

New Alternative Methods for endocrine disruptors testing: lessons learnt from applying OECD guidelines.

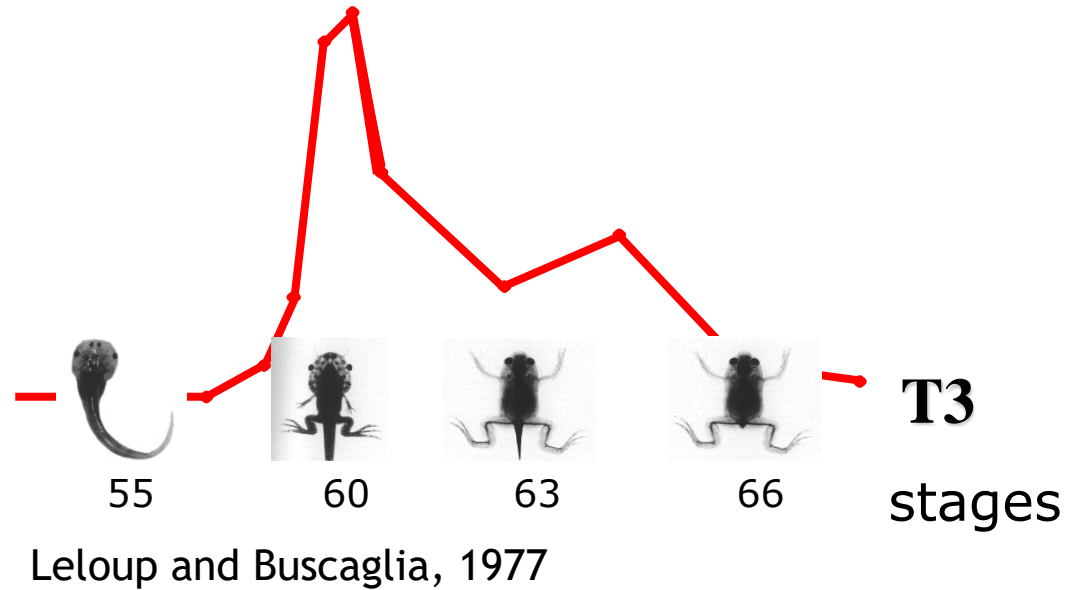
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WATCHFROG



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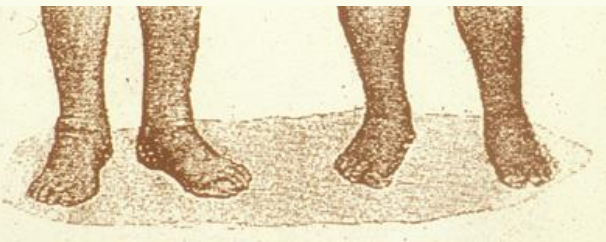
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Metamorphosis as a thyroid specific criteria



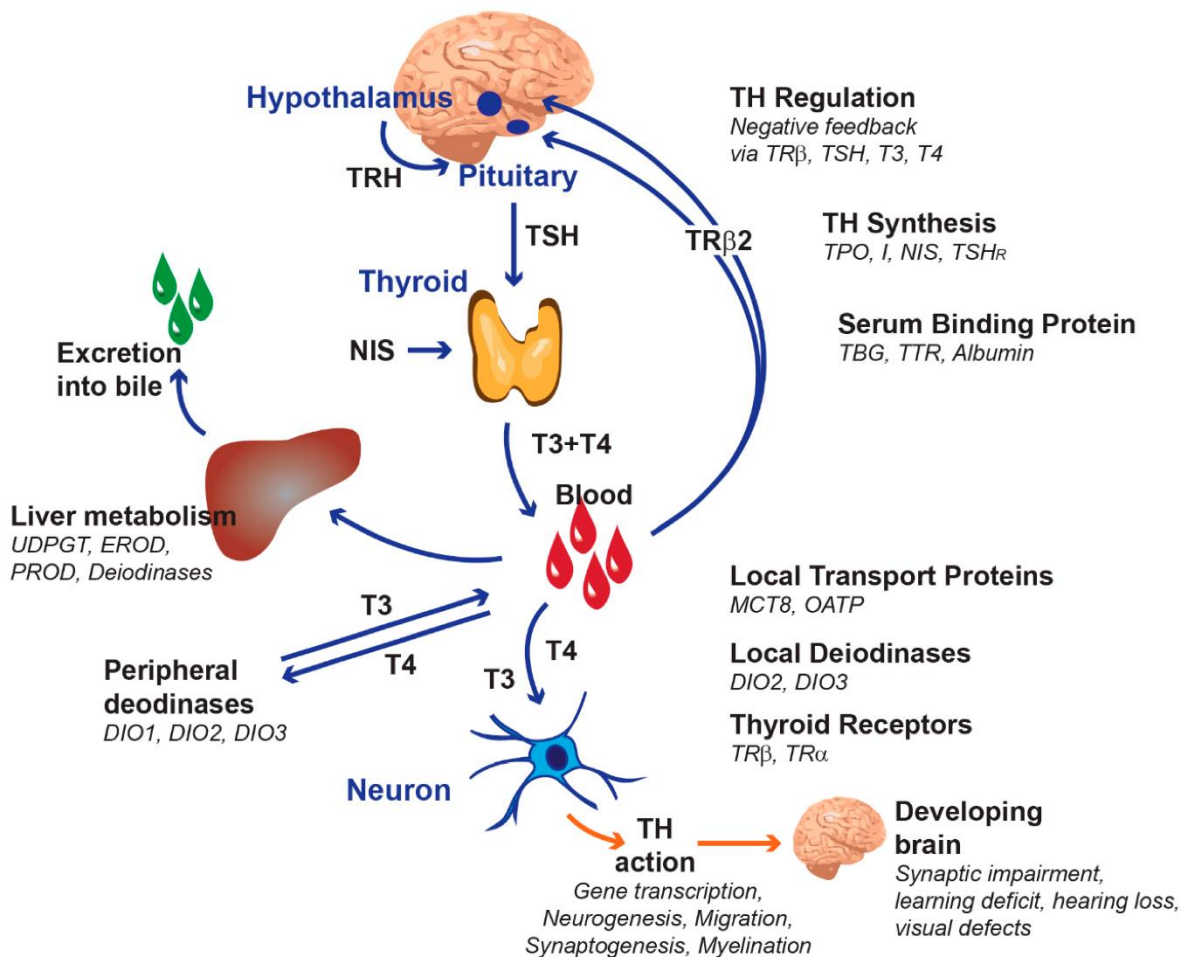
« Without a minimum of thyroid hormone at the right time during development, the tadpole remains a larva and the human baby a cretin. »

Jacques Legrand 1983





Hormonal pathways: endless targets.



In vivo:

Mammalian OECD TG 441, 407, 422 contain TH measurements
Rat extended One-Gen TG 443 with TH related endpoints (histology)

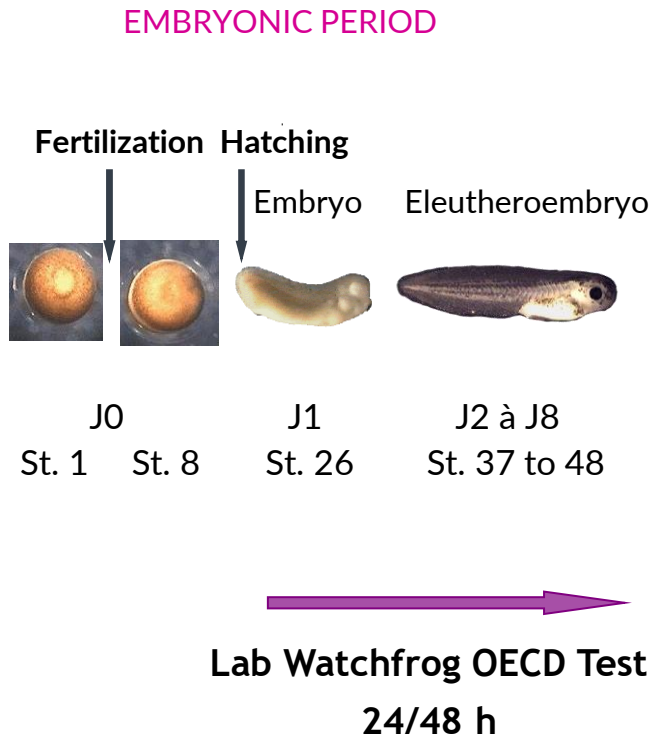
Amphibian Metamorphosis assay (TG 231)
Larval Growth and Development assay (TG 241)

In vitro:

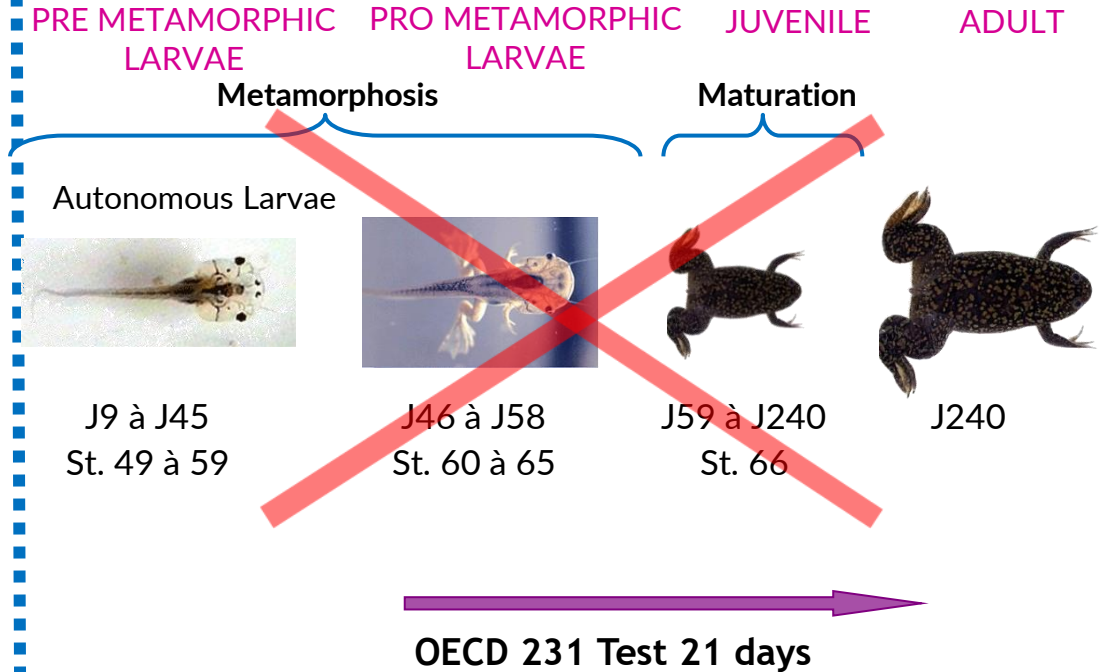
18 methods under validation (EU-NetVal)

In vitro early stages as NAMs

Non autonomous stages

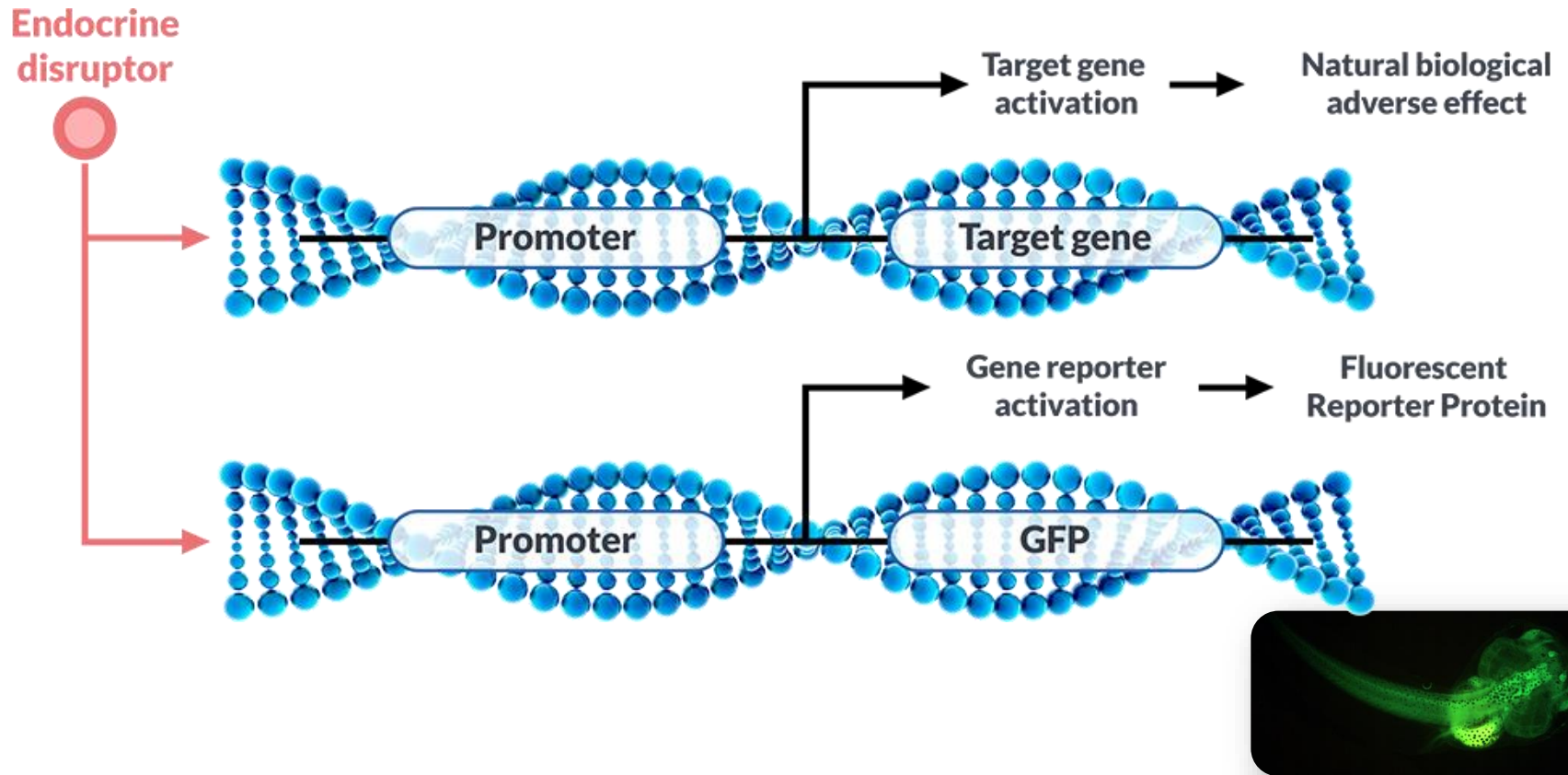


Laboratory animals



Laboratory animals Dir. 2010/63/EU: independently feeding larval forms

Fluorescence reveals physiological pathway



Comprehensive assessment of the signaling pathway

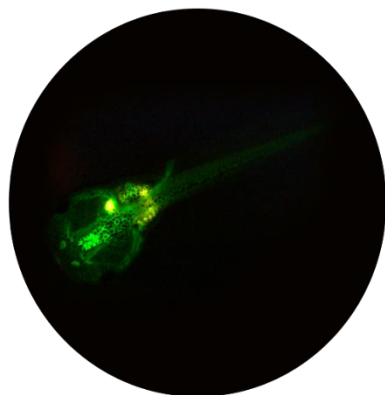
Test Range Level 3: E.A.T.S.*

OECD TG n°248

THYROID TEST - XETA®



OECD physiological criteria: Amphibian metamorphosis



*Tadpole, induced by exposition to the thyroid hormone (T3).
The fluorescence is induced in the brain.*

OECD TG n°251

ANDROGENIC TEST - RADAR®



OECD physiological criteria: Spiggin production in the Three Spined Stickleback



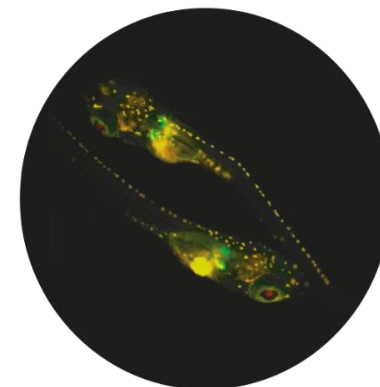
*Fish fry, induced by exposition to the androgenic hormone (17-MT).
The fluorescence is induced in the kidneys.*

TG in progress

ESTROGENS AND AROMATASE ACTIVITY TEST - REACTIV®



OECD physiological criteria: Egg production in fish, sex determination in fish



*Fish fry, induced by exposition to the estrogenic hormone (EE2).
The fluorescence is induced in the liver.*

STEROIDOGENIC EFFECTS

*EATS (Estrogenic, Androgenic, Thyroïd, Stéroid)

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Regulatory acceptance of aquatic embryos



GD 150: Describing available methods including scenarios of data interpretation « internationally agreed study protocols »















Guidance to apply methods from the assessor point of view.



Annex 440/2008 listing test methods to be applied in REACH regulation revised in march 2023.

Case study: XETA as a NAM to replace AMA

	AMA	XETA
Tested Life stages	51	45
Duration	21 d	72 h
T-MoA covered based on validation		
Modulators of TH clearance including UDP-glucuronosyltransferase modulators		
Modulators of TH metabolism, including deiodinase inhibitors		
Thyroid receptor agonist		
Thyroid receptor antagonist		
Interference with THs synthesis		
Interference with TH transporters		

XETA Annex for the Guidance document of EFSA

“It is recommended to perform a XETA, when, based on a complete dataset for mammals, no T-mediated adversity was observed”
(flowchart note 3)

<https://efsa.onlinelibrary.wiley.com/action/downloadSupplement?doi=10.2903%2Fj.efsa.2018.5311&file=efs25311-sup-0002-Annex.pdf>

Table based on data from OECD validation report.

Lessons learnt from ECHA/EFSA guidance

Combining mammalian data and tests on aquatic embryos

- Example of testing strategy established in order to cover each mode of action
- Example where showing the absence of endocrine activity using NAM could be considered as a conclusive dataset (no need to investigate adversity)
- Example where the dataset is more specific of an endocrine pathway than higher tier study using adult animals.
- Example of reducing the number of laboratory animals



Number of aquatic animals killed for ED assessment of pesticides and biocides on wildlife

	Mechanistic assays		Adversity assays	
Test	FSTRA ¹ OECD TG 229	AMA OECD TG 231	MEOGRT OECD TG 240	LAGDA OECD TG 241
Number of animals in the range-finding test	60	100	300	120
Number of animals in the definitive test ²	96	320	1,764	480
Failure rate for definitive test ³	0.1	0.1	0.5	0.2
Total number of animals in test	166	452	2,946	696
Number of animals for 475 pesticides	78,850	214,700	1,399,350	330,600
TOTAL	293,550		1,729,950	
Number of animals for 297 biocides	49,302	134,244	874,962	206,712
TOTAL	183,546		1,081,674	

From Lagadic et al. 2021

Number of adult animals replaced by XETA so far (feb. 2023)

99 active ingredients evaluated
(in progress or finalized)

44,758 adult amphibians required
for thyroid assessment

Approx **15 -20 cases eligible to XETA**
i.e., **6780 – 9040** amphibians saved

37968 – 35718 amphibians killed to
perform metamorphosis assay (TG231)



Number of saved adult amphibians could be increased:



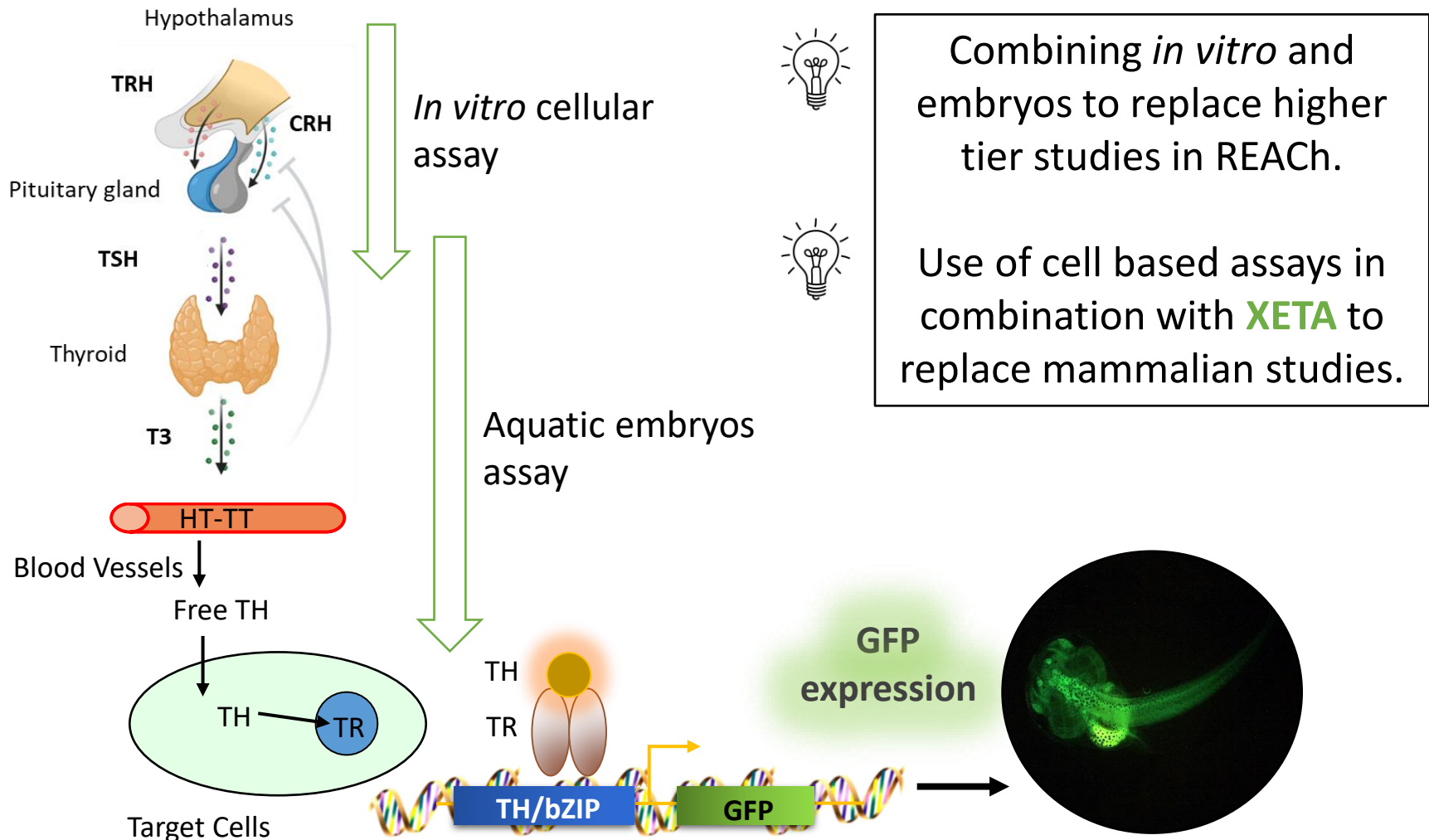
- Promote a systematic use of the XETA while in compliance with ECHA/EFSA guidance,
- Implement the possibility to combine one or two cellular assays (NIS/TPO) with XETA to fully replace metamorphosis assay.



Future perspective: replace when possible adult fish FSTRA by **RADAR/REACTiv**.

Approx. 80% of fish studies could be avoided *i.e.*, > **100,000 fish saved**.

Going a step further using XETA as a NAM





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