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Practical considerations for the inclusion of additional immunotoxicity endpoints in existing regulatory studies

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Outline

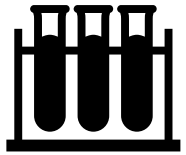
01 Practical considerations for existing immunotoxicity endpoints (EOGRTS)

02 Adding additional endpoints - example

03 Dose level selection

Immunotoxicity endpoints in regulatory studies

Immunotoxicity studies are not mandated for all chemicals but triggered by specific concerns.



However, the following endpoints are included in many repeated dose toxicity studies:

- Hematological changes
- Immune system organ weight changes; thymus, spleen, lymph nodes.
- Histopathological changes in above mentioned organs and/or bone marrow
- *Incidence of infection*

EOGRTS:

- Splenic lymphocyte subpopulation analysis – Cohort 1A; independent of DIT Cohort inclusion
- DIT Cohort (TDAR assay)

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- Splenic lymphocyte subpopulation analysis – Cohort 1A; independent of DIT Cohort inclusion
- **DIT Cohort (TDAR assay)** **June 2025 update**

Developmental Immunotoxicity (DIT)

EOGRTS

10 animals/sex/group

Determination of primary IgM antibody response to a T-cell dependent antigen (TDAR)

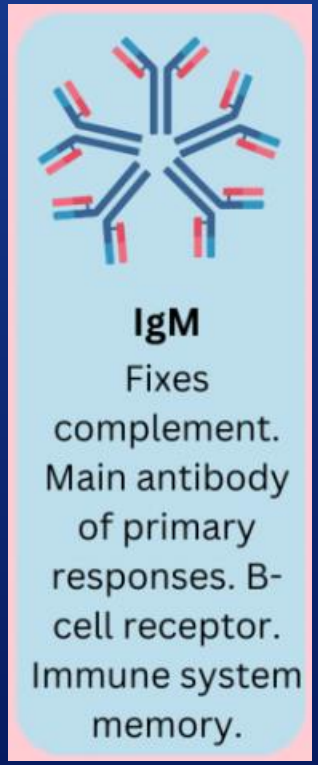
TDAR method

Antigen:

- Sheep red blood cells (SRBC)
- Keyhole limpet Hemocyanin (KLH)

Evaluation of response to immunization:

- Counting specific plaque-forming cells (PFC) in the spleen
- Determine titer of IgM antibody in serum by ELISA



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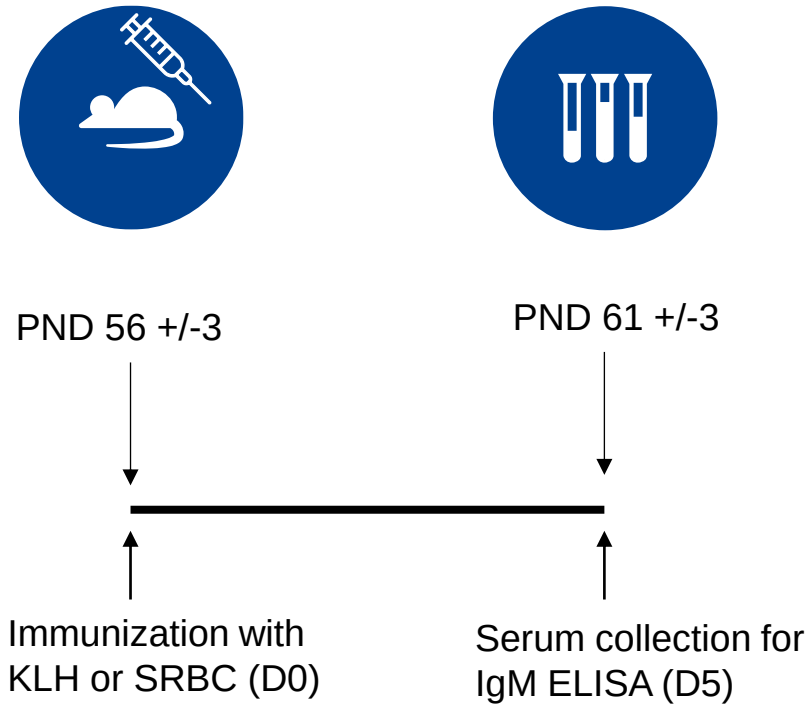
Basic TDAR assay design



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EOGRTS satellite report outcome

- Variety in used protocols (antigen used, route of administration, prime of prime/booster immunization, IgG measurement)
- Variable response to positive control compound (cyclophosphamide)
- Proficiency not always demonstrated
- Statistical analysis lacking
- Findings not discussed in relation to spenic lymphocyte subpopulation assay

Developmental Immunotoxicity (DIT)

OECD443 TG update 2025

10 animals/sex/group

Determination of primary **and secondary** IgM **and IgG** antibody response to a T-cell dependent antigen (TDAR)

TDAR method

Antigen:

- ~~Sheep red blood cells (SRBC)~~
- Keyhole limpet Hemocyanin (KLH)

Evaluation of response to immunization:

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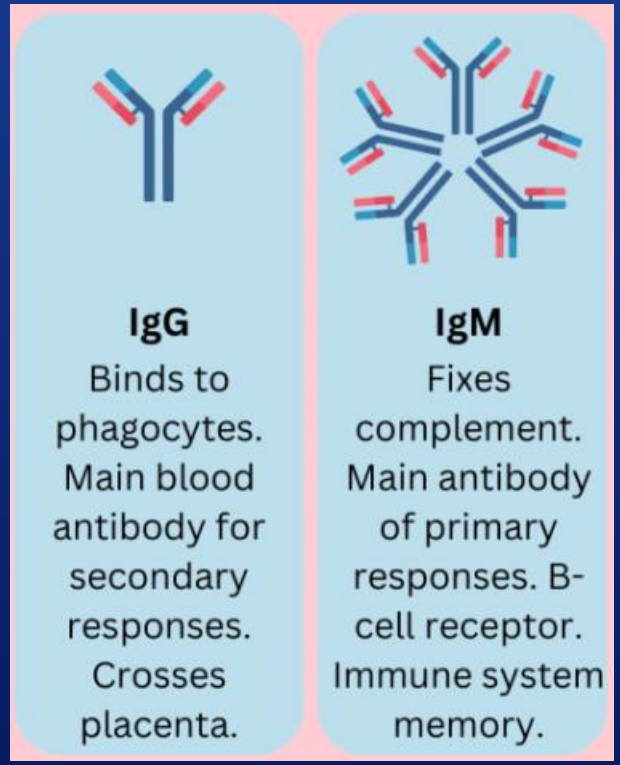

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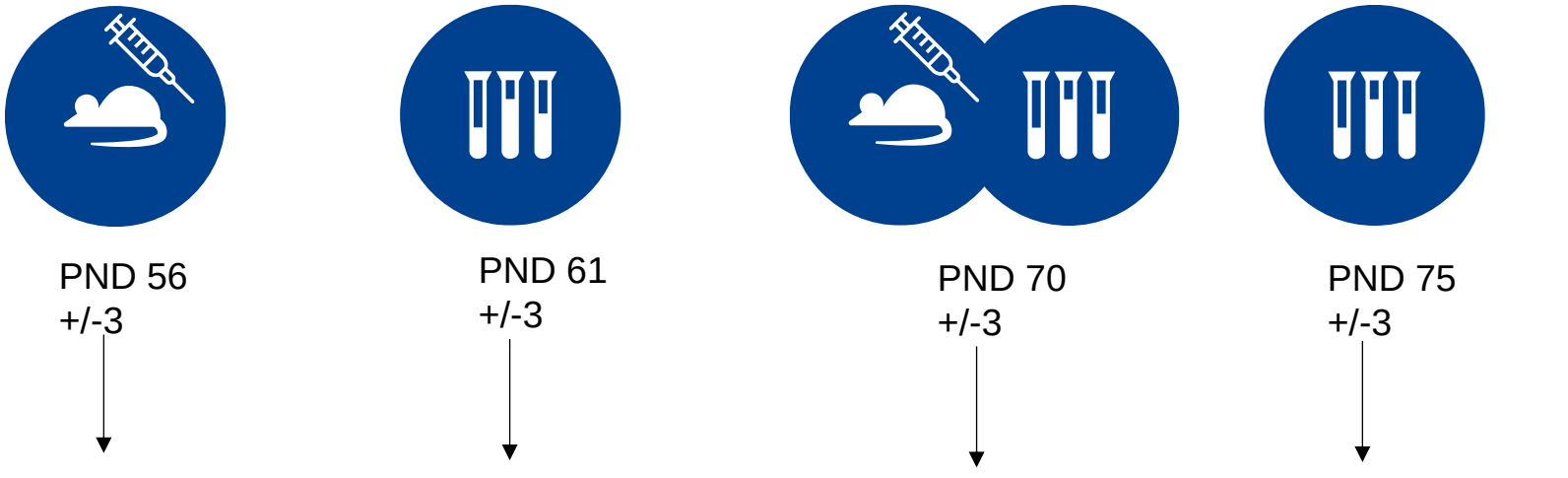
Update June 2025



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Update June 2025

Implications

- Harmonized protocol.
- More exhaustive evaluation of immune response.
- Extension of dosing period and second immunization.
- Increased volume blood collection.
- Validations and proficiency to be updated for all labs.

Considerations for adding immunotoxicity endpoint to existing studies

Scientific guidance on smoke flavouring applications

Example of proposed OECD408 enhancement with immunotoxicity endpoints

- 2021
- It was recognized that all data for Tier 1 can be obtained from the EOGRTS study, however that timelines were too short for renewals.
- Considering this legal timeline, an alternative was suggested > OECD408 including several immunotoxicity endpoints.

Suggested immunotoxicity endpoints

	Standard	Enhancement
At term (necropsy):		
Histopathology (lymphatic organs, including bone marrow cellularity	X	
Weighing lymphoid organs	X	
In blood:		
Immunoglobulin isotypes		X
Complement assays: total serum haemolytic activity or individual components		X
C-reactive protein (CRP)		X
Total and differential white blood cell count	X	
In spleen:		
Phenotypic analysis of spleen cells (CD4 and CD8 T cells, regulatory T cells, B cells, natural (NK) cells, macrophages		X
Natural killer cell functional analysis		X
Phagocytic activity		X
Mitogen stimulation assays for B and T cells		X

Overall considerations

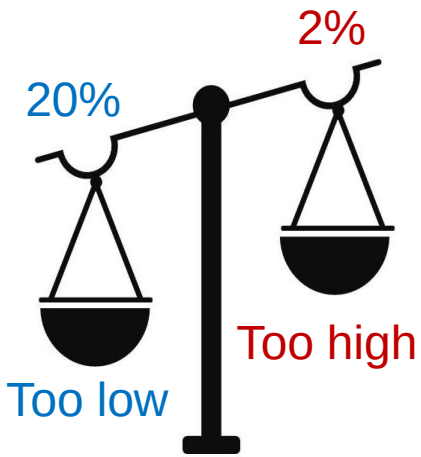
- Logistics and validation
- Proficiency
- Historical control data
- Tiered approach?
- *Dose level section*

Finding ECHA EOGRTS Review Project – dose level

- 11/55 studies (20%) did not use adequate dose levels (according to ECHA).
- 80% of the studies evaluated by ECHA were **appropriately dosed**!

- Meanwhile we know that there is no need to ‘push’ the high dose, however dose level selection should be considered when adding immunotoxicity endpoint to regulatory studies.

- Differentiate between direct and indirect immunotoxicity?



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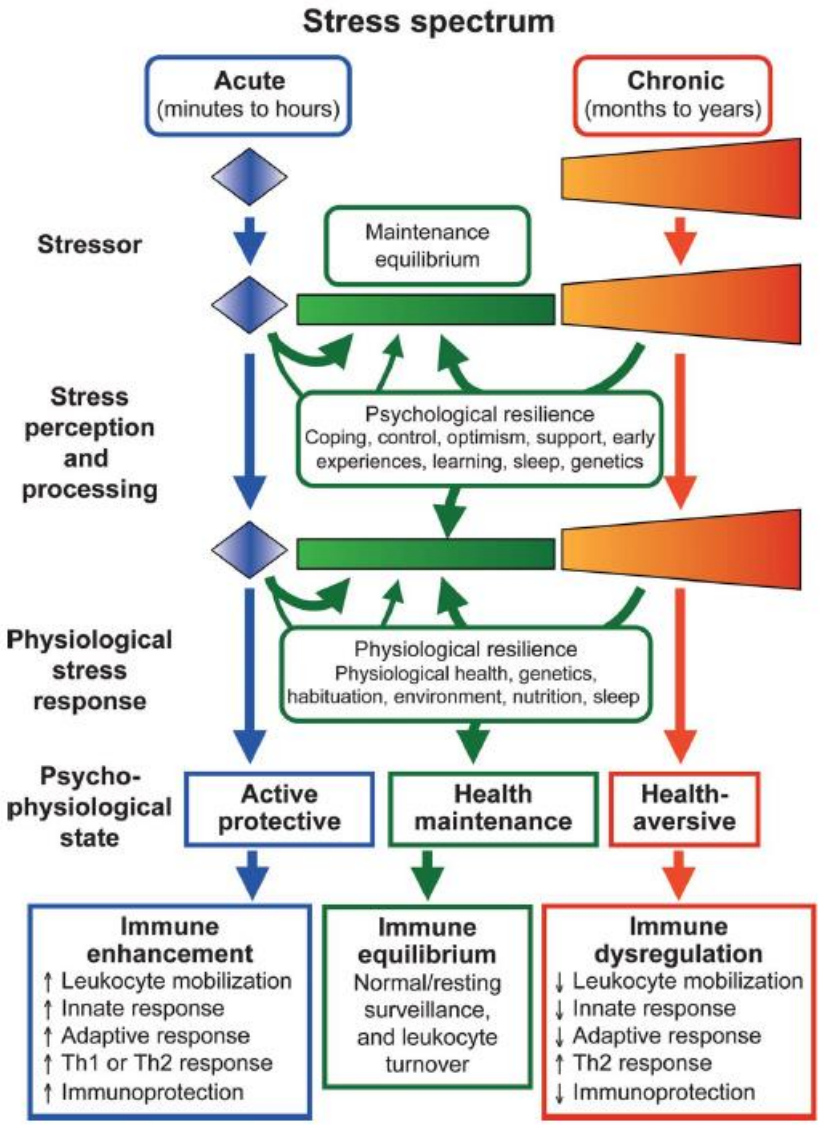

Everds et al. 2013
Interpreting Stress Responses during Routine Toxicity Studies: A Review of the Biology, Impact, and Assessment

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