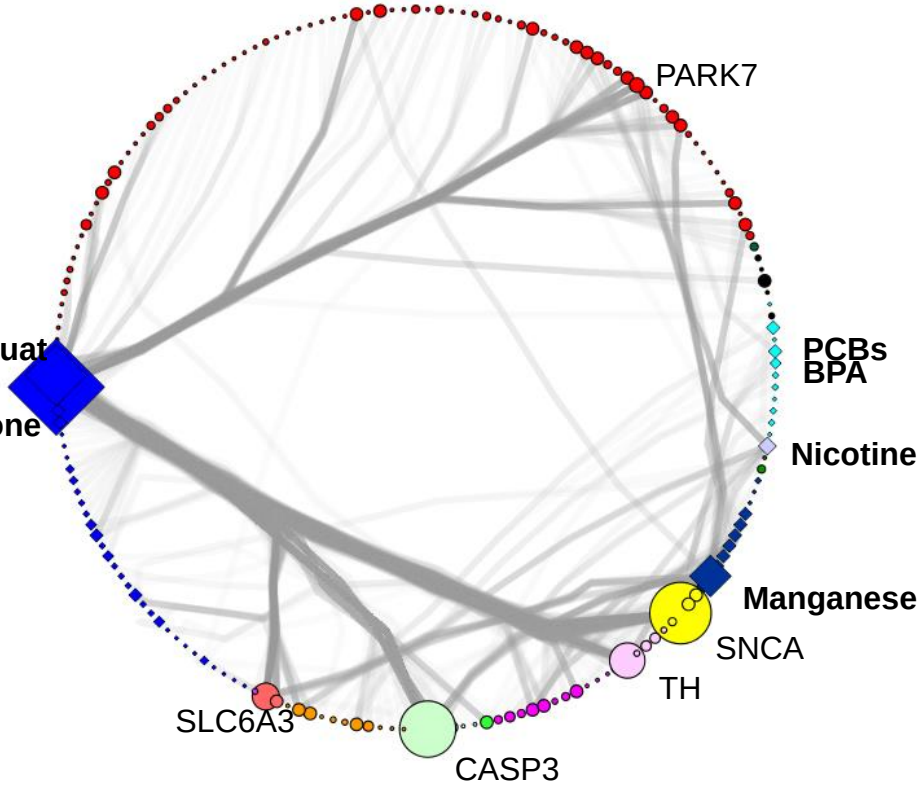
Nisha Sipes
BSB

Chemical Classes

- Pesticides
- Nicotine
- Metals
- Other

Biological Pathways

- Apoptosis
- Adenosine Receptor
- Axon guidance/synaptic
- Dopamine
- Dopamine Receptor
- Immune System
- α-synuclein/Lewy body
- Neuronal survival/activity
- Other
- Other receptors
- Mitochondria/ox phos
- Transcription factor
- Transporter
- Ubiquitin

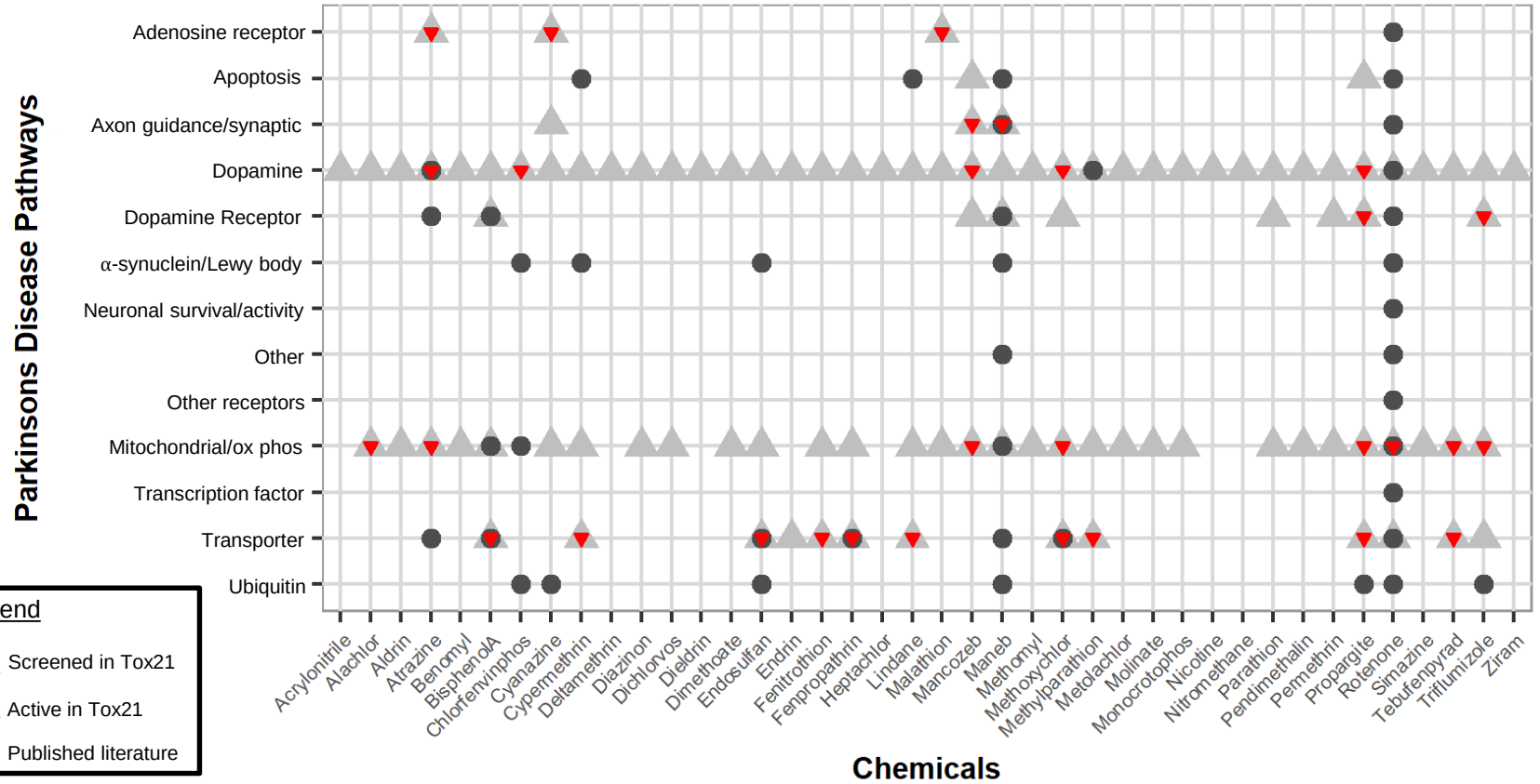


Cytoscape

Identifying Chemical-Assay Combinations

ToxCast and Tox21 Data

Sipes
SB



NTP Parkinson's Disease Project

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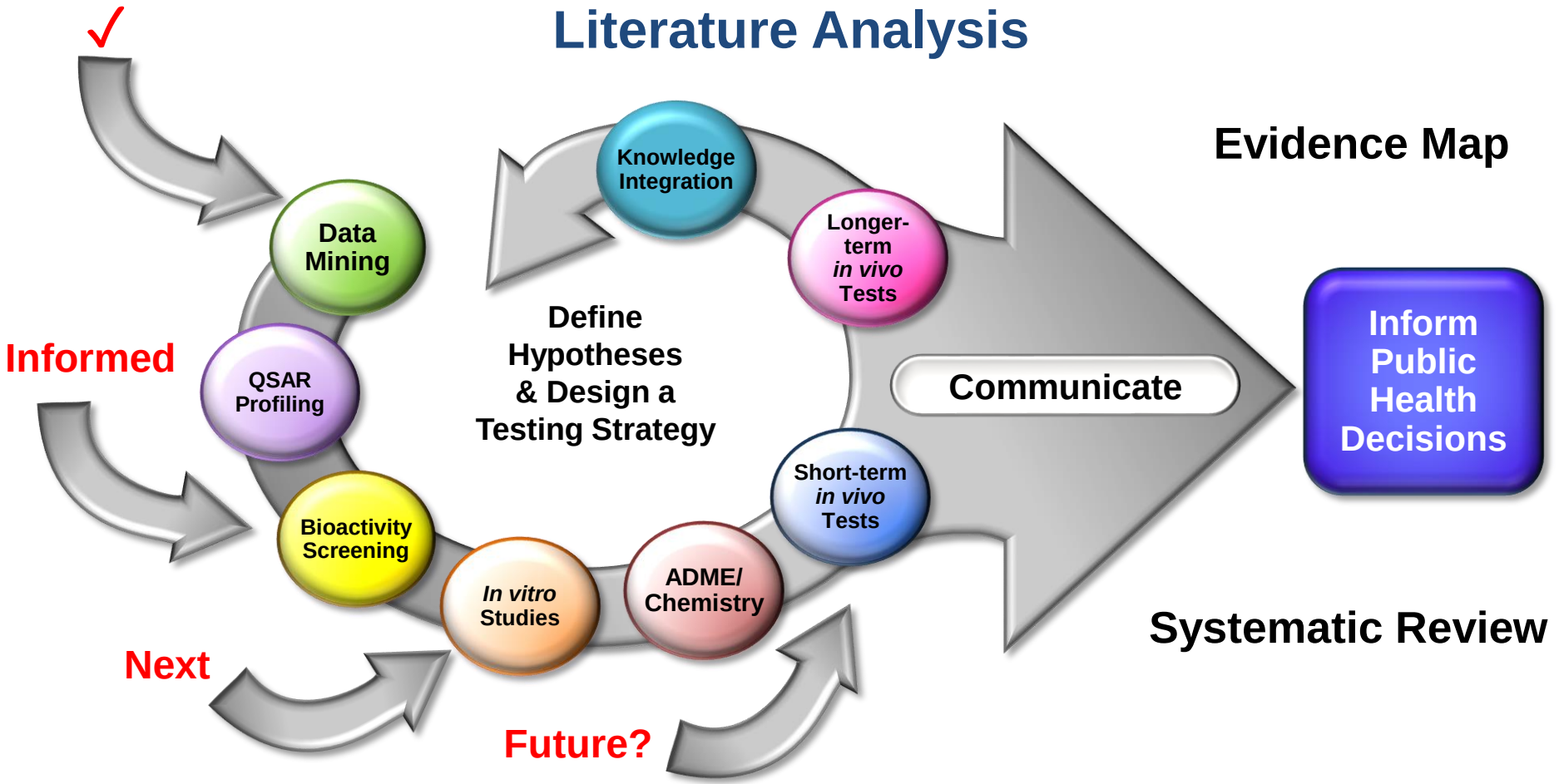

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OHAT

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- Ana Antonic, University of Melbourne
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- Austin Wray and Aaron Niman, US EPA

Peer Review

- Chris McPherson, Paraquat Scoping Report
- Nisha Sipes, Paraquat Scoping Report

…on behalf of OHAT

Thank you

Questions?