Scientific conference on the use of epidemiological findings in regulatory pesticide risk assessment
Parma 21 November 2017

The AOP conceptual framework as a tool to support biological plausibility of epidemiological studies.

Practical example: Inhibition of the mitochondrial complex I of nigrostriatal neurons leading to Parkinsonian motor deficits

Karine ANGELI
ANSES-DEPR

BACKGROUND

- In Europe, plant protection products are principally regulated by **Regulation (EC) No 1107/2009**.
- Human health risk assessment for pesticide active substances mostly relies on **experimental toxicology and pathology data collected from laboratory animals**.
- According to Regulation No 1107/2009, and as indicated in Regulation 283/2013 setting out data requirements for active substances submitted dossier shall include scientific peer-reviewed literature notably **"relevant epidemiological studies shall be submitted, where available"**

BACKGROUND

- Abundance of epidemiological studies investigating possible associations: pesticide exposure/health effects

- **Associations between pesticide exposure and Parkinson's disease (PD) is consistently reported in meta-analysis published in the scientific literature**

➡Sufficient evidence to conclude on an association between pesticides exposure and PD

➡Not enough to conclude on a causal relationship with specific active substances.

➔ 2014 PPR Panel of EFSA requested to prepare Scientific Opinion: Investigation into experimental toxicological properties of plant protection products having a potential link to Parkinson's disease (and childhood leukaemia).

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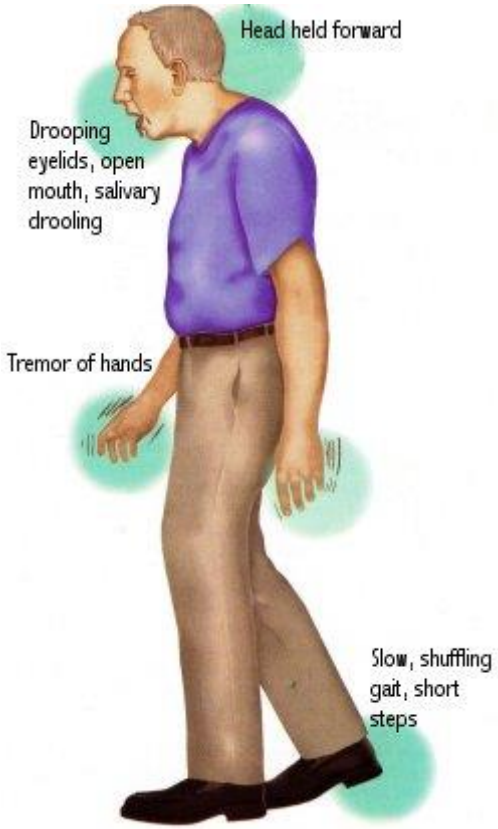
PARKINSON'S DISEASE

- **PD: Chronic progressive neurodegenerative disorder, complex multifactorial disease**

environmental, lifestyle and genetic risk factors

- **Parkinson motor deficits:** bradykinesia, rigidity, resting tremor, postural instability

possibly associated with vegetative symptoms and cognitive decline.



- Selective degeneration **dopaminergic neurons in the substantia nigra pars compacta (SNpc)**

- **Histopathology: Lewy body (LB)**

cytoplasmic protein-rich inclusions

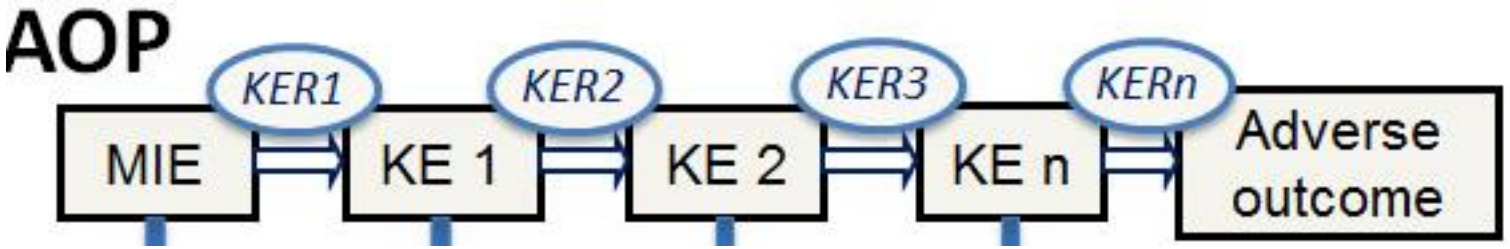
INVESTIGATION OF BIOLOGICAL PLAUSIBILITY

- Limitations of regulatory studies to inform on specific & complex human health outcomes as PD.
- Experimental data investigating plausible mechanisms available in the open scientific literature.
- **In order** to support /refute **the biological plausibility of epidemiological studies** linking pesticides exposure to PD **Adverse Outcome Pathway (AOP) conceptual framework tested.**

INVESTIGATION OF BIOLOGICAL PLAUSIBILITY

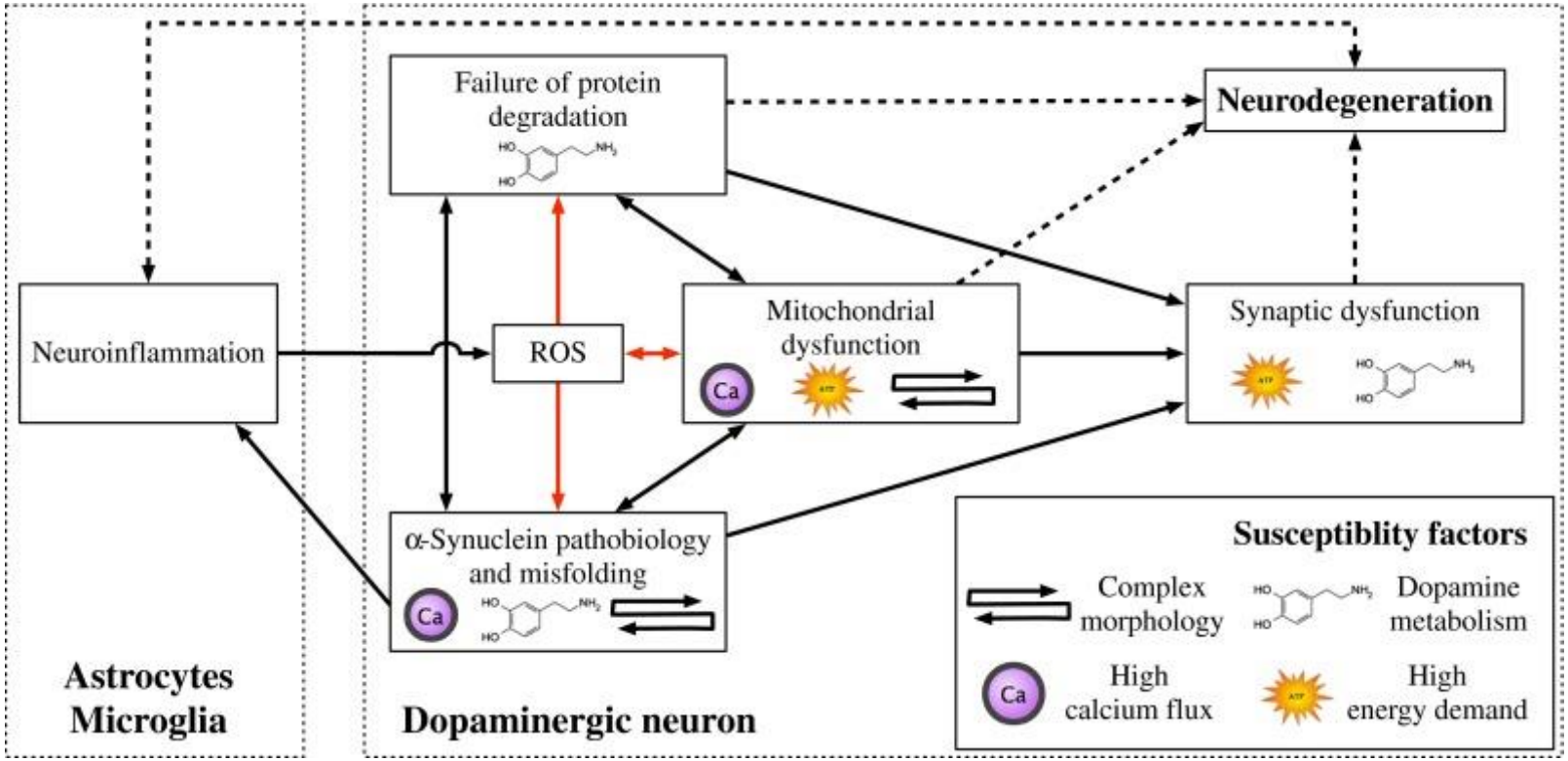
- AOP describes the chain of events leading from the first interaction of any chemical with a target (molecular initiating event (**MIE**)) to an adverse outcome (**AO**), generally an apical endpoint in accepted regulatory toxicity testing.

- **AOP use as a tool** for the review, organization and interpretation of all the available experimental data coming from different fields ➔ mechanistic based assessment of biological plausibility of epidemiological outcomes



DEVELOPING AN AOP FOR PD

- Systematic review (EN-955, 2016)+ experts knowledge on the state of experimental parkinsonism research



Fujita et al. 2014
Pathways implicated in PD and their relationship to susceptibility factors of SNpc DA neurons. Map of pathogenesis of PD

DEVELOPING AN AOP FOR PD

- AOP not initially developed for a complex disease. From available experimental data ➔Multiples AO, KE and AOPs possible for PD

AO, e.g.: bradykinesia, rigidity, resting, tremor, postural instability, cognitive decline, degeneration of DA neurons of SNpc…

KE, e.g: Complex I inhibition, Mitochondrial dysfunction, ALDH inhibition, Oxidative stress, Impaired proteostasis, neuroinflammation…

- AOP = tool pragmatically simplifying complex biological events ➔**single sequence of key events connecting the MIE to an AO**

➔1 AOP may explain only a part of the supposed interaction pesticides/ PD risk.

➔AOP: not specific to one compound ("compound-agnostic"), focussed on biology pathway

DEVELOPING AN AOP FOR PD

- Selection of Adverse Outcome: **Parkinsonian motor deficits**

relatively specific, found in all cases of PD, transferable to animal models

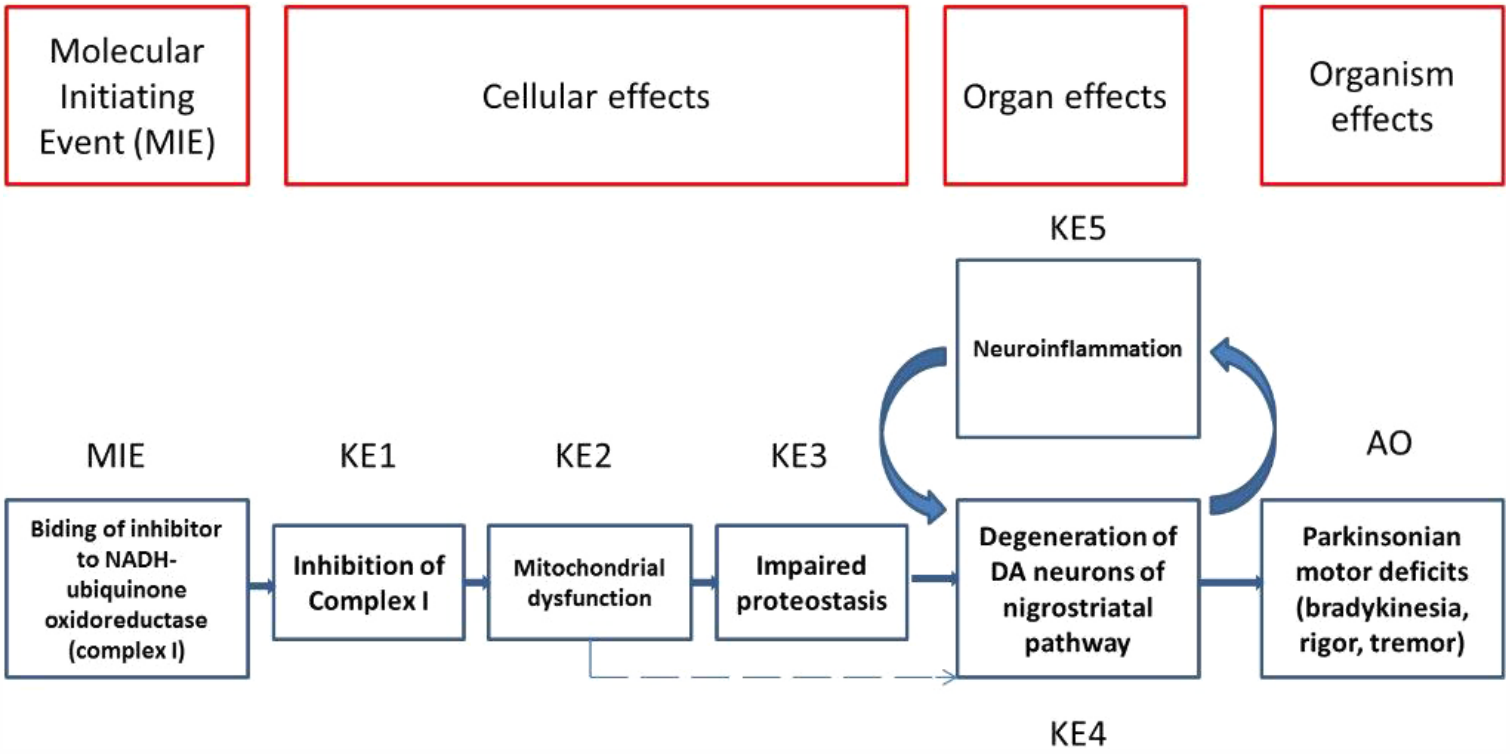
- Proposed relevant AOP for both Parkinsonian motor deficits and pesticides as risk factors: **Inhibition of the mitochondrial complex I of nigrostriatal neurons leads to parkinsonian motor deficits**

- Tool chemicals: **MPTP/MPP+ and rotenone** based on abundant documentation

Rotenone: numerous data from rodent models and well characterized molecular target

MPTP/MPP+ : same molecular target, evidence it plays a key role in human disease pathology.

PROPOSED AOP



Inhibition of the mitochondrial complex I of nigrostriatal neurons leads to parkinsonian motor deficits

DOSE-RESPONSE AND TEMPORALITY TABLE

Dose/Concentration	KE1 Inhibition of C I	KE2 Mitochondrial dysfunction	KE3 Impaired proteostasis	KE4 Degeneration of DA neurons of nigrostriatal pathway	AO Parkinsonian motor symptoms
Rotenone					
5-10 nM *in-vitro*	+ 4-72 hrs	+ 4-72 hrs	+ 24 hrs	-	-
20-30 nM *ex-vivo*, rat brain concentration	++ 4-72 hrs	++ 4-72 hrs	++ 24 hrs	++ 5 weeks	+++ 5 weeks
100 nM *in-vitro*	+++ 4-72 hrs	+++ 4-72 hrs	+++ 24 hrs	Above MTD	Above MTD
MPTP					
1 mg/kg infusion mouse	-	-	+ 4 weeks	+ 4 weeks	No effect
5 mg/kg infusion mouse	-	-	++ 4 weeks	++ 4 weeks	+++ 4 weeks
20-30 mg/kg mouse infusion or ip injection 4 times every 2 hours ***Brain con: 47µM 12µM***	+++ 4 hrs	+++ 4hrs	+++ 4 weeks	+++ 1-4 weeks	+++ 4 weeks

WOE-BIOLOGICAL PLAUSIBILITY, COHERENCE & CONSISTENCY

Support for Biological Plausibility of KERs	Is there a mechanistic relationship between KEup and KEdown consistent with established biological knowledge?
MIE => KE1 Binding of inhibitor to complex I leads to complex I inhibition	**STRONG**
KE1 => KE2 Inhibition of complex I leads to mitochondrial dysfunction	**STRONG**
KE2 => KE3 Mitochondrial dysfunction results in impaired proteostasis	**MODERATE**
KE2 => KE4 Mitochondrial dysfunction leads to the degeneration of dopaminergic neurons of the nigrostriatal pathway	**STRONG**
KE3 => KE4 Impaired proteostasis leads to degeneration of DA neurons of the nigrostriatal pathway	**MODERATE**
KE4 <=> KE5 Degeneration of DA neurons of the nigrostriatal pathway leads to neuroinflammation	**MODERATE**
KE4 => AO Degeneration of DA neurons of the nigrostriatal pathway leads to parkinsonian motor symptoms	**STRONG**

WOE - ESSENTIALITY

Support for Essentiality of KEs	Are downstream KEs and/or the AO prevented if an upstream KE is blocked ?
KE1 Inhibition of complex I	**STRONG**
KE2 Mitochondrial dysfunction	**STRONG**
KE3 Impaired proteostasis	**MODERATE**
KE4 Degeneration of DA neurons of nigrostriatal pathway	**STRONG**
KE5 Neuroinflammation	**MODERATE**

WOE-EMPIRICAL SUPPORT

Support for Biological Plausibility of KERs	Does the empirical evidence support that a change in the KEup leads to an appropriate change in the KE down? Does KEup occur at lower doses and earlier time points than KE down and is the incidence of KEup higher than that for KE down?
MIE => KE1 Binding of inhibitor to complex I leads of complex I inhibition	**STRONG**
KE1 => KE2 Inhibition of complex I leads to mitochondrial dysfunction	**STRONG**
KE2 => KE3 Mitochondrial dysfunction results in impaired proteostasis	**STRONG**
KE2 => KE4 Mitochondrial dysfunction leads to the degeneration of dopaminergic neurons of the nigrostriatal pathway	**STRONG**
KE3 => KE4 Impaired proteostasis leads to degeneration of DA neurons of the nigrostriatal pathway	**STRONG**
KE4 <=> KE5 Degeneration of DA neurons of the nigrostriatal pathway leads to neuroinflammation	**MODERATE**
KE4 => AO Degeneration of DA neurons of the nigrostriatal pathway leads to parkinsonian motor symptoms	**STRONG**

QUANTITATIVE CONSIDERATIONS

Dose/Concentration	KE1 Inhibition of C I	KE2 Mitochondrial dysfunction	KE3 Impaired proteostasis	KE4 Degeneration of DA neurons of nigrostriatal pathway	AO Parkinsonian motor symptoms
Rotenone					
Rotenone **20-30 nM rat brain concentration**	**Approx.** **53%**	**Approx** **20-53%** (decrease in respiration rate)	**Approx** **20-60%** (decrease in UPS (26S) activity)	**Neuronal loss** (50% of animal affected)	**Motor impairment** (100% of animals with neuronal loss)
MPTP					
MPP+ **12-47 µM rat brain concentration**	**Approx.** **50-75%**	**Approx.** **38%** (reduction in phosphorylating respiration)	**Approx.** **60%** (decrease in UPS activity)	**Approx.** **50% of neuronal loss**	**Motor impairment**

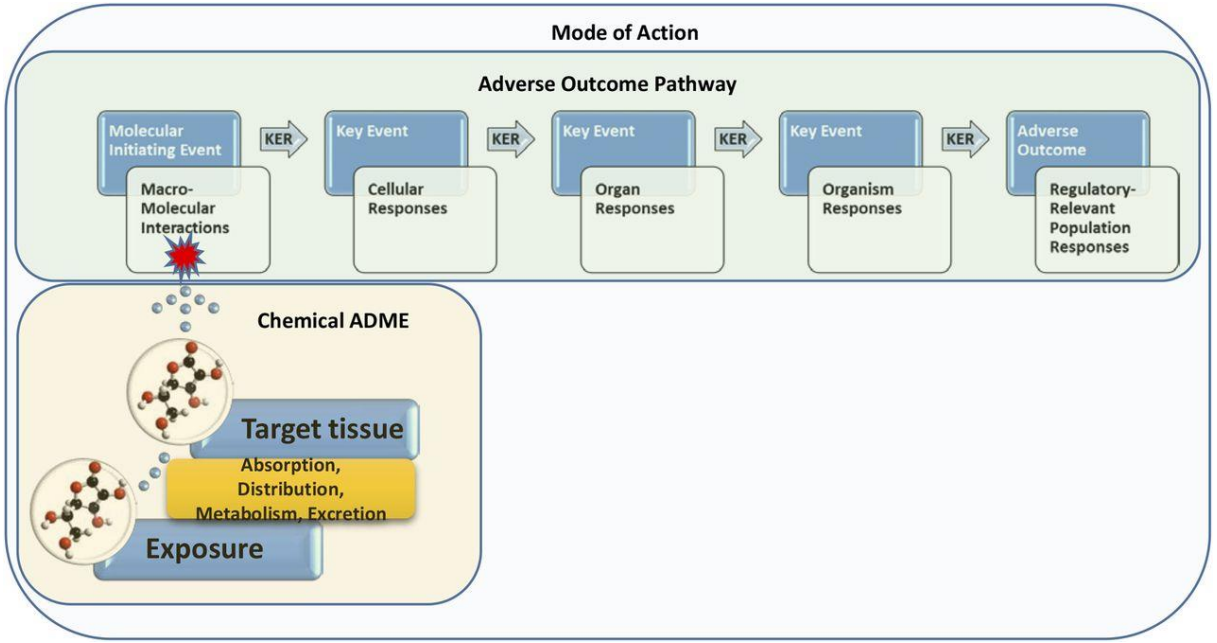
submitted to the AOP-Wiki peer-review by the OECD

INVESTIGATION OF BIOLOGICAL PLAUSIBILITY

- **The WoE supports a qualitative AOP.** Data gaps to move to a quantitative AOP

➔Biologically plausible that a pesticide (or chemical) affecting this AOP can be associated with the AO and ultimately with PD e.g.: known CI inhibitor

! Specific **ADME data** on this given compound needed

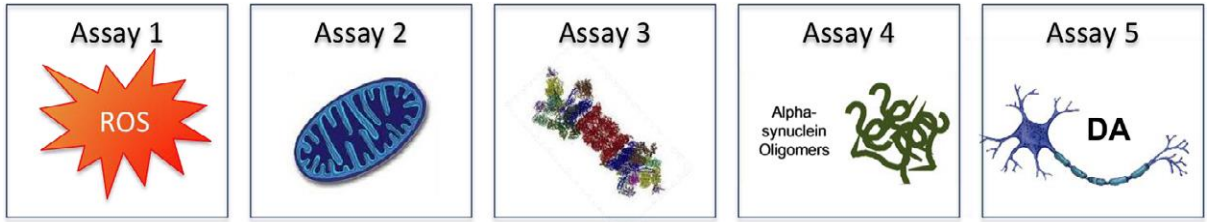


Stephen W. Edwards, 2016

INVESTIGATION OF BIOLOGICAL PLAUSIBILITY

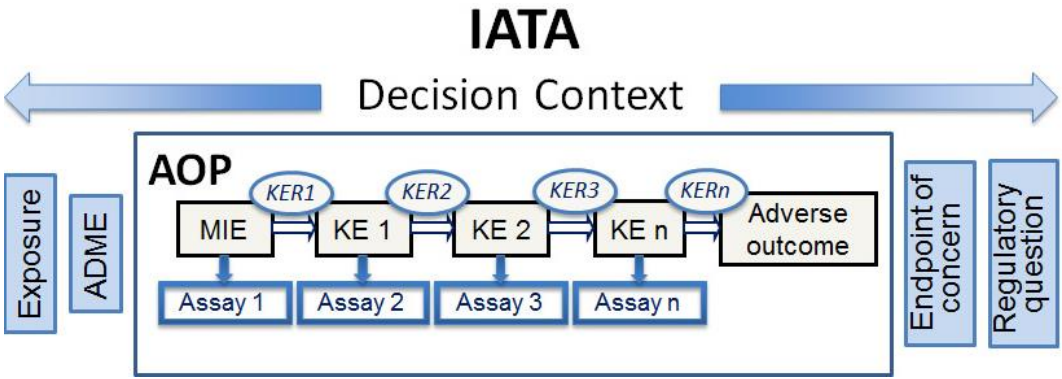
i.e.: known CI inhibitors

- Testing strategy should consider all KEs followed by selection of the most predictive assays.



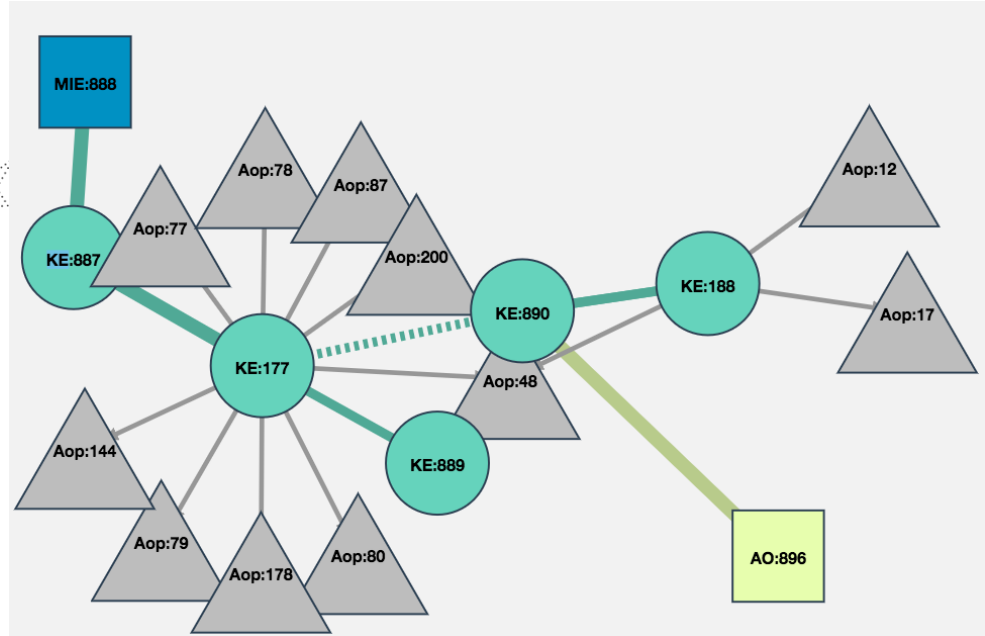
- Optimization of the design of *in vivo* test: dosing schedule proper stereology protocols and detailed neuropathology assessment with inclusion of special stain procedure

- AOP-informed IATA



INVESTIGATION OF BIOLOGICAL PLAUSIBILITY

- This AOP: only a part of the supposed interaction pesticides/ PD risk
- 1 other AOP developed: Redox-cycling of a chemical initiated by electrons released by the mitochondrial respiratory chain leading to parkinsonian motor deficits
- Other AOPs to be developed to allow linking of many different pesticides (or chemicals) to various symptoms of PD.
- May share common key events ➔ **AOP network**



https://aopwiki.org/aops/3

CONCLUSION

- **AOP concept = promising tool to address biological plausibility of association pesticides/PD in epidemiological studies.**
- **Development of AOPs relevant for PD ➡ identification of individual pesticides possibly linked to this adverse outcome.**
- **AOP networks ➡ identification of mechanistically driven cumulative assessment group for PD.**

Thank you for your attention