Integration of Endocrine Modalities into an Existing Mechanism of Action-based *in silico* Scheme for use in Environmental Risk Assessment

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LJMU Mechanistic Profiling Scheme

Structural profiling tool enabling assignment of putative mechanism of toxic action to screened substances:

- **Coverage and detail enhanced relative to schemes of Verhaar (1992) and Russom (1997)**
- **Anchored at level of molecular initiating event (MIE)**
- **Considerate of mammalian, arthropod, fish, plant, and microorganism species**

Development of an Enhanced Mechanistically Driven Mode of Action Classification Scheme for Adverse Effects on Environmental Species

Maria Sapounidou, David J. Ebbrell, Mark A. Bonnell, Bruno Campos, James W. Firman, Steve Gutsell, Geoff Hodges, Jayne Roberts, and Mark T. D. Cronin*

Environ. Sci. Technol., 2021, 55, 1897-1907

Construction of an In Silico Structural Profiling Tool Facilitating Mechanistically Grounded Classification of Aquatic Toxicants

James W. Firman,* David J. Ebbrell, Franklin J. Bauer, Maria Sapounidou, Geoff Hodges, Bruno Campos, Jayne Roberts, Steve Gutsell, Paul C. Thomas, Mark Bonnell, and Mark T. D. Cronin

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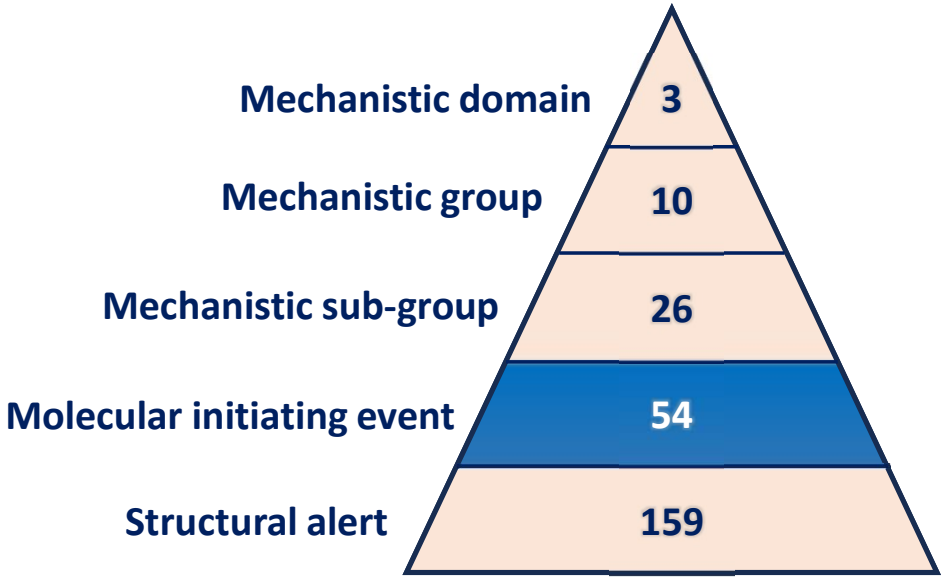


Profiling scheme: Organisation and present scope

Focus has remained upon acute manifestation of toxicity

Mechanistic domain overview:

1. Narcosis	**"Baseline" toxicity** ***Cell membrane accumulation***	**Group** **Sub-group** **MIE** **SA**	***2*** ***4*** ***4*** ***22***
2. Reactive	**Non-specific chemical reactivity** ***Biomolecule modification***	**Group** **Sub-group** **MIE** **SA**	***3*** ***7*** ***15*** ***63***
3. Specific	**Defined bioactivity** ***Receptor/enzyme interaction***	**Group** **Sub-group** **MIE** **SA**	***5*** ***15*** ***35*** ***74***

Representative pathways:

- **1.2. Enhanced narcosis**
  - **1.2.1. Polar narcosis**
    - **1.2.1.1. Membrane phospholipid accumulation**
      - **2.2.1.1.11. Quaternary ammonium**
- **2.2. Electrophilic reactivity**
  - **2.1.2. Hard electrophilicity**
    - **2.1.2.1. Acylation**
      - **2.1.2.1.1. Carboxylic anhydride**
- **3.2. Bioenergetic dysfunction**
  - **3.2.1. Oxidative phosphorylation**
    - **3.2.1.2. Inhibition of mETC complex III**
      - **3.2.1.2.2. Bixafen-like**

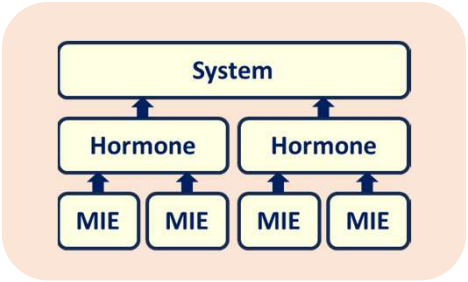
However, chronic endpoints – such as those associated with endocrine activity – are also of great significance

Expansion of scheme coverage: Endocrine activity

Gather key information upon endocrine pathophysiology

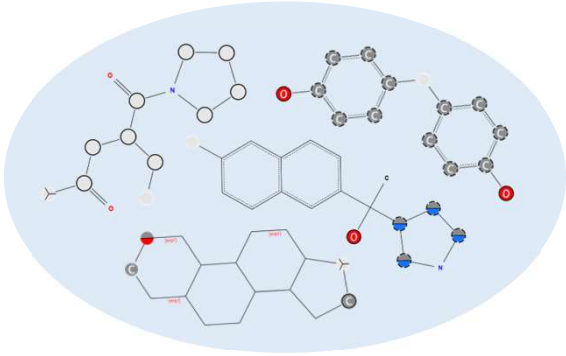
- Essential systems and pathways
- Established/credible AO causality
- Range of appropriate taxa

Organise knowledge within formalised framework



- Series of event-aligned tiers
- Standardisation of terminology
- Relates MIE to downstream effect

Devise structural alerts which characterise MIEs



- Sourcing of MIE-active substances
- Definition of key chemical features
- Formulation of alert rules

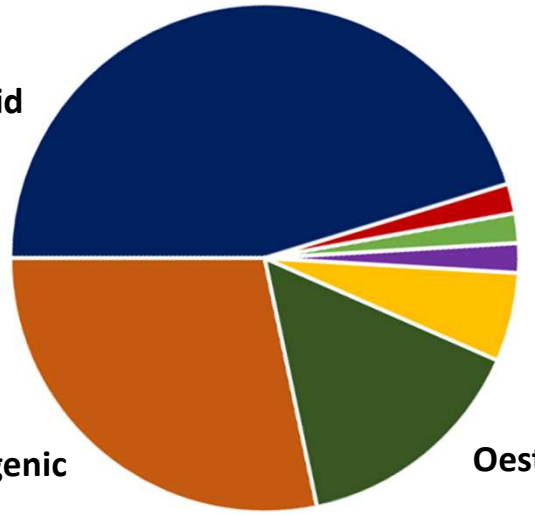
Sourcing of knowledge relating to endocrine activity

AOP Wiki

www.aopwiki.org

Broader literature further consulted

54 appropriate AOPs



Neither list exhaustive

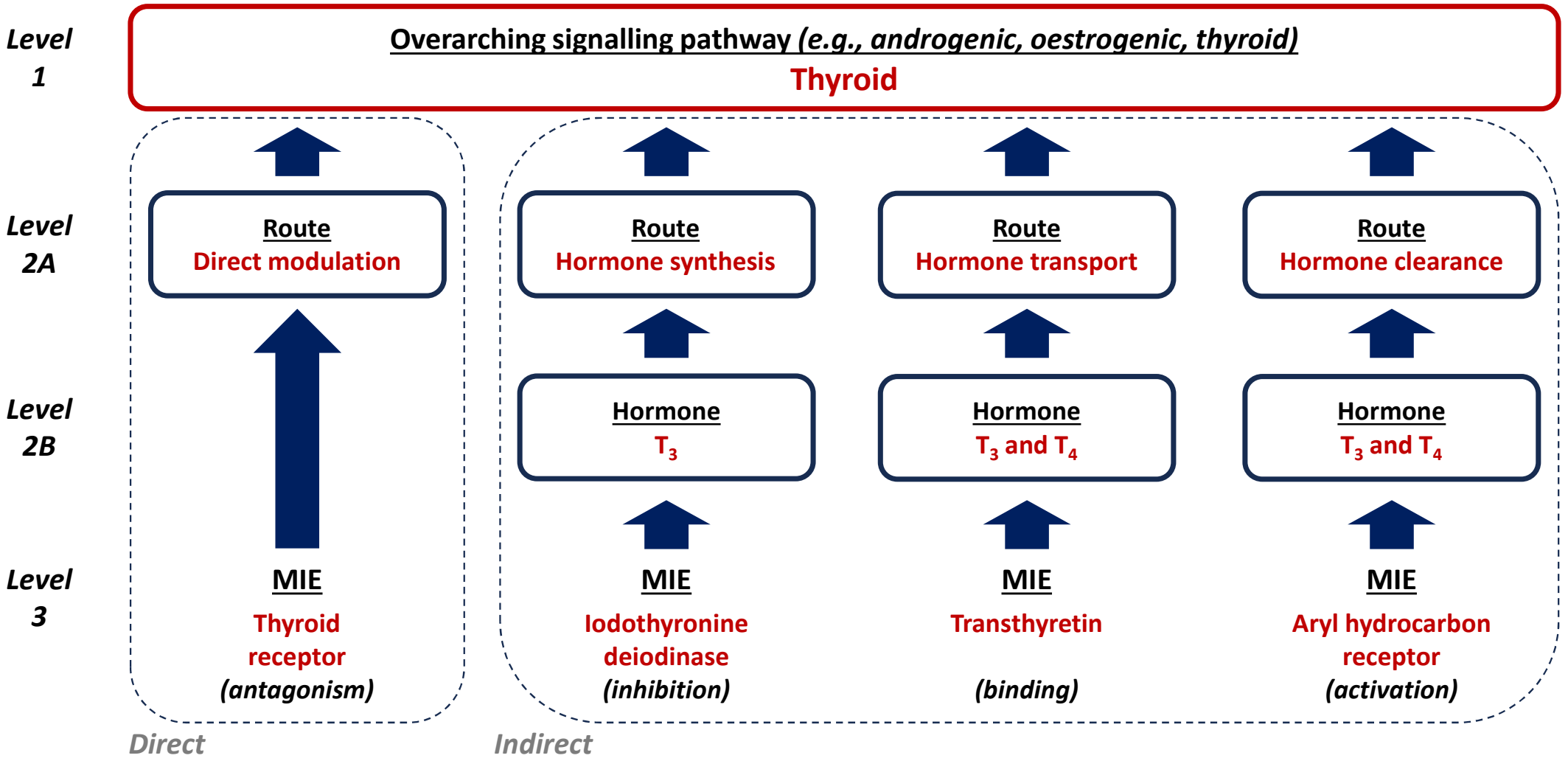
Endpoints encountered

Reproductive impairment
Neurodevelopmental abnormality
Carcinogenicity

Impacted species and taxa

Mammalian (inc. Homo sapiens)
Fish
Arthropod

Overview of endocrine activity ontology framework



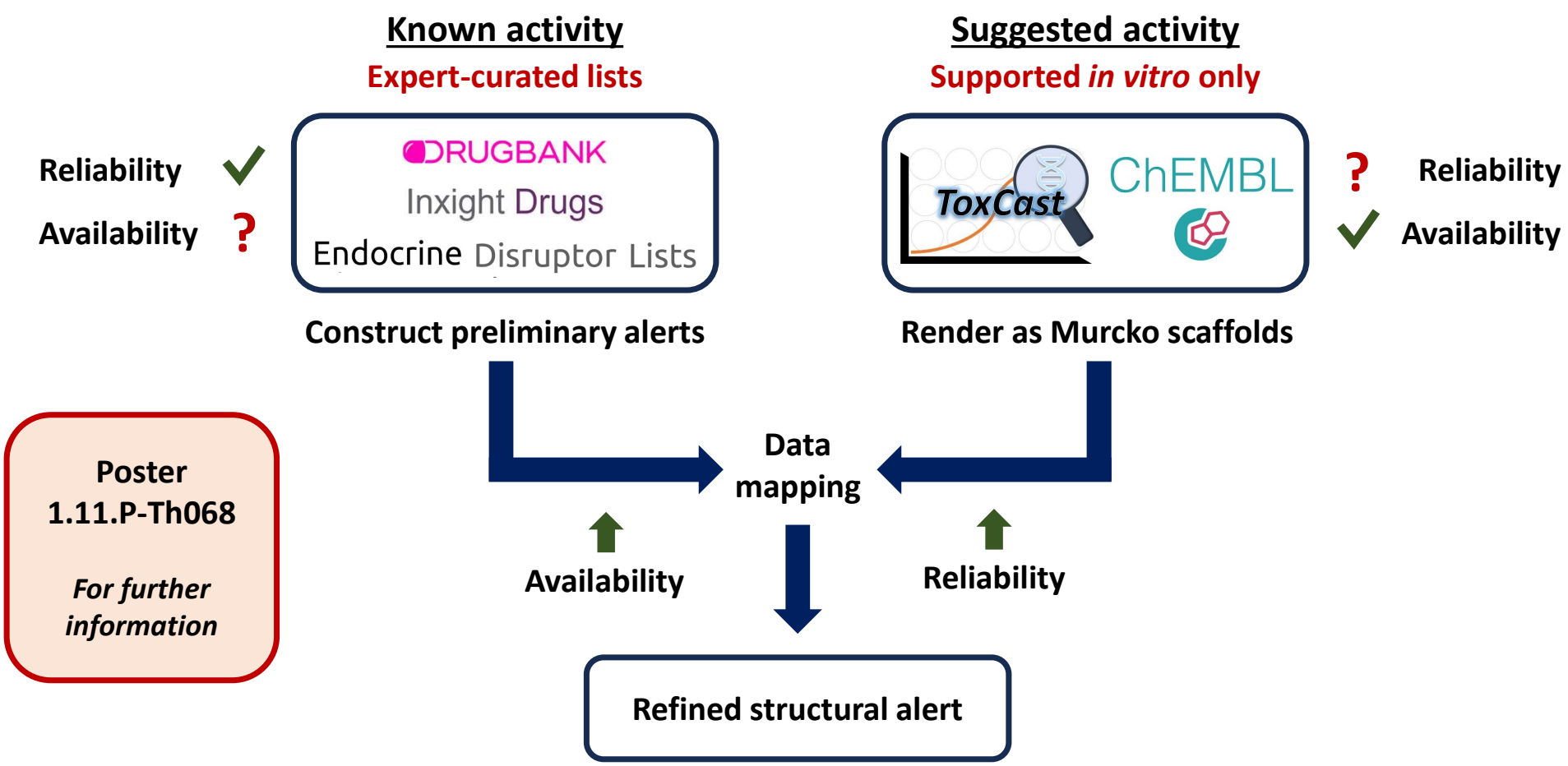
Molecular initiating event targets

28 distinct, endocrine-associated target sites identified:

MIE target site	Route impacted	Signalling pathway
11β-Hydroxysteroid dehydrogenase	Hormone synthesis	Androgenic
Androgen receptor	Direct modulation	Androgenic
Aromatase	Hormone synthesis	Oestrogenic
Aryl hydrocarbon receptor	Hormone clearance	Thyroid
Dual oxidase	Hormone synthesis	Thyroid
Ecdysone receptor	Direct modulation	Ecdysteroid
Glucocorticoid receptor	Direct modulation	Glucocorticoid
Iodothyronine deiodinase	Hormone synthesis	Thyroid
Iodotyrosine deiodinase	Hormone synthesis	Thyroid
Juvenile hormone receptor	Direct modulation	Juvenile hormone
Luteinising hormone receptor	Direct modulation	Luteinisation
Na+/I- symporter	Hormone synthesis	Thyroid
Octopamine receptor	Direct modulation	Octopaminergic
Oestrogen receptor	Direct modulation	Oestrogenic

MIE target site	Route impacted	Signalling pathway
Pendrin	Hormone synthesis	Thyroid
PPAR-α	Hormone synthesis	Androgenic
PPAR-γ	Hormone synthesis	Oestrogenic
Pregnane X receptor	Hormone clearance	Thyroid
Progesterone receptor	Direct modulation	Progestogenic
Steroid 11β-hydroxylase	Hormone synthesis	Androgenic
Steroid 17α-monooxygenase	Hormone synthesis	Androgenic
Steroid 5α-reductase	Hormone synthesis	Androgenic
Succinate dehydrogenase	Hormone synthesis	Thyroid
Thyroid hormone receptor	Direct modulation	Thyroid
Thyroid peroxidase	Hormone synthesis	Thyroid
Thyrotropin hormone receptor	Hormone synthesis	Thyroid
Thyrotropin-releasing hormone receptor	Hormone synthesis	Thyroid
Transthyretin	Hormone transport	Thyroid

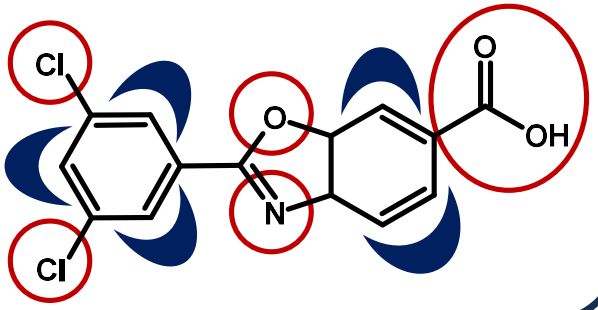
Recovery and classification of MIE activity data



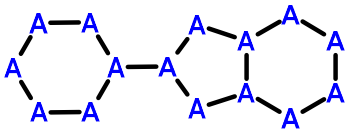
Alert-construction case study: Transthyretin binding

Single known active:

Tafamidis



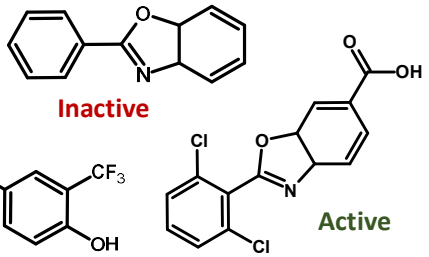
Murcko scaffold



ChEMBL:

424 matches

Examples

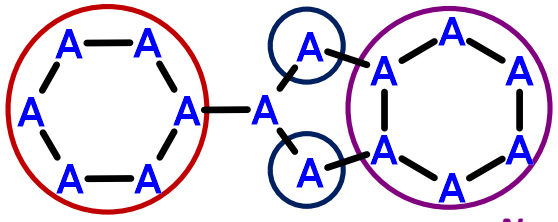


Required? **Substitution?**

Scaffold heteroatoms?

Permissible replacements?

Refined alert

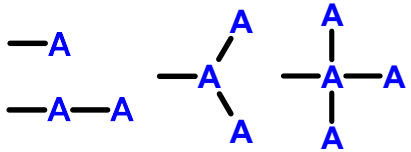


"Small" group, any position

Scaffold atoms of any form

"Small" group, optional

No substituent restrictions



Further enhancement?

Application of molecular modelling…

Ongoing and intended future developments

Expansion and refinement of ontology framework:

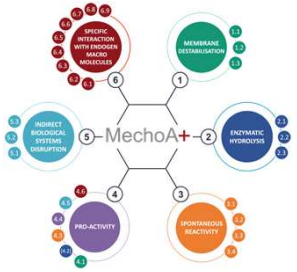
- Sourcing of additional MIE targets (particularly non-mammalian)
- Establishment of interplay between downstream adverse effects
- Enhanced confidence in taxonomical and species applicability

Refinement of structural alerts and rules:

- Identification of further candidates for alert development
- Continued refinement of those existing (assisted through molecular modelling)

Rule integration and implementation:

- As part of broader LJMU scheme (OECD QSAR Toolbox)
- Within KREATiS MechoA system



Poster 1.11.P-Th069

For further information

Thank you for your attention

Further information:

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