

Dying for change: A roadmap to refine the fish acute toxicity test after 40 years of applying a lethal endpoint

**RSPCA meeting on Refining severe disease models
and procedures, Karolinska Institute, Stockholm, August 24th-25th 2022**



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The most widely used OECD Test Guideline

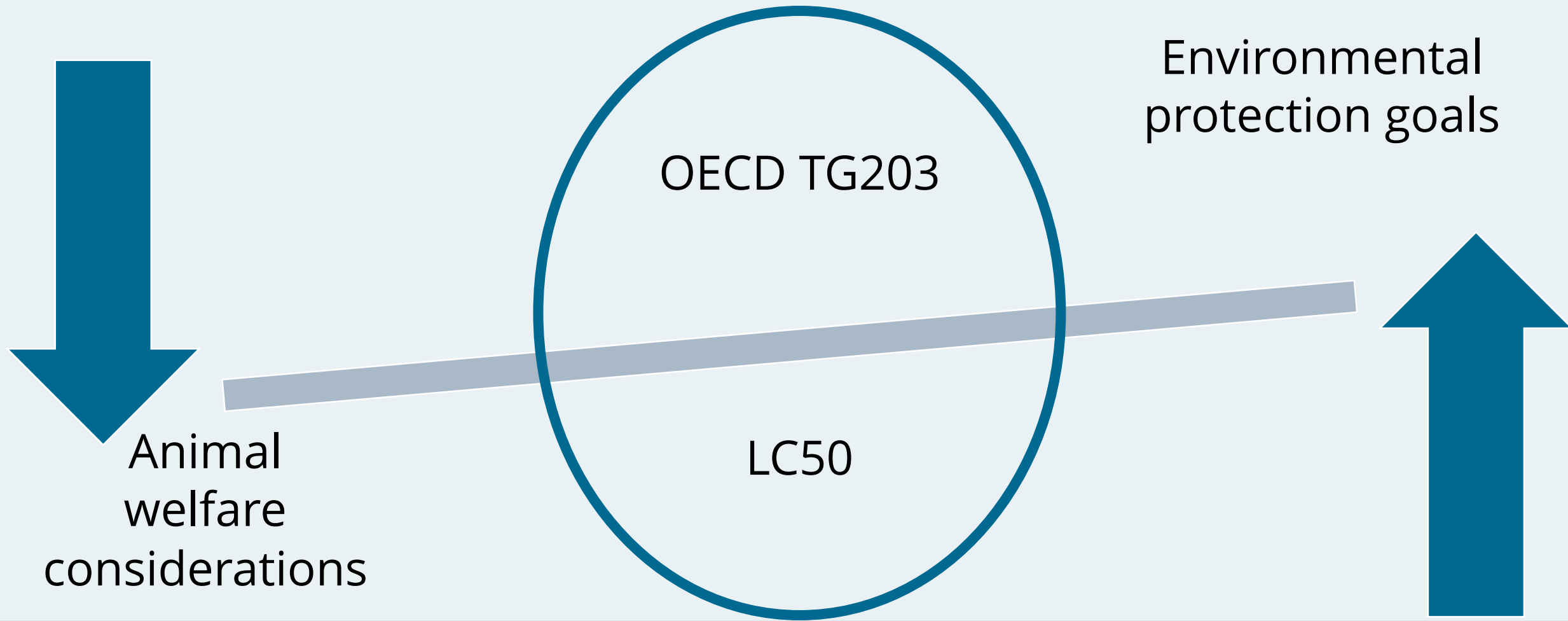
TG203



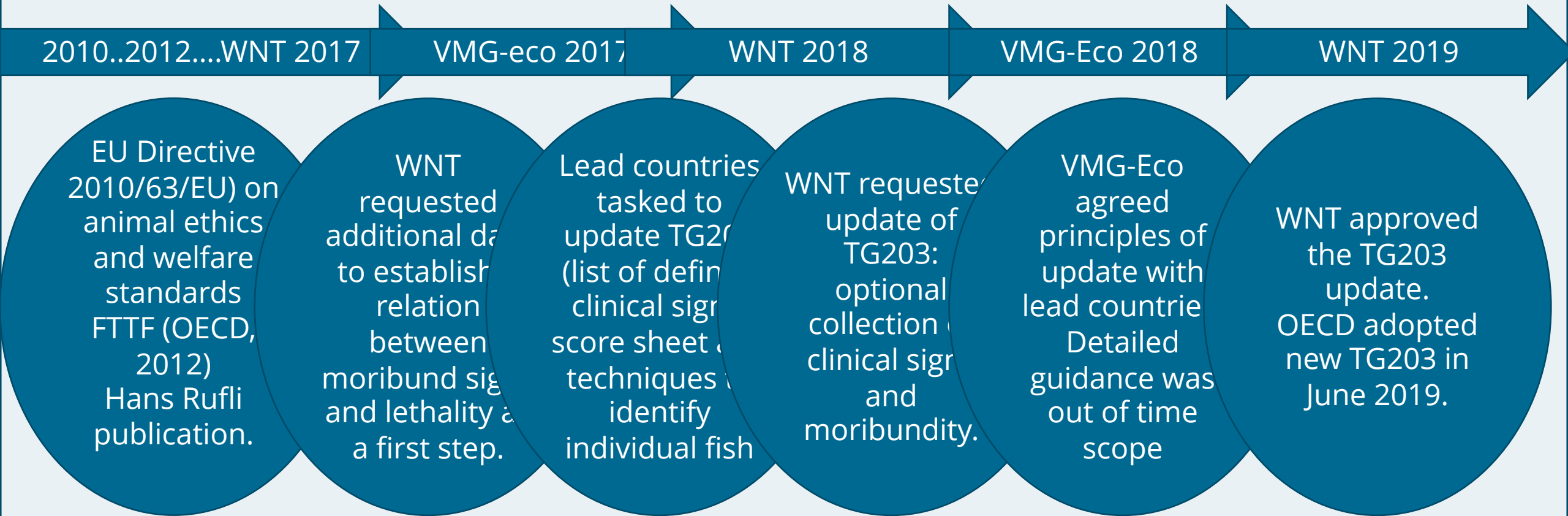
- Introduced in 1981
- Reviewed in 1992 (less fish/tank) and 2019
- Fish are exposed to range of chemical concentrations for 96hours
- Dead fish are counted and used to derive the LC50 for acute toxicity



What is the problem with TG203?



Background history....



The current state of play

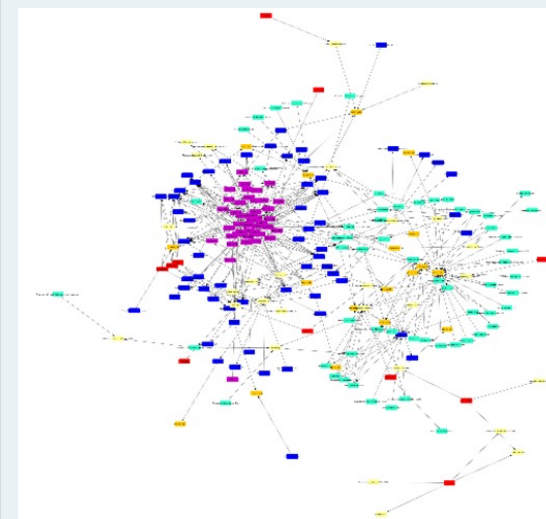
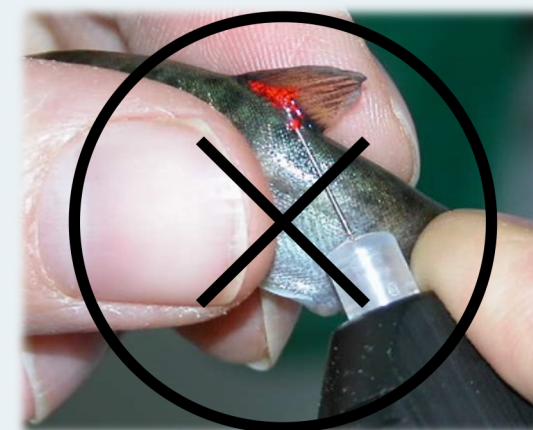
TG update led to protocol modernisation – making significant advances on the 1992 version, including the recommendations of the OECD Fish Toxicity Testing Framework (OECD, 2012)

- Consideration of alternative methods (QSAR, read across, embryos, fish cell lines etc)
- Additional guidance on range finding, **threshold approach**
- Clearer explanation on the 0% and 100% mortality
- Clarification on test tank replication, flow-through conditions, maximum fish-load, fish health, inclusion of estuarine and marine species, fish length and weight
- Harmonisation regarding solvents and difficult-to-test-substances
- Guidance on statistical evaluation, inadequate data, analysis of controls
- Improved harmonisation with US EPA 850.1075

Enhanced description and standardised recording of visible abnormalities (also referred to as sublethal clinical signs) that fish may display during the exposure.

- Mortality remains the endpoint-no addition of moribundity
- No individual fish marking
- Voluntary use of score sheet
- Encouragement to provide information on chemical (KoW, MoA, etc)

Clinical sign \ Score		1	2	3
Distribution	Loss of schooling / shoaling behaviour			
	Dense schooling / shoaling behaviour			
	Vertical distribution -surface			
	Vertical distribution - bottom			
Equilibrium & buoyancy	Abnormal horizontal orientation			
	Abnormal vertical orientation			
	Loss of buoyancy control			
Observed behaviours	Hypoactivity			
	Hyperactivity			
	Spiral swimming			
	Hyperventilation			
	Hypoventilation			
	Irregular ventilation			
	Increased ventilation depth			
	Convulsions			
	Coughing			
	Gulping			
	Gasping			
	Surface escape / avoidance behaviours			
	Bottom escape / avoidance behaviours			
	Irritated skin behaviours			
	Aggression and cannibalism			
Appearance	Tetany			
	Skin colour - darkening			
	Skin colour - lightening			
	Skin colour - mottled			
	Oedema			
	Haemorrhagic areas - petechias			
	Haemorrhagic areas - haematomas			
	Exophthalmia			
	Mucus secretion			
	Faecal (anal) casts			
Provoked behaviour - responses to	Visual and tank knocking stimulus - over reactive			
	Visual and tank knocking stimulus - under reactive			
	Tactile stimulus - under reactive			
Additional observations				



Defra-funded workshop, London, March 2020

Bring together multi-sectoral, international stakeholders to identify the knowledge gaps impeding standardised recording of sublethal clinical signs of toxicity in fish, and ultimately move away from using mortality as the endpoint in TG203 Fish, Acute Toxicity Testing

- To share feedback from CROs on the TG203 2019 update
- To identify training & guidance needs for reporting of clinical signs of toxicity in fish internationally
- To identify a mechanism for data collection and analysis in support of humane endpoints in TG203
- To identify a roadmap on moving away from mortality as an endpoint in fish



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Workshop salient points 1/3

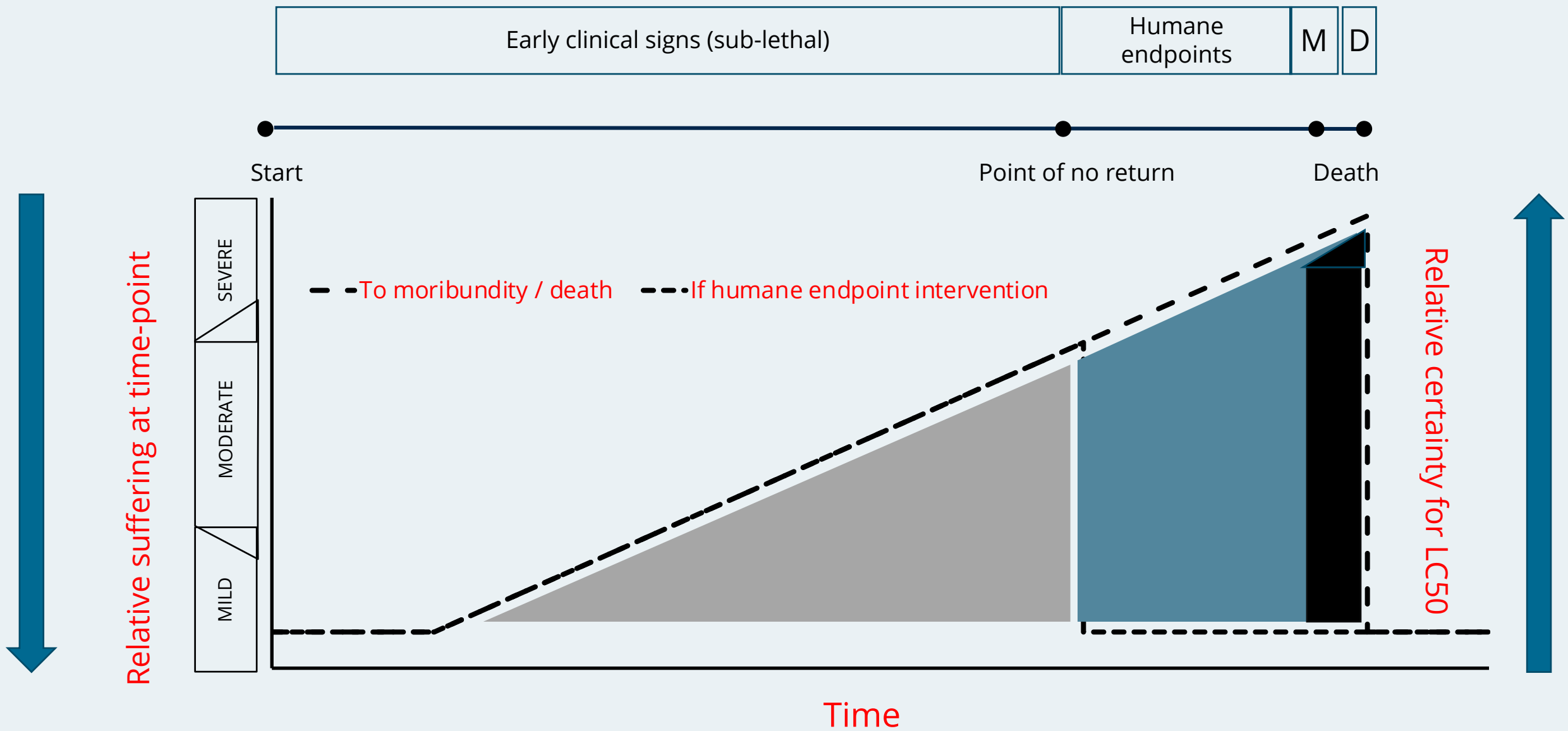
- Aiming to define and apply moribundity will have little welfare gains
- If lethality (LC50) remains a regulatory need

The **point of no return** should be the focus of training and guidance to allow application of humane endpoints whilst maintaining the lethality as endpoint. International effort is needed but experience already exists in some countries/CRO's, so material exists.

- If evident toxicity becomes the regulatory need

The predictive ability of early clinical signs, to define evident toxicity can be assessed via data mining. This will require more detailed guidance and training, perhaps targeted via research programmes but will build toxicity pathway knowledge and benefit predictive toxicology.

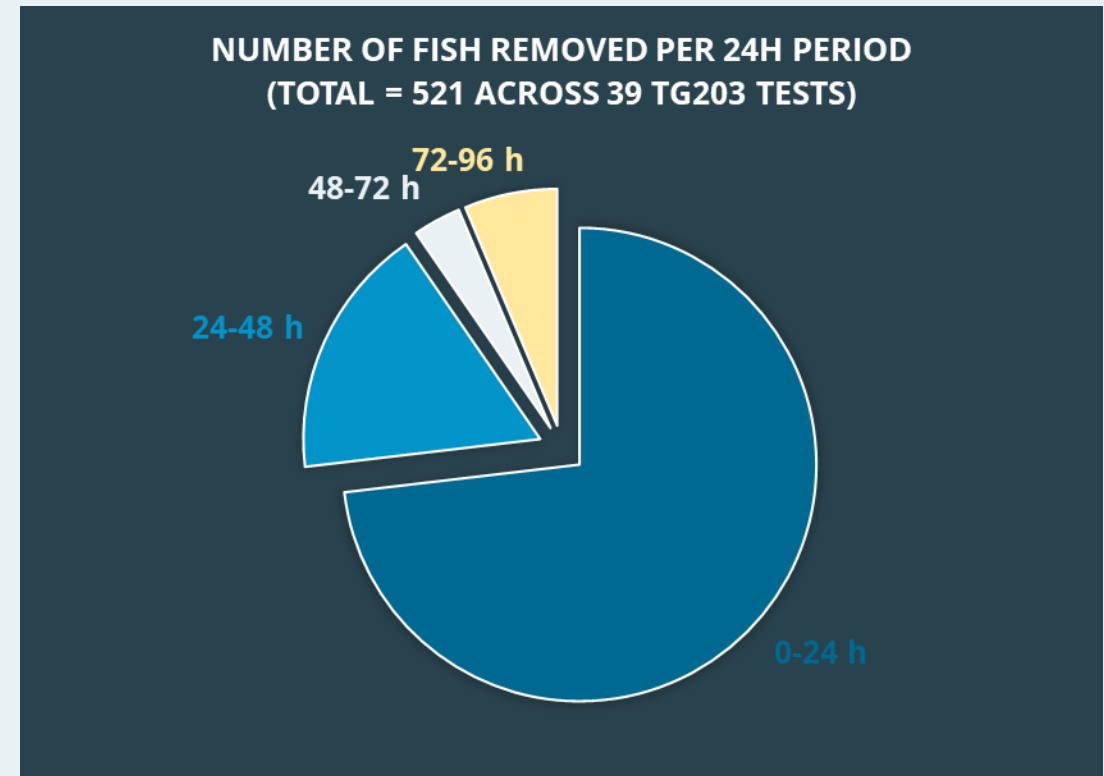




Workshop salient points 2/3

Reducing test duration and effect on LC50

Historic data analysis can provide the evidence needed to assess this effect wider as data collected with in TG203 allow this calculation. Importantly, if any knowledge on the mode of action, may reveal the underlying mechanism for a slower onset of mortality, where it exists.



Workshop salient points 3/3

- Data mining via reporting templates and summary tables

TG203 is a very data rich test. The reporting templates along with the summary tables, if used as suggested, could provide a solid database to allow mining.

Data mining can contribute significantly to reduction (i.e. read across), refinement (i.e. threshold approach) and even replacement (use weight of evidence, waiving need for testing).



SHORT TERM (6-18 months)	MEDIUM TERM (2-3 years)	LONG TERM (>3years)
Publication of workshop output with recommendations ✓	Development of a training manual (OECD working group including international experts and CROs) X	Prospective data collection and analysis (OECD working group) WIP
Identify funding and data repository mechanisms (stakeholder, NC3Rs, OECD, funding bodies) ✓	Harmonised reporting template (Regulators) ✓	Review scope of acute toxicity testing (Regulators) WIP
Historic data collection (LC50s and observations) and analysis (Networks to reach CROs, OECD working group) WIP	Use data to build predictive TKTD models and AOPs (Industry, Academia) WIP	Refinement of TG203 (humane endpoints and shorter duration) (OECD) X
Call for identifying existing training material (WNT, current training providers, FELASA, SETAC) X	Review assessment factors (Regulators) WIP	Replace or eliminate the need to conduct TG203 WIP!!!!



Summary thoughts

- Small steps already made towards reduction and refinement, although defining moribundity in fish is an unresolved issue (i.e. death still required)
- NAMs (New Approach Methods that may involve animals but are not considered sentient and Non-Animal Methods) are a very active field-gaining confidence in NAMs is further required
- Computational methods hold a great promise if existing knowledge and information is mined and organised adequately (i.e. seqAPASS)
- An Adverse Outcome Pathway for acute toxicity at OECD Wiki would be very beneficial-most acutely toxic chemicals act via narcosis)
- In parallel, a regulatory sift is not out of question: TG236 (Fish Embryo Toxicity Test) and TG249 (Fish Cell Line Acute Toxicity - The RTgill-W1 cell line assay) provide an excellent alternative platform especially when combined.



All in all an exciting time to be involved in achieving the step change needed for fish welfare in regulatory testing!!

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