



Scientific Opinion on the Investigation into Experimental Toxicological Properties of PPPs Having a Potential Link to Parkinson's Disease and Childhood Leukaemia

Susanne Hougaard Bennekou

Senior Advisor, the Danish EPA,
The EFSA PPR Panel

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Ntzani EE, Chondrogiorgi M,
Ntritsos G, Evangelou E, Tzoulaki I

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EXTERNAL SCIENTIFIC REPORT

Literature review on epidemiological studies linking exposure to pesticides and health effects¹

Evangelia E Ntzani, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I

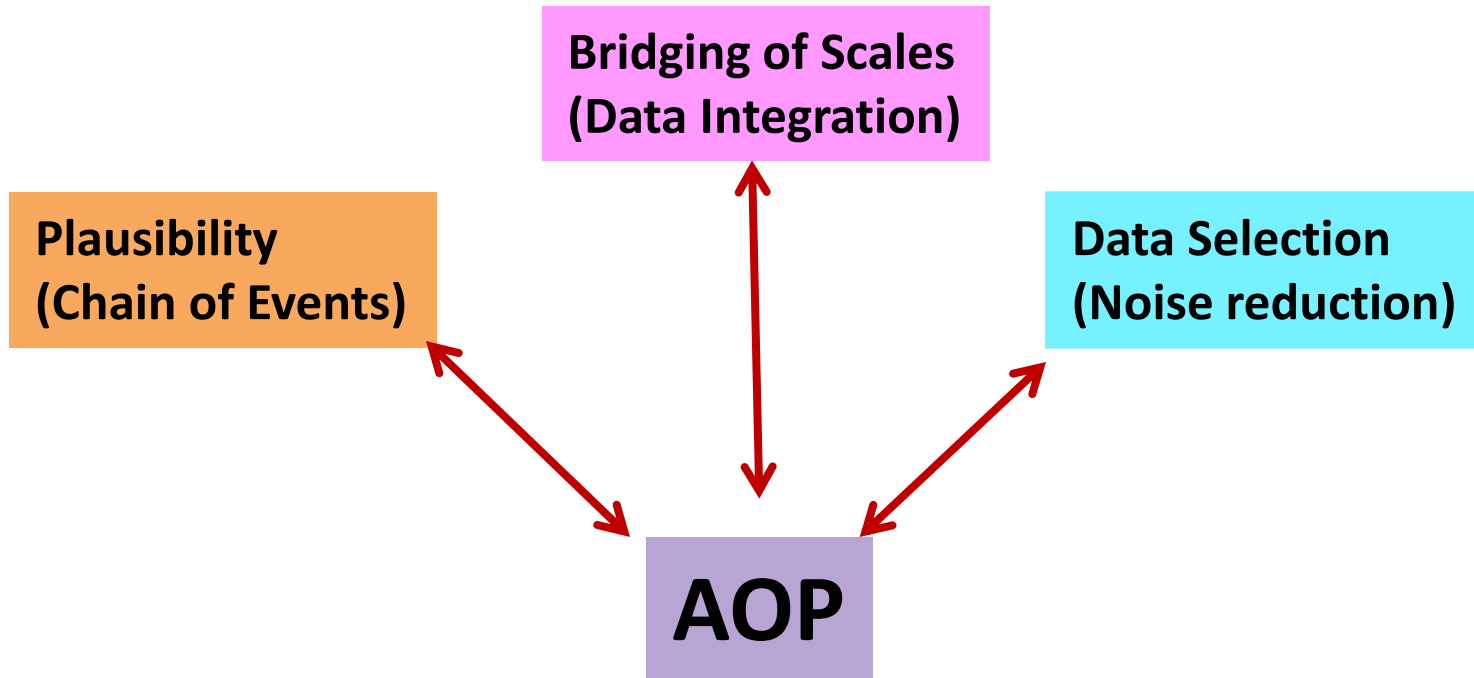
Department of Hygiene and Epidemiology, University of Ioannina Medical School, Ioannina, Greece



OPINION of ANSES
on the INSERM collective expert appraisal report
“Pesticides. Health effects”

An association between exposure to pesticides and the risk of developing Parkinson’s disease (PD) and childhood leukemia

Epidemiological data have intrinsic weaknesses – does not
allow conclusions but still concern
What is the biological plausibility?



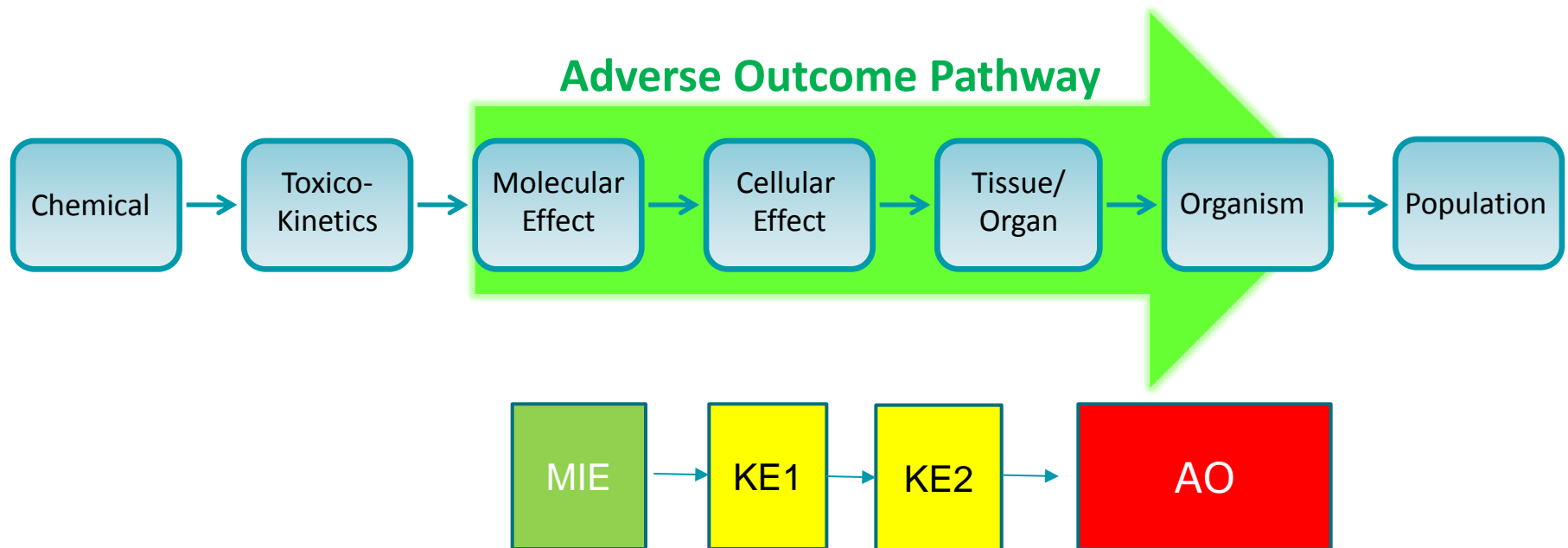
How can we use AOP

to select the information that may link pesticides to PD/CHL

to structure information relevant for a potential link of pesticides to PD/CHL

to select/perform studies addressing a potential link of pesticides to PD/CHL

Structuring of a chain of events



MIE: Molecular initiating event

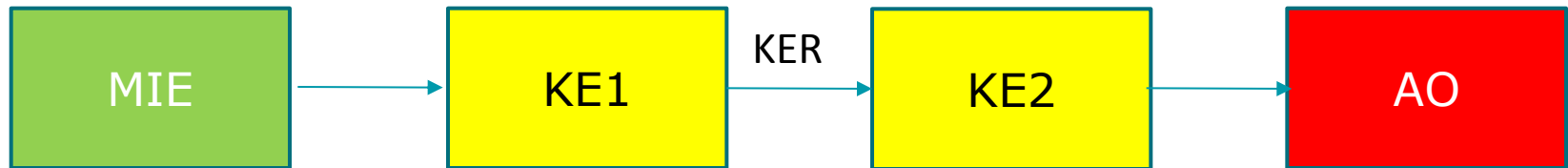
KE: Key event

AO: Adverse outcome

An AOP cannot account for ADME/exposure
- these are specific properties of substances

An AOP is characterizing hazard, not risk

Construction principles of AOP



MIE: compound agnostic (biology-focussed), preceded by ADME events (local dose relevant for MIE)

KE: must be an **essential** process (necessary, but not sufficient)
has an activation threshold (**measurable**, generally observable)
may be shared between AOP

AO: is not a complex disease (like PD), but a distinct apical endpoint

KER: The KE relationship links two blocks. It is AOP specific

Parkinsons Disease - Characteristics

Symptoms – a neurodegenerative progressive disease

- Tremor – shaking of limbs, often hands
- Slowed movement
- Rigid muscles
- Impaired posture and balance
- Loss of automatic movement
- Speech and writing changes
- Non-motor symptoms: Loss of sense of smell, sleep and mood disorder, hypotension, constipation, cognitive problems etc

Risk - aging (2-4% risk for person over age 60)

Pathology

- Lewis bodies in the substantia nigra,
- loss of dopaminergic neurons, of dopamine in striatum
- Effects in other areas of CNS (motor nucleus of vagus nerve, hypothalamus etc) and in the autonomic NS

Genes

- Specific mutations in certain genes (parkin, PINK1) - uncommon
- Certain gene variations can increase the risk of PD – each contributing a little

Environment

- Exposure to certain toxins or environmental factors – paraquat, rotenone, agent orange, MPTP

Regulatory Studies

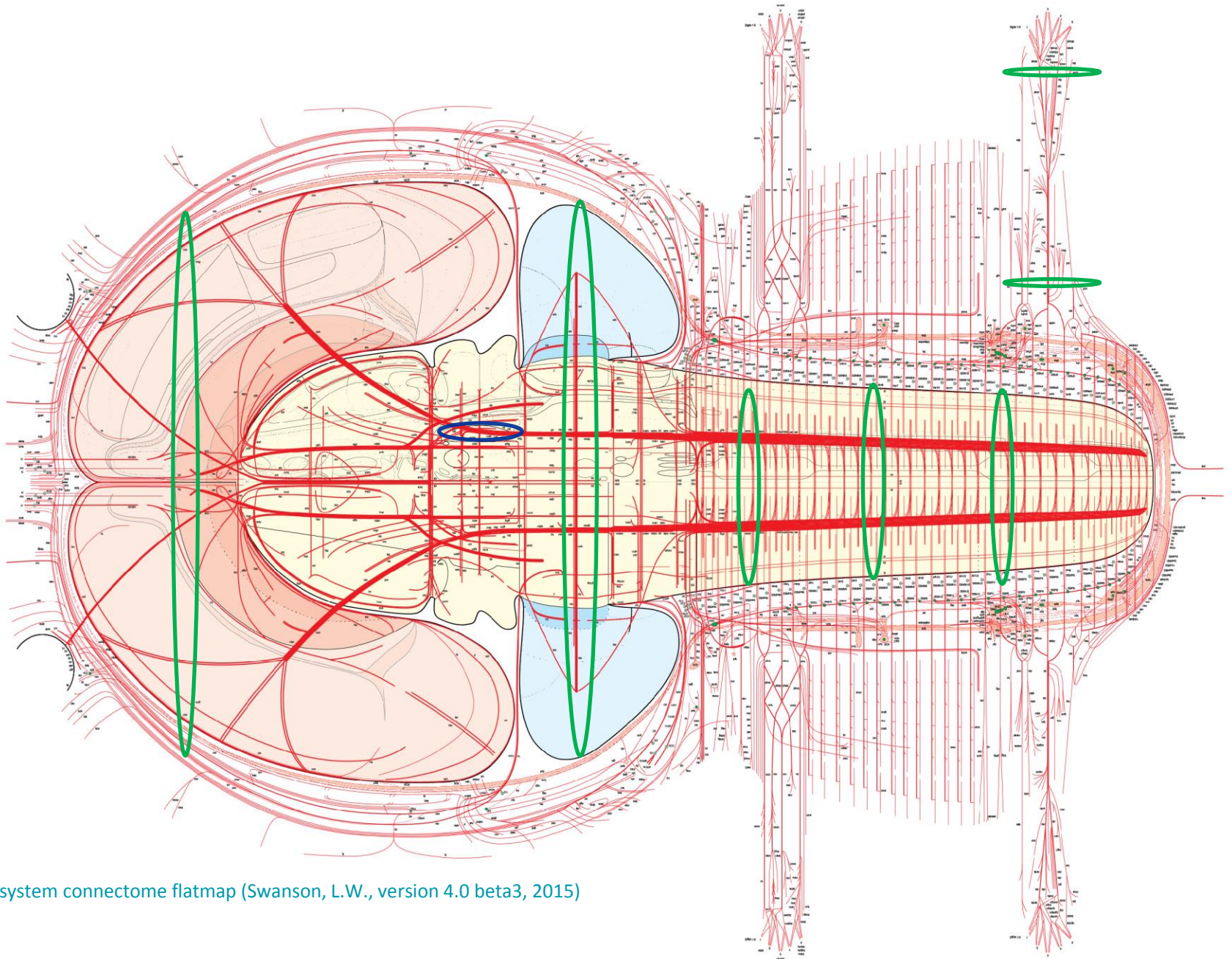
Regulation 1107/2009 on placing PPP's on the market

- Neurotoxicity should be evaluated to identified and characterized hazard of active substances and to perform RA in order to approve or not an active substance
- Also important since one of the criteria to categorized active substances:
 - ➔ Criteria as candidate for substitution (article 24)
 - ➔ Criteria for basic substances (article 23)

Regulation 283/2013 setting out the data requirements for a.s.

- Potential neurotoxic effects shall be carefully addressed and reported in "general" toxicity studies (Short-term, long term, generational studies...)
- Neurotoxicity studies

	Test procedure	Detailed clinical observations	Functional tests	Pathology
Repeated dose 28-day oral toxicity study in rodents OECD 407 (2008)	Animal: Rat young adults 10 (5M&5F)/group 3 doses tested + 1 control group Exposure: 28 days	In the home cage and open field (see OECD 424) Frequency: -prior to first exposure - weekly	Sensory reactivity Limb grip strength Motor activity Frequency: once	Brain weight Histopathology of representative sections of: Brain (cerebrum, cerebellum and medulla/pons), Spinal cord Peripheral nerve
Repeated dose 90-day oral toxicity study in rodents OECD 408 (1998)	Animal: Rat young adults 20 (10M&10F)/group 3 doses tested + 1 control group Exposure: 90 days	In the home cage and open field (see OECD424) Frequency: -prior to first exposure - Weekly	no	Brain weight Histopathology of representative sections of: Brain (cerebrum, cerebellum and medulla/pons), Spinal cord (at three levels: cervical, mid-thoracic and lumbar), Peripheral nerve (sciatic or tibial)
Repeated dose 90-day oral toxicity study in non-rodents OECD 408 (1998)	Animal: generally Dog 8 (4M&4F)/group 3 doses tested + 1 control group Exposure: 90 days	In the home cage and open field (see OECD424) Frequency: -prior to first exposure - weekly	Sensory reactivity Limb grip strength Motor activity Frequency: once may be omitted when data on functional observations available from other studies and daily observations not revealing functional deficits.	Brain weight Histopathology of representative sections of: Brain (cerebrum, cerebellum and medulla/pons), Spinal cord (at three levels: cervical, mid-thoracic and lumbar), Peripheral nerve (sciatic or tibial).
Chronic Toxicity Studies OECD 452 (2009)	Animal: Rodent young adults 40 (20M&20F)/group Non rodent young adults 8 (4M&4F)/group 3 doses tested + 1 control group Exposure: 52 weeks	In the home cage and open field (see OECD424) Frequency: -prior to first exposure - end of the first week - then monthly	Optionally for chemicals where previous repeated dose 28-day and/or 90-day toxicity tests indicated the potential to cause neurotoxic effects.	Brain weight Histopathology of representative sections of: Brain (cerebrum, cerebellum and medulla/pons), Spinal cord (at three levels: cervical, mid-thoracic and lumbar), Peripheral nerve (sciatic or tibial)



Rat nervous system connectome flatmap (Swanson, L.W., version 4.0 beta3, 2015)

Regulatory Studies

Neurotoxicity end points in acute and repeat dose studies:
All potentially adverse effects should be investigated to establish NOAEL including Neurotoxicity.

Triggers (strengthened in 1107/2009)

Specific neurotoxicity in rodent shall be performed

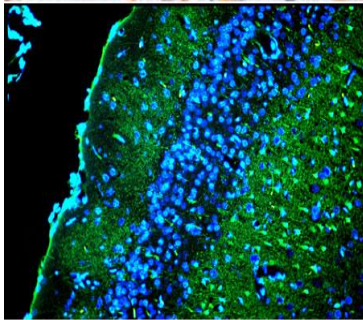
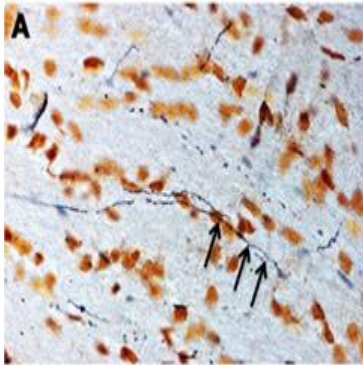
- if there is indication of neurotoxicity
- active substance is structurally related to known neurotoxic compound
- has known neurotoxic pesticidal MoA

Delayed neurotoxicity shall be carried out if the active substance is similar to compounds that cause delayed polyneuropathys like organophosphates

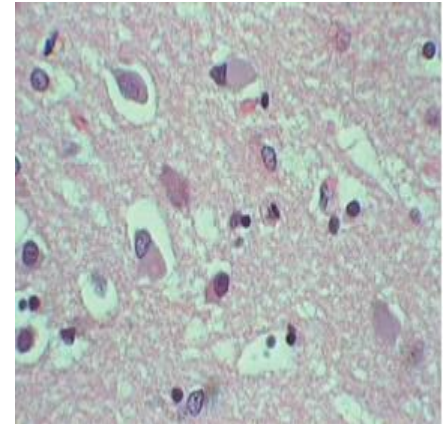
Developmental Neurotoxicity test if there is an indication of such effect in previous studies

Conclusion: specific neurotoxicity studies are not routinely required

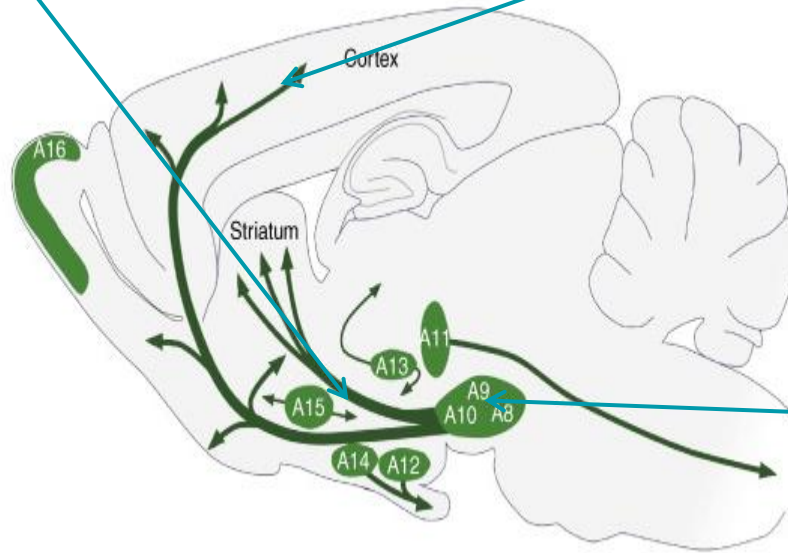
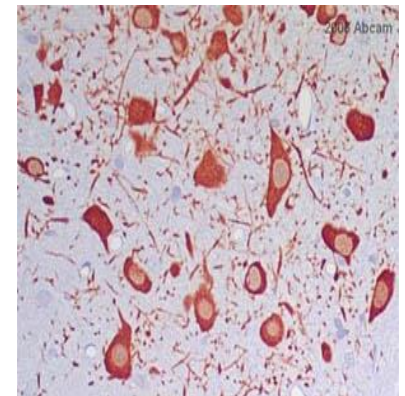
Degenerating axons



Lewy Bodies



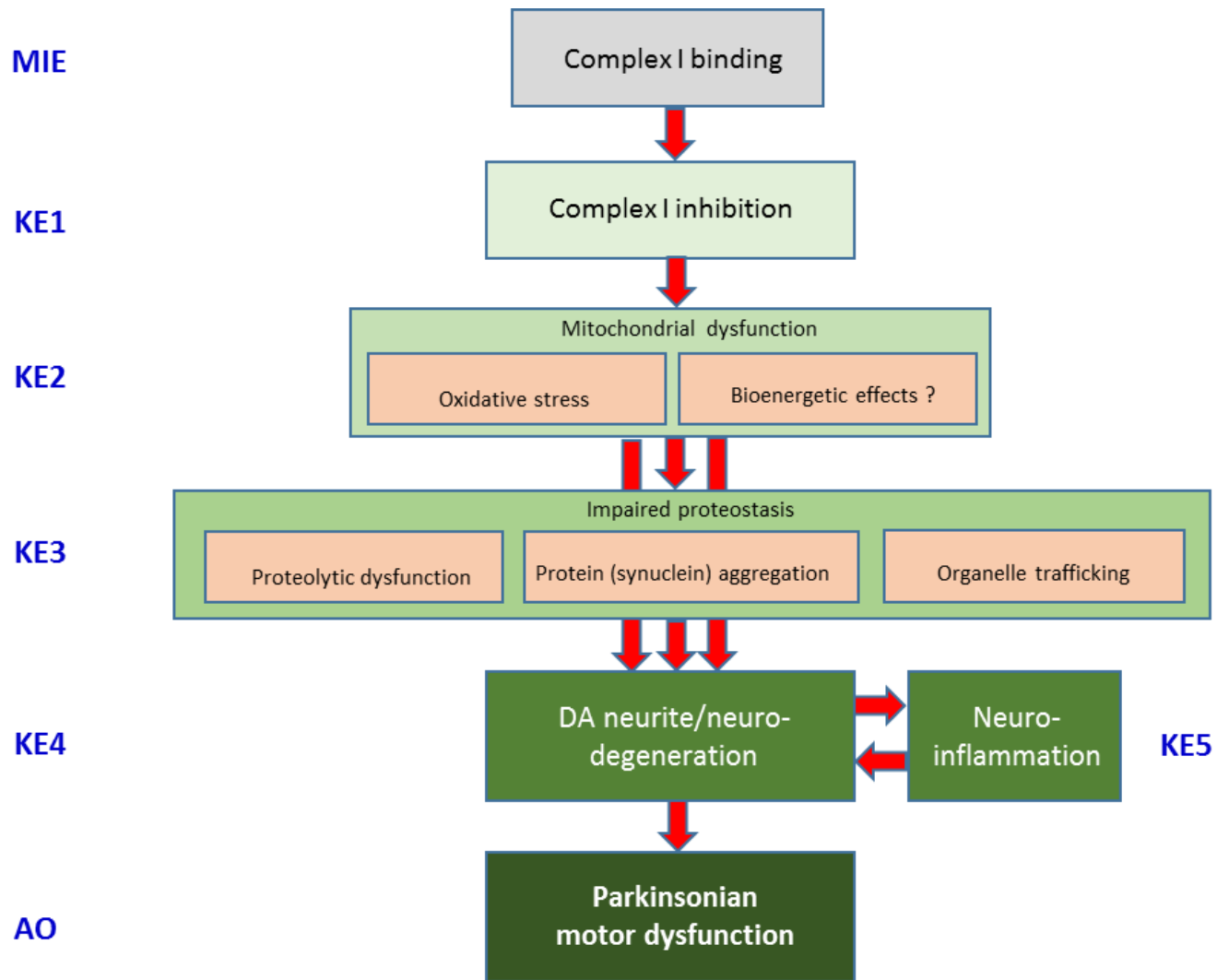
Dopaminergic neurons

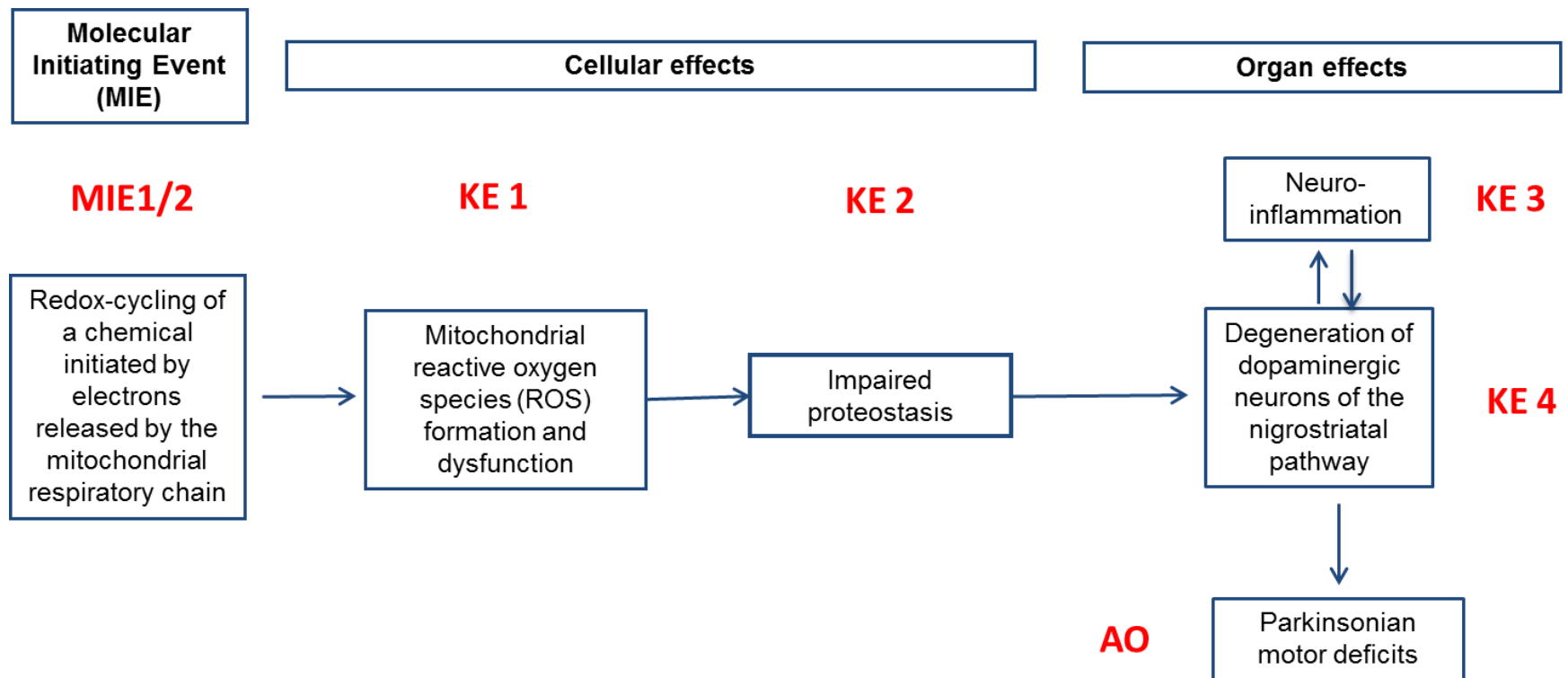


Björklund & Dunnet Trends in neuroscience 2007

Endpoints relevant for identification of PD

- Substantia nigra is in the rostral part of the midbrain – is not investigated in standard studies but is in neurotoxicity 424 and 426 studies
- Lewis bodies are detected by immunostaining – is not carried out in routine studies
- Only indicator of PD in routine studies is motor activity in short term repeat studies
- Only in case of suspected neurotoxicity specialised tests are carried out
- **CONCLUSION: The hazard will not be picked up in routine studies**





Childhood leukemia

- Most common cancer in children and teens -1 out of 3 – still a rare disease
- 3 out of 4 are acute lymphoblastic leukemia (ALL) – rest mostly acute myeloblastic leukemias
- Epidemiological studies: the association is stronger than for Parkinsons disease
- No distinction on which pediatric leukemia

ALL: neoplasm of immature lymphoid progenitors – mostly the B-cell lineage >70% (T-cell lineage 30%)

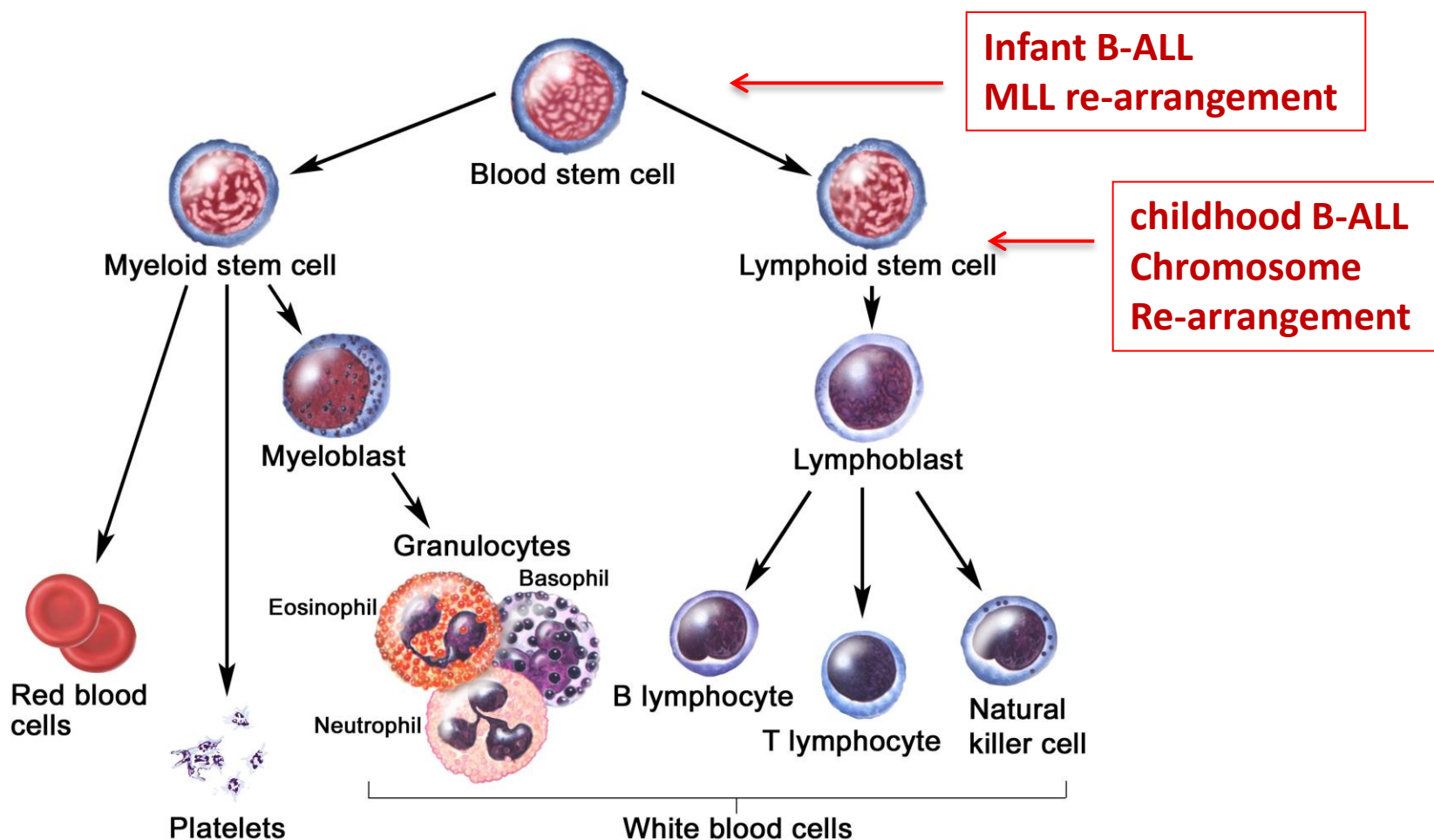
Infant B-ALL

- 10% of the ALL
- Debut before age of 1 year
- 5 year survival <11%
- “one-hit” model - silent mutational landscape
- Developmental disease ?

Childhood B-ALL

- Debut 2-4 years
- cure rate ~90%
- “two-hit” model – typical mutational landscape

CHILDHOOD LEUKEMIA



Regulation

Regulation 1107/2009 on placing PPP's on the market

Carcinogenicity, genotoxicity and hematological endpoints as other toxicity endpoints should be evaluated to identify and characterize hazard of active substances and to perform RA in order to decide on approval of an active substance.

Regulation 283/2013 setting out the data requirements for a.s.

- ➔ Genotoxicity and carcinogenicity studies always required
- ➔ Hematological endpoints addressed and reported in "general" toxicity studies (Short-term, long term, generational studies)

Endpoints relevant for identification of Pediatric leukemias

Regulatory requirements vs Leukaemia:

Genotoxicity is consistently addressed in regulatory dossier

Other putative MoA, no data available in “routine” data package

Carcinogenicity studies: limit of rodent animals

Different classification schemes used in rodent

Distinction between lymphoma and leukemia often difficult particularly in mouse

Rat : - relatively resistant to chemical induced leukaemia
 - Fischer 344: high incidence of Leukemia (LGLL) usefulness?

Mouse: - high incidence of Lymphoid neoplasms
 - malignant and non-malignant myeloproliferation difficult to determine

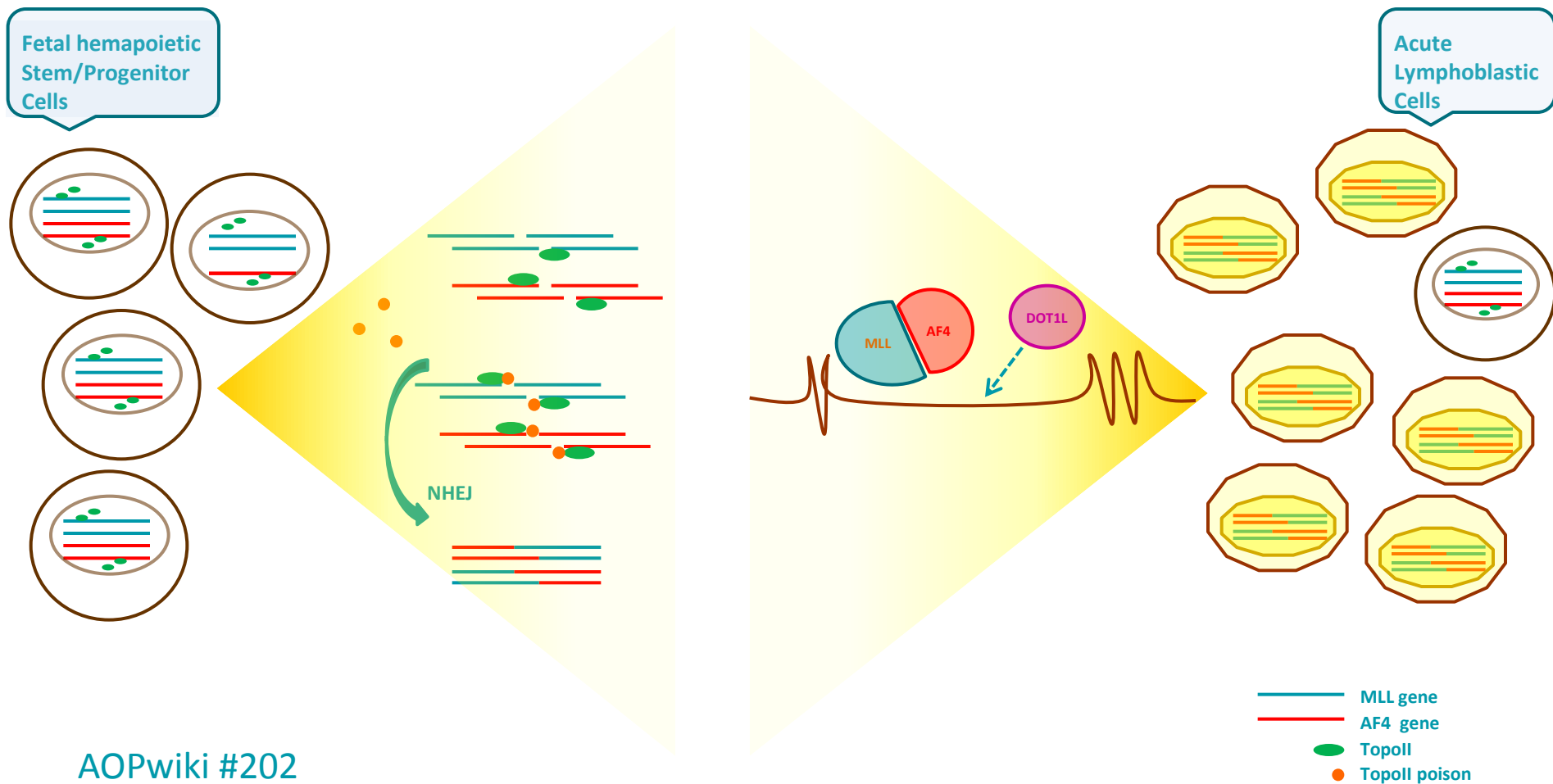
Regulatory requirements vs Childhood Leukaemia:

Is genotoxicity tested in the relevant cells?

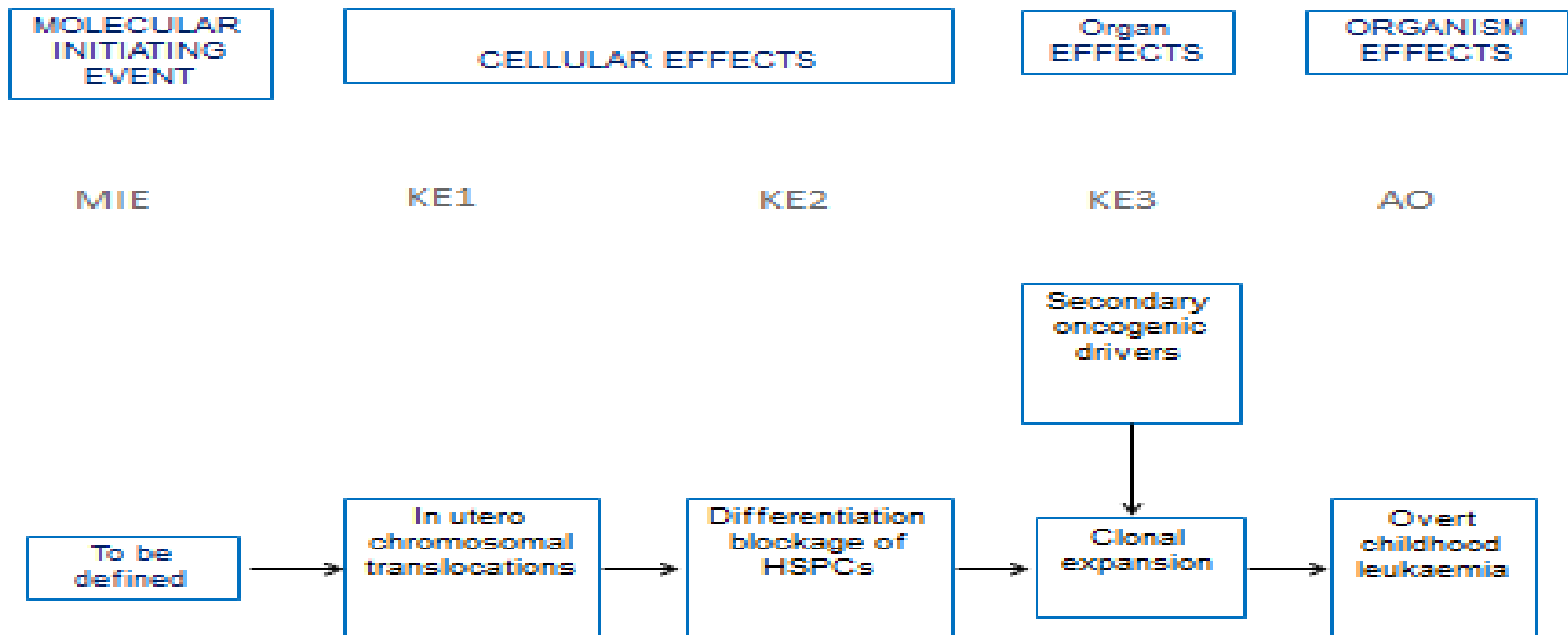
Specific windows of exposure: only 1-generation study) where haematology/histopathology of haematopoietic organs are performed on animals exposed in utero and during juvenile period.

- However, up to now not frequently submitted (recent guideline)
- Low number of animals examined for those parameters (low power)

Adverse Outcome Pathway (AOP): *In utero* DNA Topoisomerase II Inhibition Leading to Infant Leukemia



Putative AOP: Childhood Leukemia



Conclusion Childhood leukemia

Does the current testing paradigm detect the hazard?

- **In vitro genotoxicity: different sensitivity between cells?**

Yes, it is plausible. The etiology of especially infant leukemia suggests it

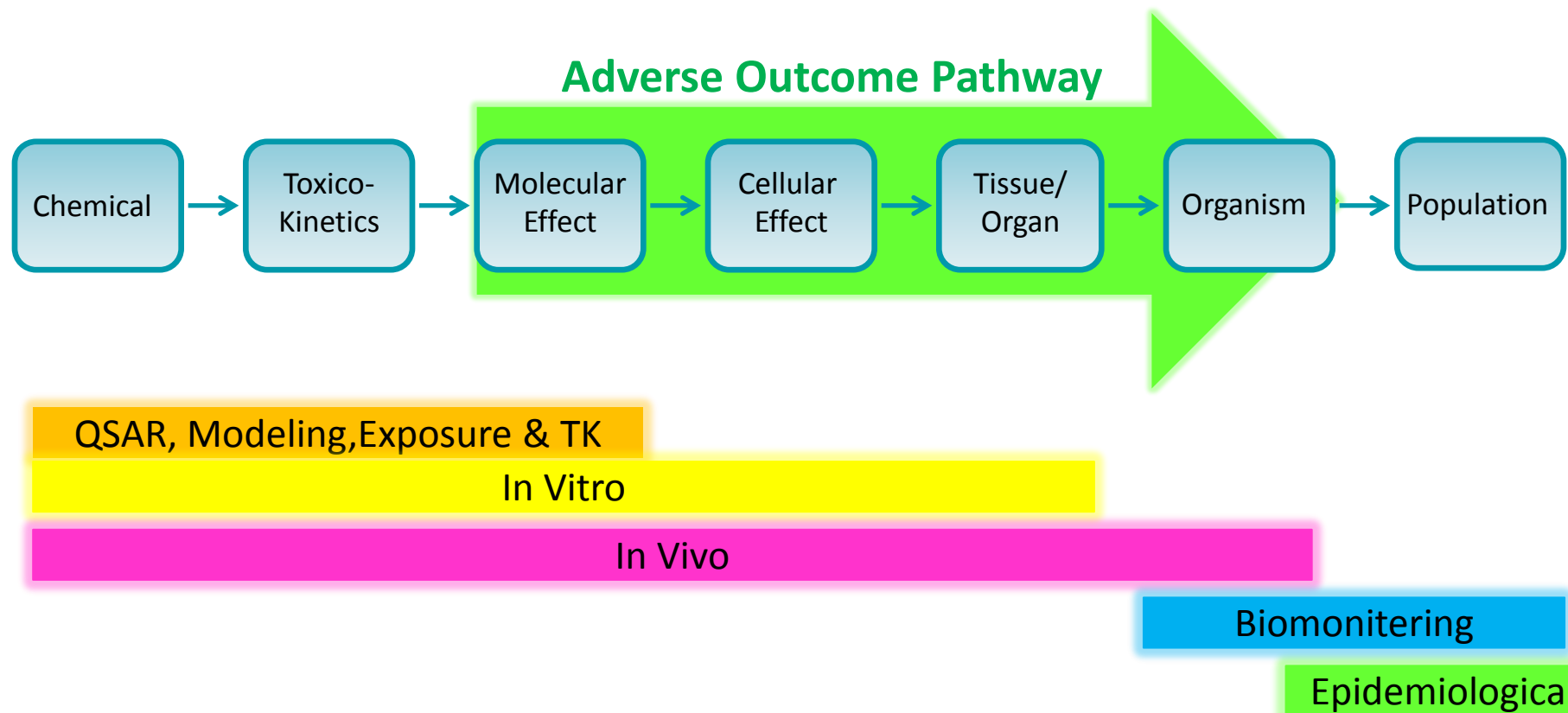
- **In vivo genotoxicity: sensitivity poor**
- **Carcinogenicity study design**
 - does not cover the relevant window of exposure
 - The model does not include a second hit (the model for Childhood leukemia)– this has been captured in experimental models (Martin-Lorenzo november 2015)

No, the hazard is probably not captured

Overall Conclusion

- AOP framework is useful in RA to explore if an AO is biologically plausible or not – AOP contributes to hazard identification and characterisation. BUT chemical specific RA needs the aid of MoA and/or IATA framework
- The prototype AOPs support that pesticides affecting the MIE's and the pathways are risk factors for PD and IFL.
- The systematic review and meta-analysis indicate that pesticides as a risk factor in PD and IFL could be linked to other AOPs
- The AOP on CHL is not bringing definitive evidence of biological plausibility – circumstantial evidences indicate it. Mainly epidemiological data
- The AOP framework is an appropriate tool to understand if chemical hazards relevant to human diseases (PD & CHL's) can be explored and detected in standard reg. studies. Some endpoints of reg. studies can inform on some KE, the mechanistic understanding of the apical endpoints indicate that the studies have limitations (design/sensitivity)

AOPs Final Note





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Overall Assessment – Weight of Evidence

	Biological Plausibility	Empirical Support
MIE=>KE1 (Binding of inhibitor to NADH-ubiquinone oxidoreductase leads to inhibition of complex I)	Strong	Strong
KE1=>KE2 (Inhibition of complex I leads to mitochondrial dysfunction)	Strong	Strong
KE2=>KE3 (Mitochondrial dysfunction results in impaired proteostasis)	Moderate	Strong
KE2=>KE4 (Mitochondrial dysfunction leads to degeneration of DA neurons of the NS pathway)	Strong	Strong
KE3=>KE4 (Impaired proteostasis leads to degeneration of DA neurons in the NS pathway)	Moderate	Strong
KE4⇔KE5 (Neuroinflammation)	Moderate	Moderate
KE4=>AO (Degeneration of DA neurons of the NS pathway leads to parkinsonian motor symptoms)	Strong	Strong

Overall Assessment – Weight of Evidence

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 the NS pathway)	Strong
KE5 (Neuroinflammation)	Moderate