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dépasser les frontières

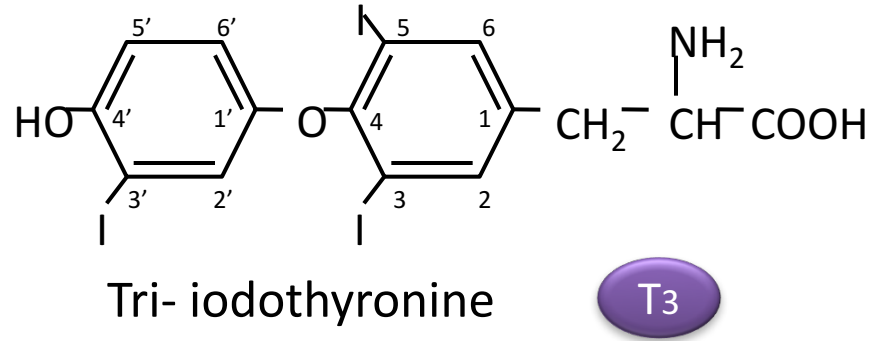
Deciphering thyroid hormone disruption during development

Dr Jean-Baptiste Fini

CNRS/ MNHN Sorbonne Universités Paris,
France

Webinar 14.11.18

Thyroid Hormones are essential in vertebrates



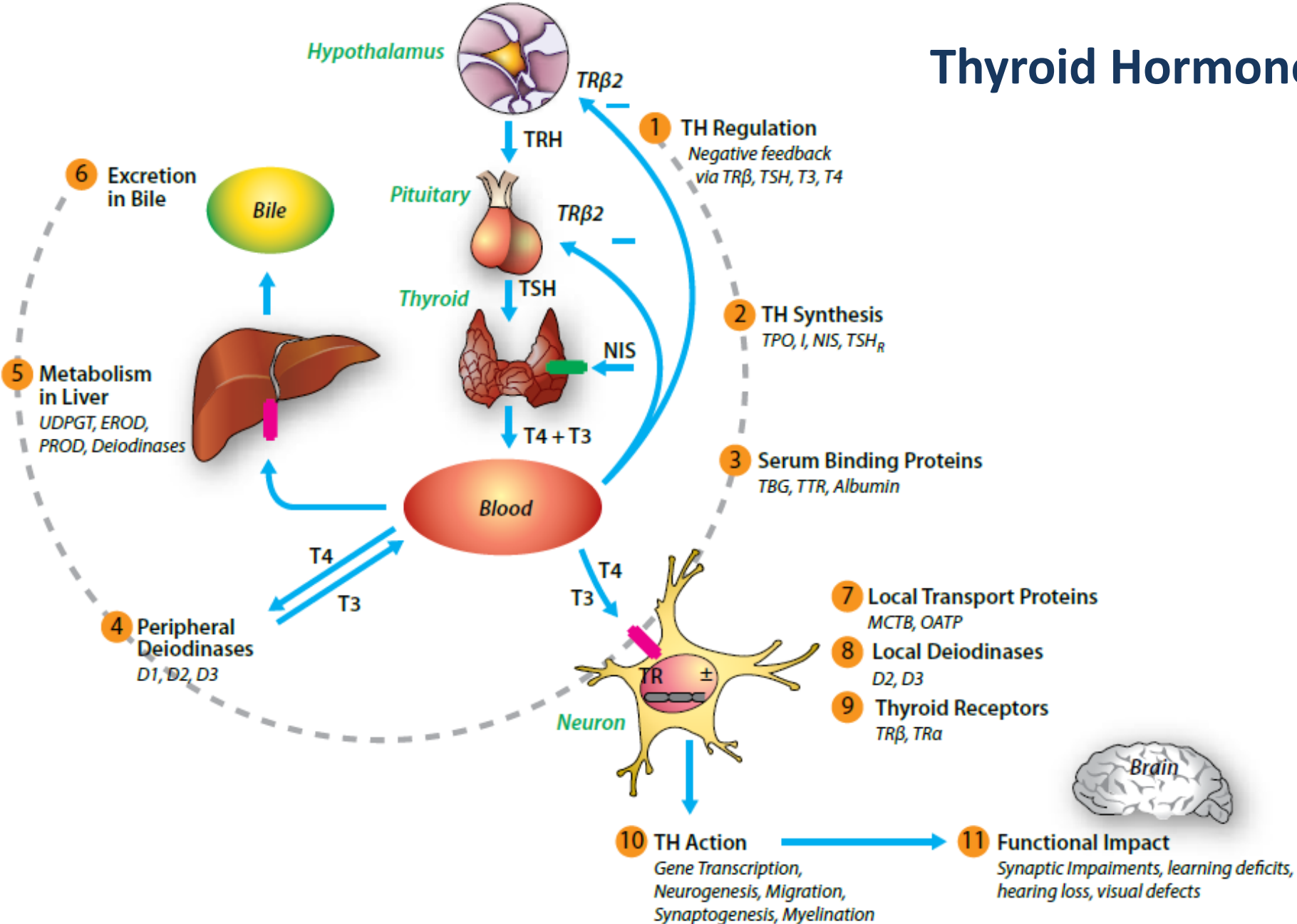
Thyroid hormone signalling conserved throughout 450 million years of vertebrate evolution

Amphibian metamorphosis, bird hatching, mammals brain maturation are all TH-dependent processes

Thyroid hormone is the most complex **halogenated** molecule synthesized by **vertebrates**

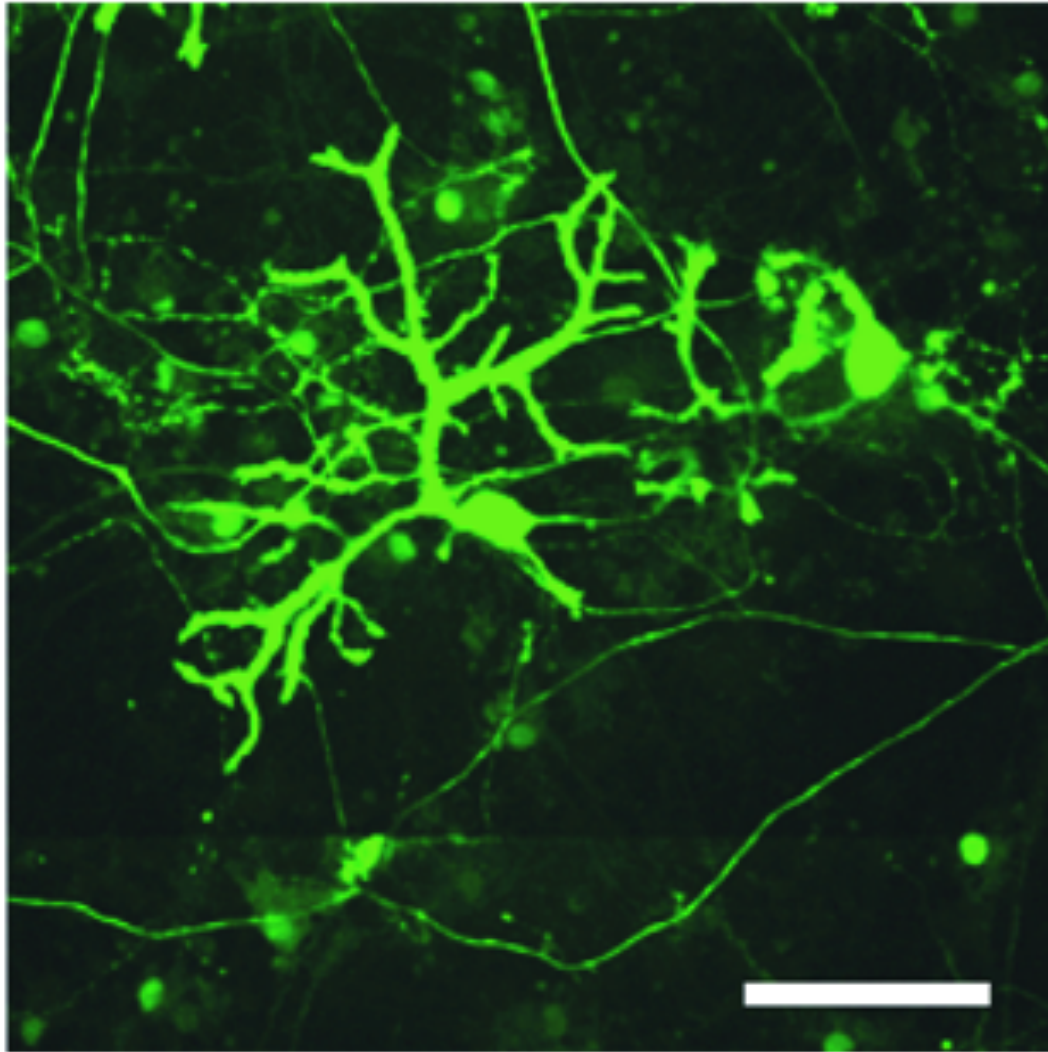
Only one to contain **iodine**, both **iodine and thyroid** hormone are essential for **brain development**

Thyroid Hormone axis

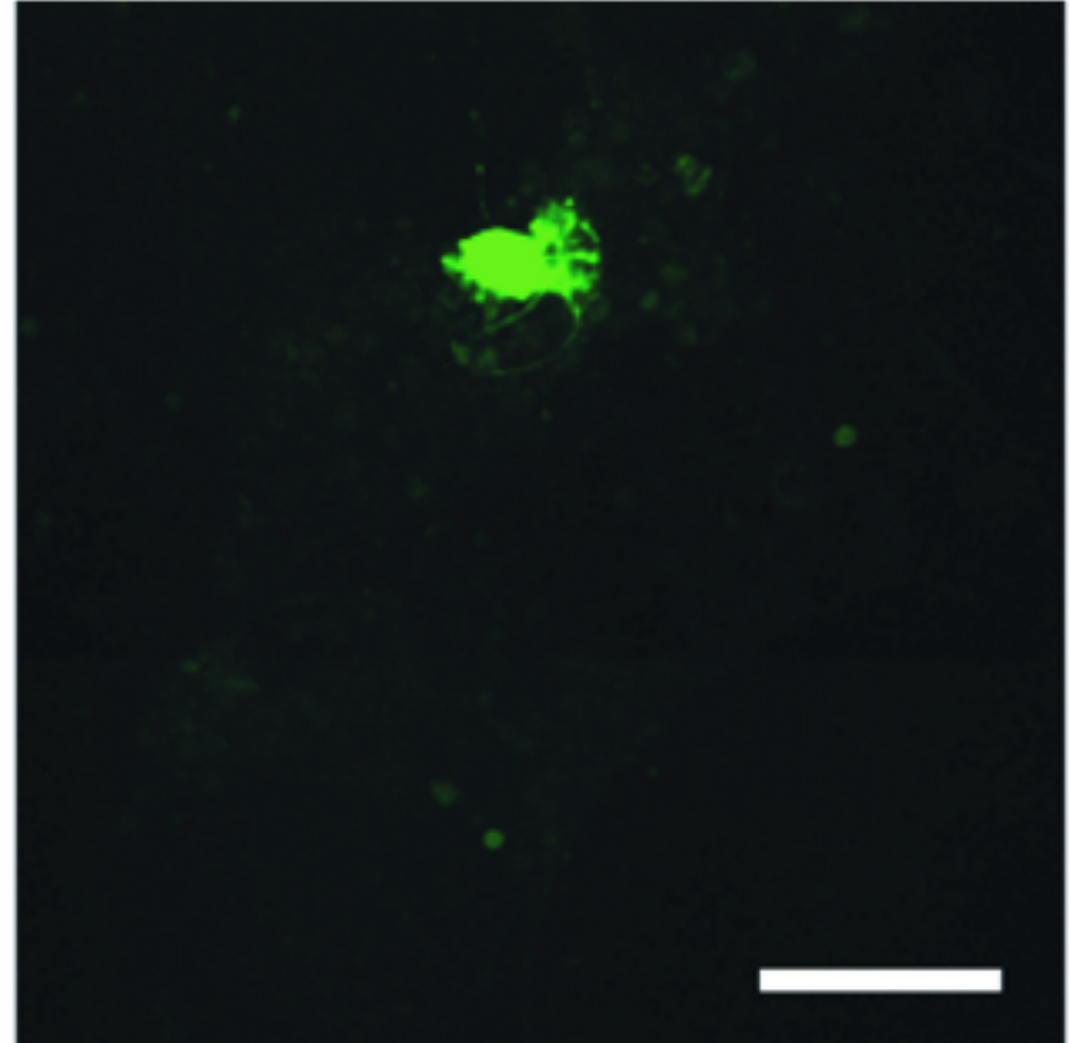


From Gilbert et al., 2011 (OMS2012)

Thyroid hormone is essential for Purkinje cells arborization



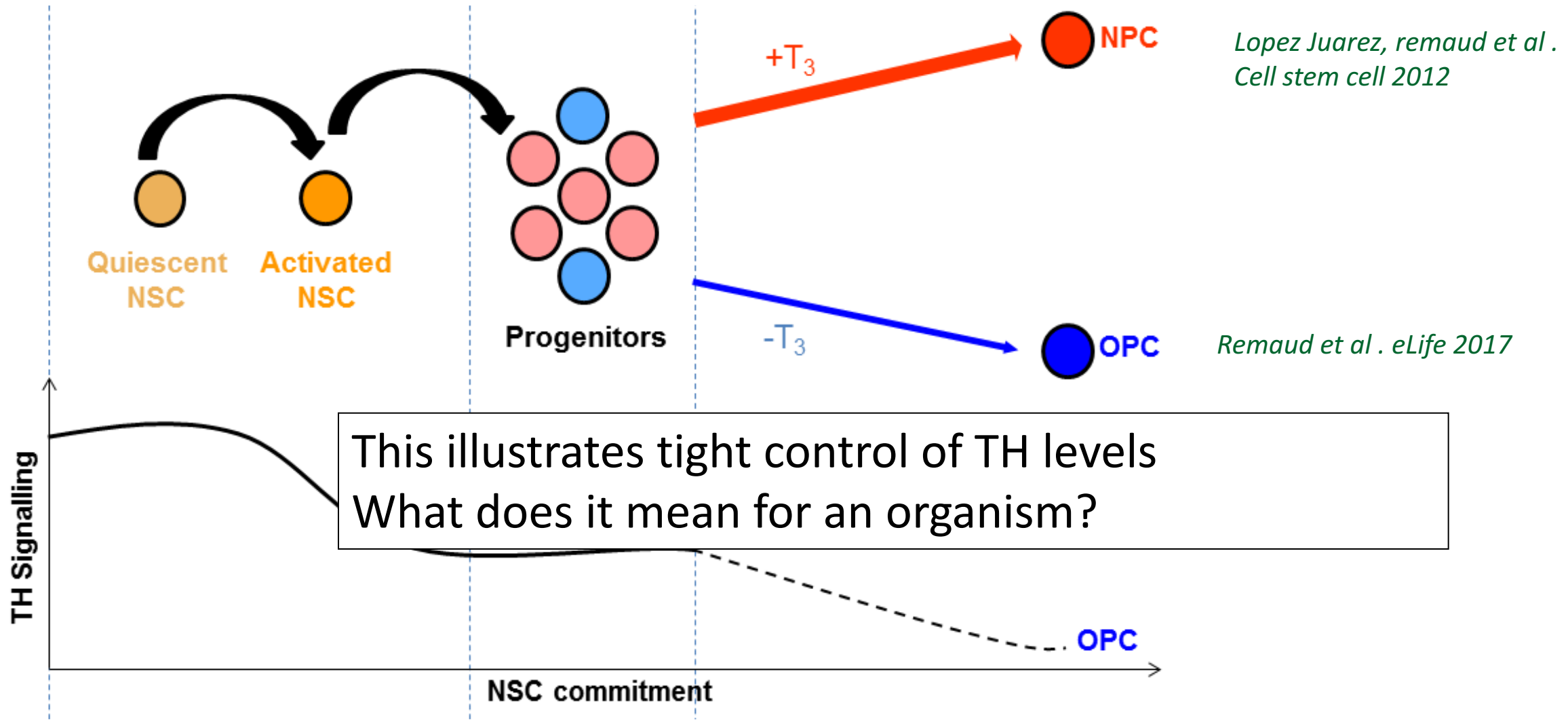
Presence of T4



Absence of T4

Ibhazehiebo et al. 2011, Environ Health Perspectives

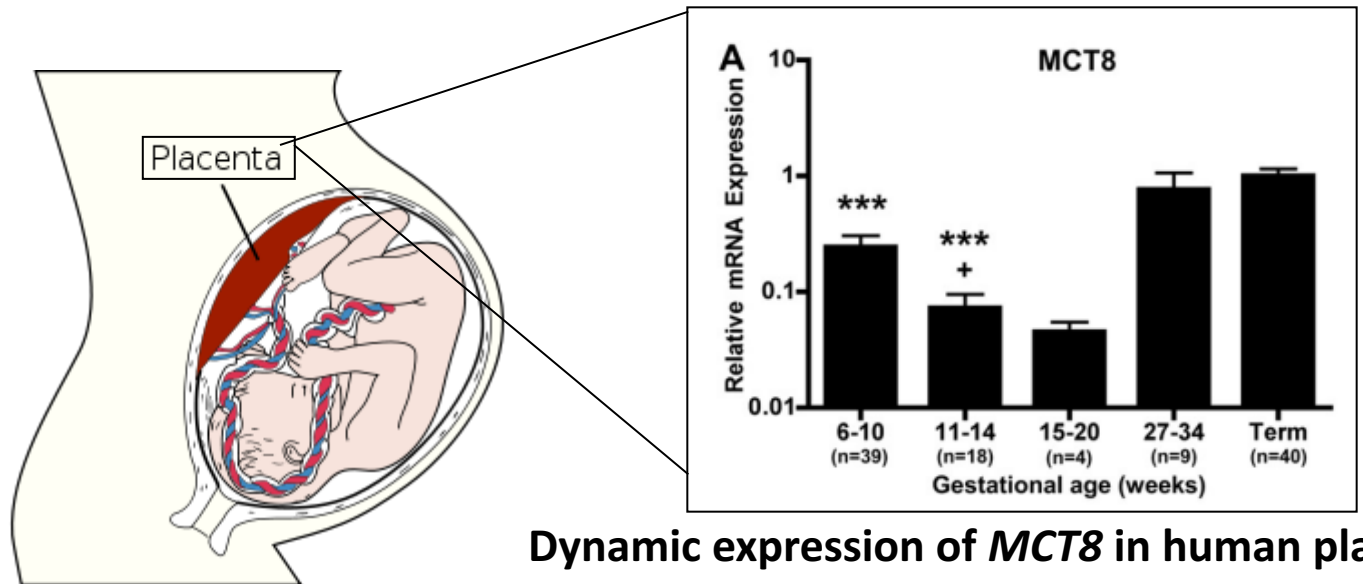
Thyroid hormone is essential for neural progenitor commitment



Mutation of MCT8, the only thyroid hormone transporter expressed on human neurons, causes Allan Herndon Dudley Syndrome



This illustrates a need for tight control of maternal levels of thyroid hormone in early pregnancy



Dynamic expression of *MCT8* in human placenta

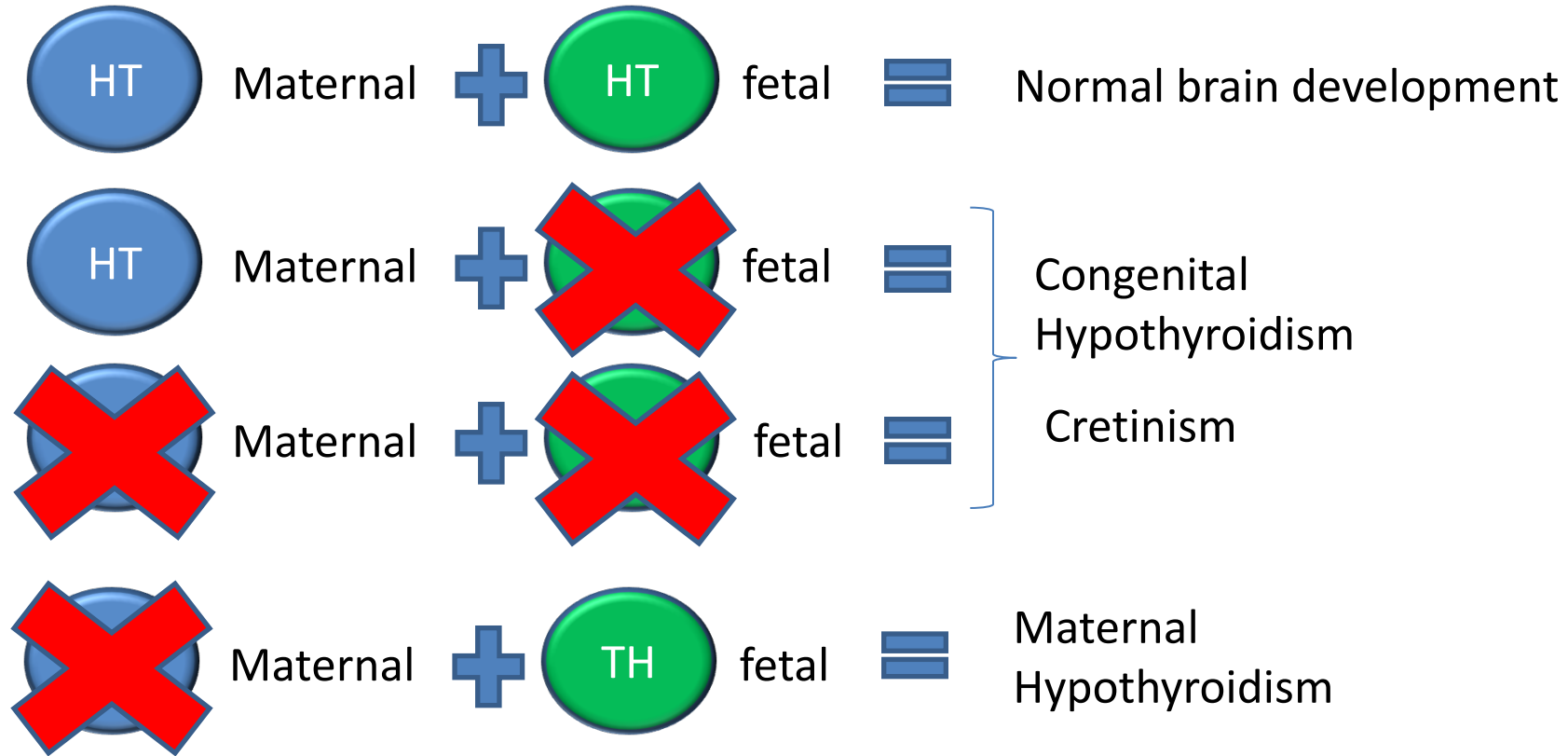
Rodrigues F et al 2014; L.S. Loubiere et al. 2010

All babies in western countries are tested at birth for their Thyroid Stimulating Hormone

If >17 mIU/L sign of congenital hypothyroidism but...

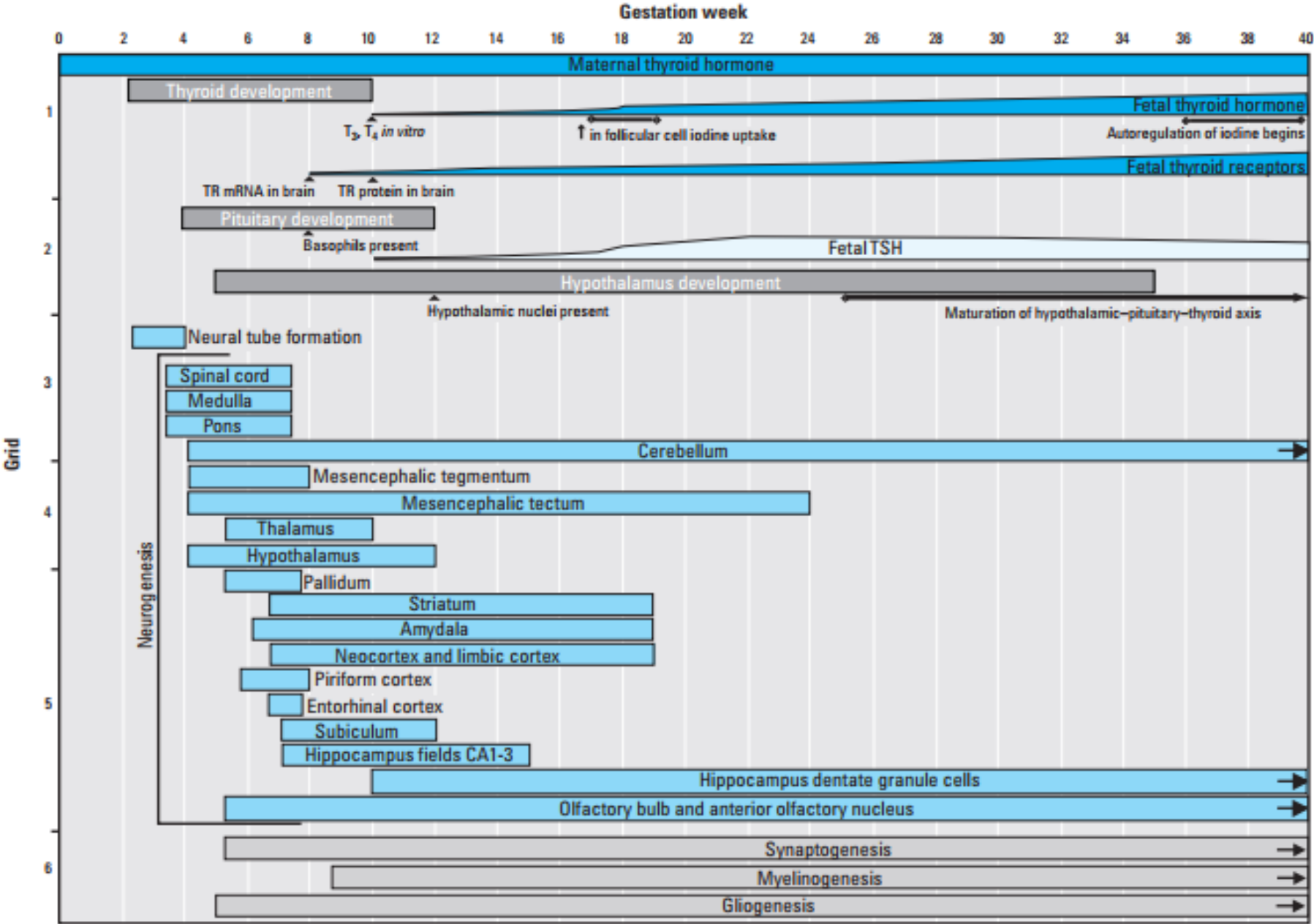


Crucial role for thyroid hormone in normal brain development



Maternal hypothyroidism/disruption consequences?

Fetal brain development is tightly orchestrated



Howdeshell 2002 EHP

OCTOBER 4, 2010

Environment Special:
The oceans—why 70%
of our planet is in danger

The Facebook Movie:
The secret history of
social networking

TIME

**How the
first nine
months
shape
the rest
of your life**

The new science
of fetal origins

BY ANNIE MURPHY PAUL



THREE

www.time.com

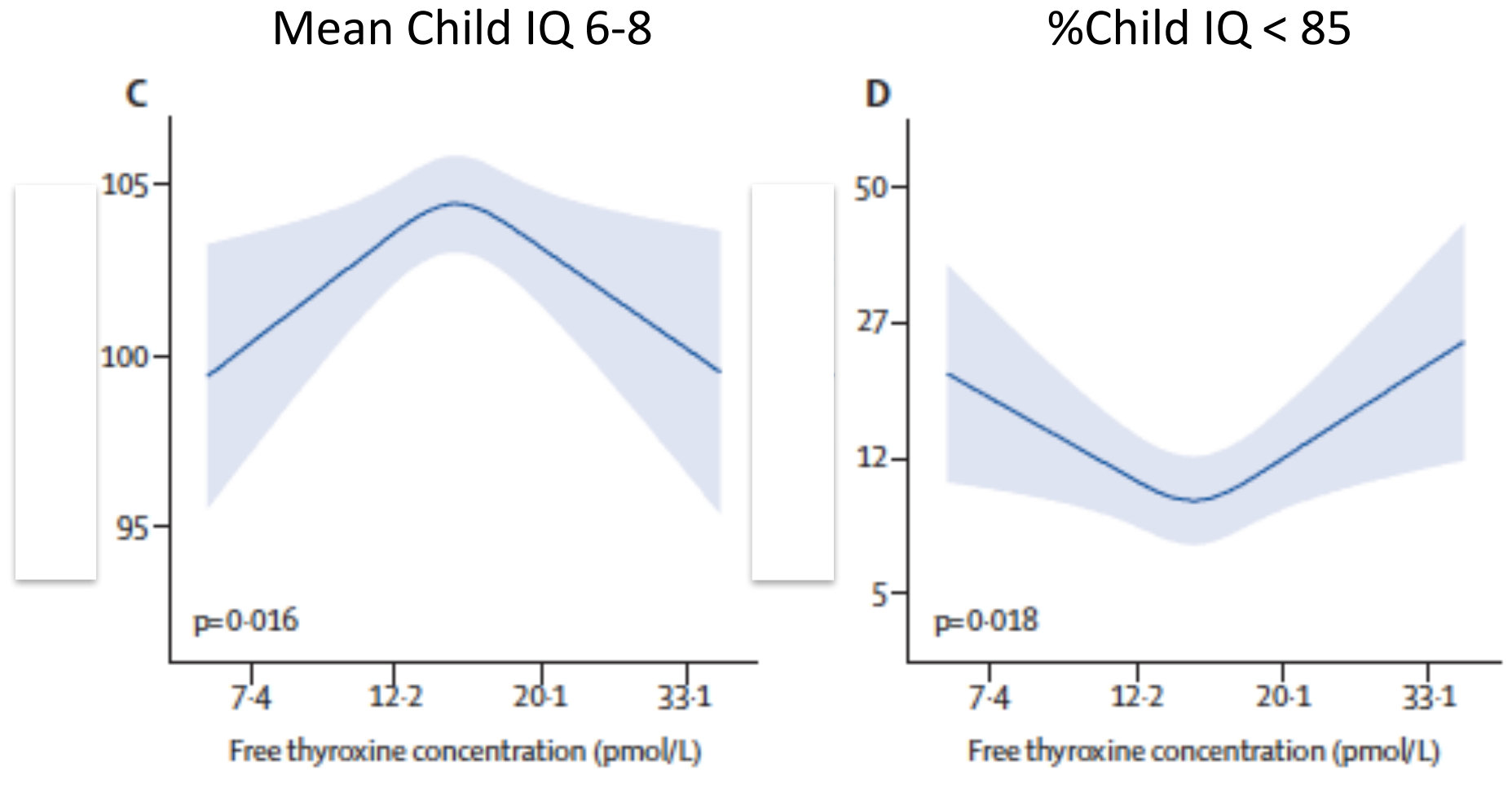
Association of maternal thyroid function during early pregnancy with offspring IQ and brain morphology in childhood: a population-based prospective cohort study

Tim I M Korevaar, Ryan Muetzel, Marco Medici, Layal Chaker, Vincent WV Jaddoe, Yolanda B de Rijke, Eric A P Steegers, Theo J Visser, Tonya White, Henning Tiemeier, Robin P Peeters

*Tim Korevaar ...Robin Peters,
Lancet Diabetes Endocrinology 2016
4, 35-43*

- Generation R Study (Rotterdam)
- Maternal thyroid hormone/TSH levels > 18 weeks of pregnancy
- Child IQ at 6 -8 yrs old
- MRI scans at 8 years old

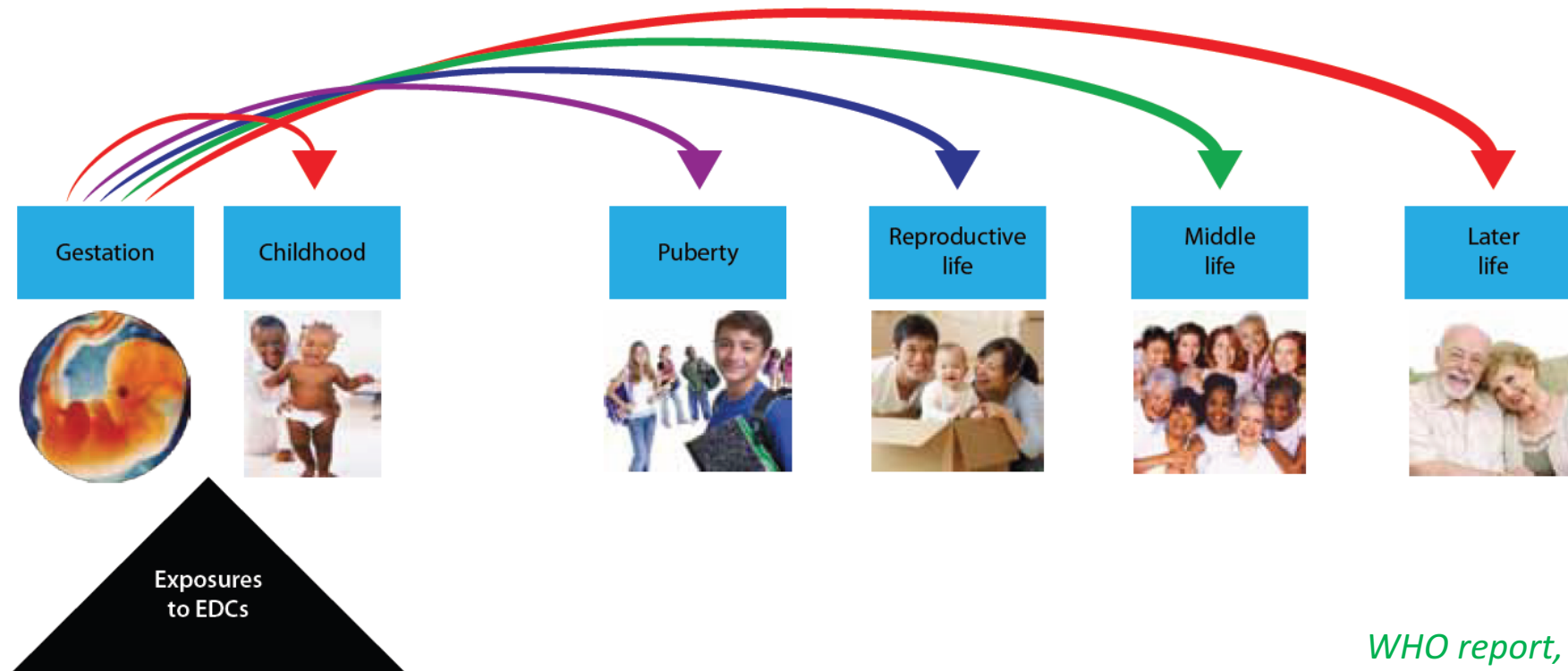
Too much thyroid hormone is as bad as too little



Koreevar et al., Lancet Diabetes Endocrinol 2016

Hypo and hyperthyroxinemia also associated with lower white matter density and reduced cortical volume

Central concept: developmental origin of adult health and disease



WHO report, 2012

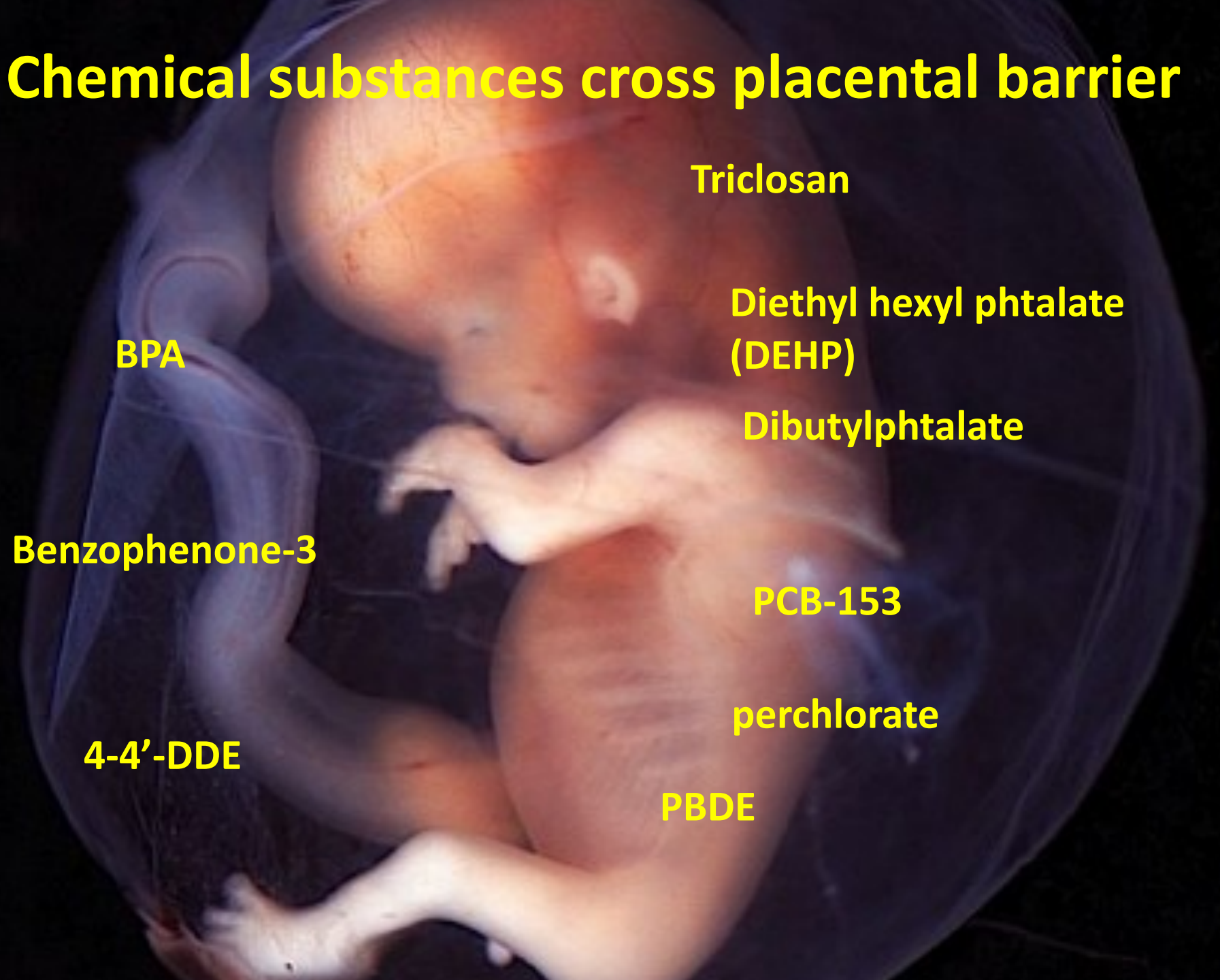
- Fetal development is a critical developmental window, **including for thyroid hormone availability**

Is there direct fetal exposure to chemical?

“...exposure to multiple chemicals is inevitable... we live in a chemical soup...”

Linda Birnbaum (NIEHS)

Chemical substances cross placental barrier



BPA

Triclosan

Diethyl hexyl phthalate (DEHP)

Dibutylphthalate

Benzophenone-3

PCB-153

perchlorate

4-4'-DDE

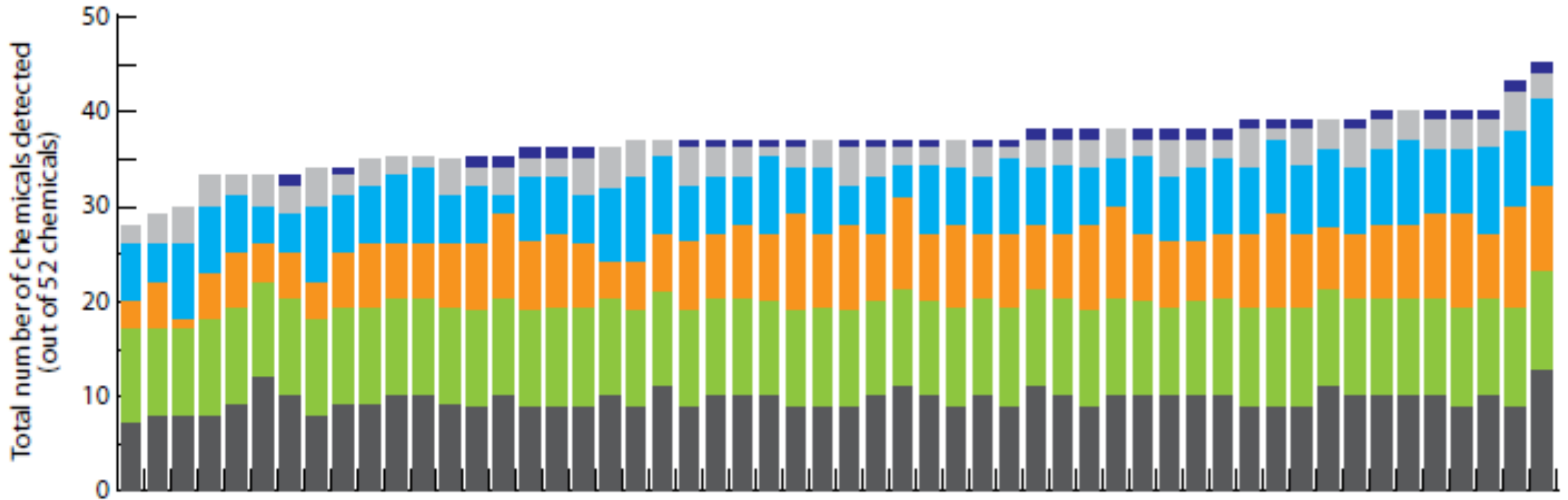
PBDE

Testing thyroid hormone disrupting potency ?

- Mixture « Amniotic » vs single compounds
- Mixtures « Neurodevelopment » and « Growth »

Strategy #1: Starting from exposure

On average pregnant women carry
33 compounds out of **52** analysed, **15** are ubiquitous



Pregnant women in NHANES subsample B
(N=54; each bar represents one study participant)

- Cotinine (1 total measured)
- Metals (4 total measures)
- OC Pesticides (13 total measured)
- PBDEs (11 total measured)
- PAHs (10 total measured)
- Phthalates (13 total measured)

Mixture made of the 15 common contaminants at levels found in human amniotic fluid



Phenolic compounds

- Bisphenol A (BPA)
- Triclosan $0.7 \cdot 10^{-7} \text{ M}$
- Benzophenone-3

Phthalates

- DBP
- DEHP $10^{-7} - 10^{-6} \text{ M}$



Organochlorine pesticides

- HCB 10^{-11} M
- 4-4' DDE 10^{-9} M



FLAME
RETARDANT



Polyaromatic hydrocarbons

- 2-Naphthol

Perfluorinated compounds

- PFOS 10^{-8} M
- PFOA 10^{-8} M



Halogenated compounds

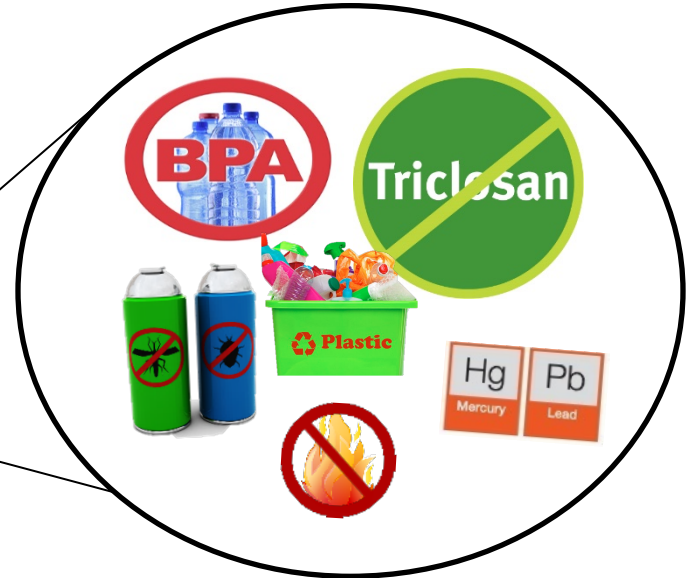
- PCB-153
- BDE-209
- Sodium perchlorate 10^{-8} M

Heavy metals

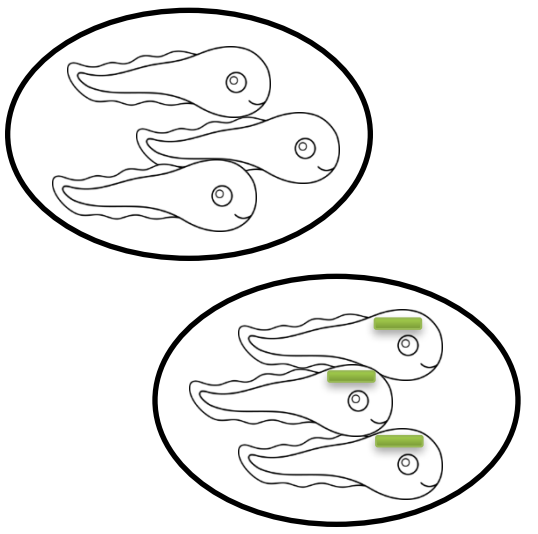
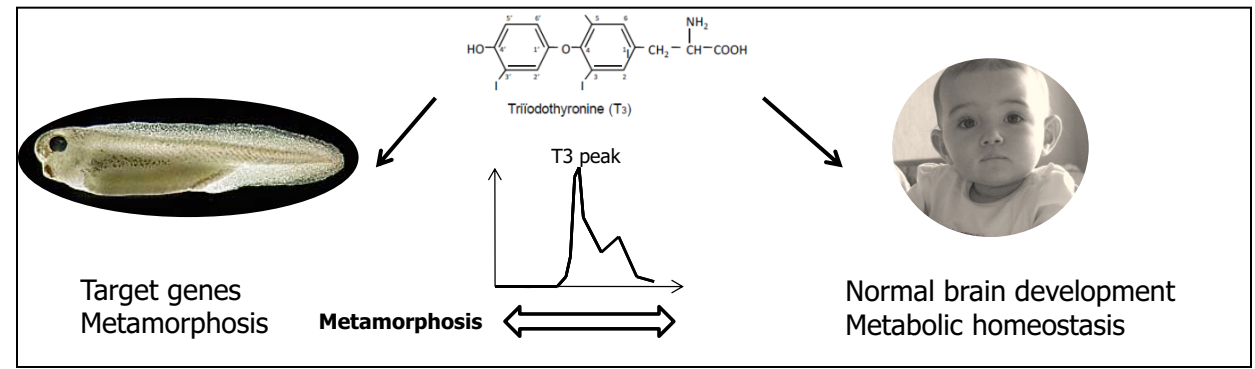
- Methyl Mercury 10^{-7} M
- Lead



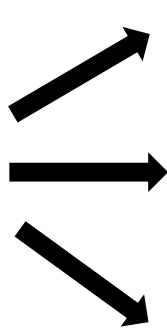
Which model to use?



“Representative” Amniotic mixture



3 day exposure

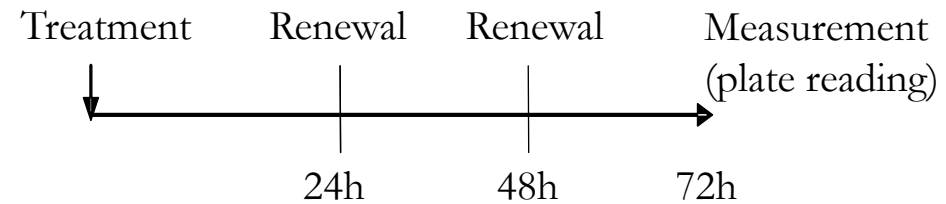
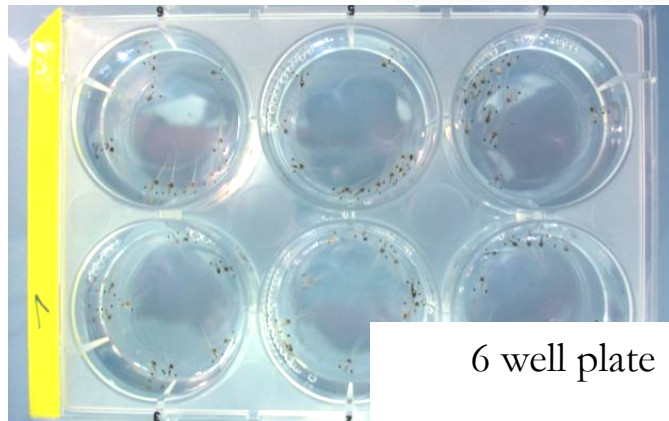
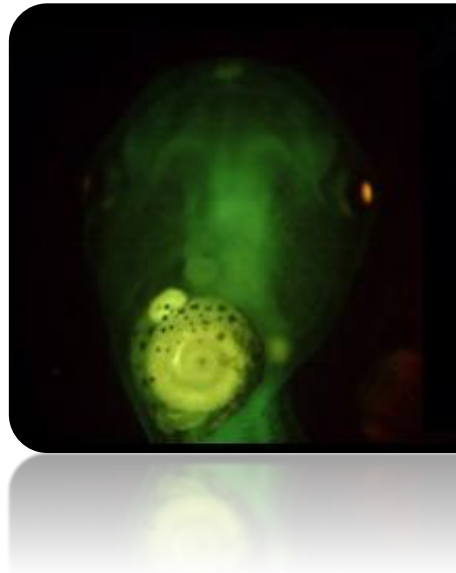


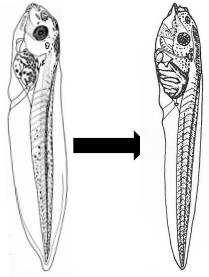
Xenopus Embryonic Thyroid assay (OECD validation)

Immunohistochemistry and 3D reconstruction

Behaviour analysis

Xenopus embryonic thyroid assay (XETA)



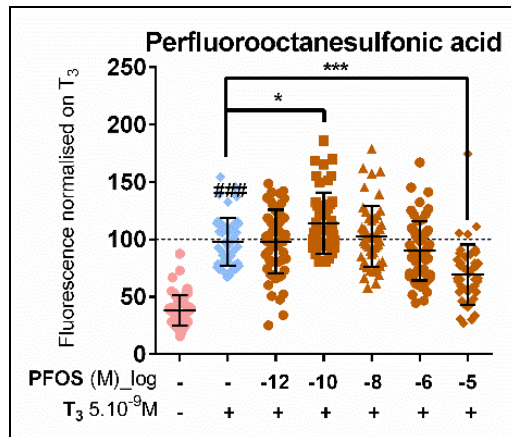
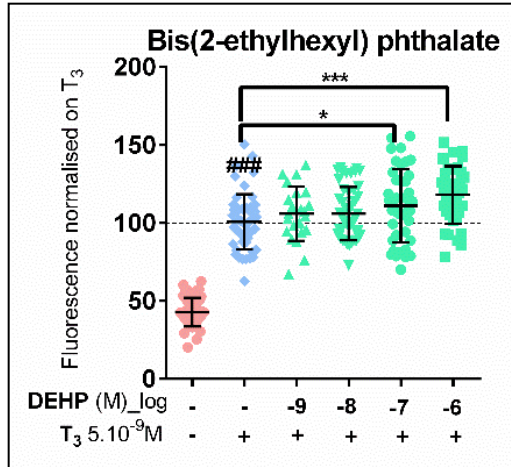


GFP
measurement

Chemical mixture dose-dependently disrupts thyroid hormone signalling

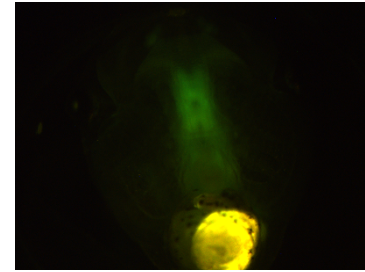
NF45
3 d treatment

Effects of single common chemicals

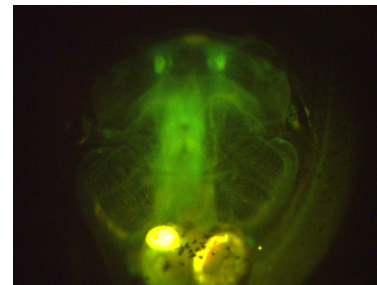


Chemical mixture of 15 common chemicals

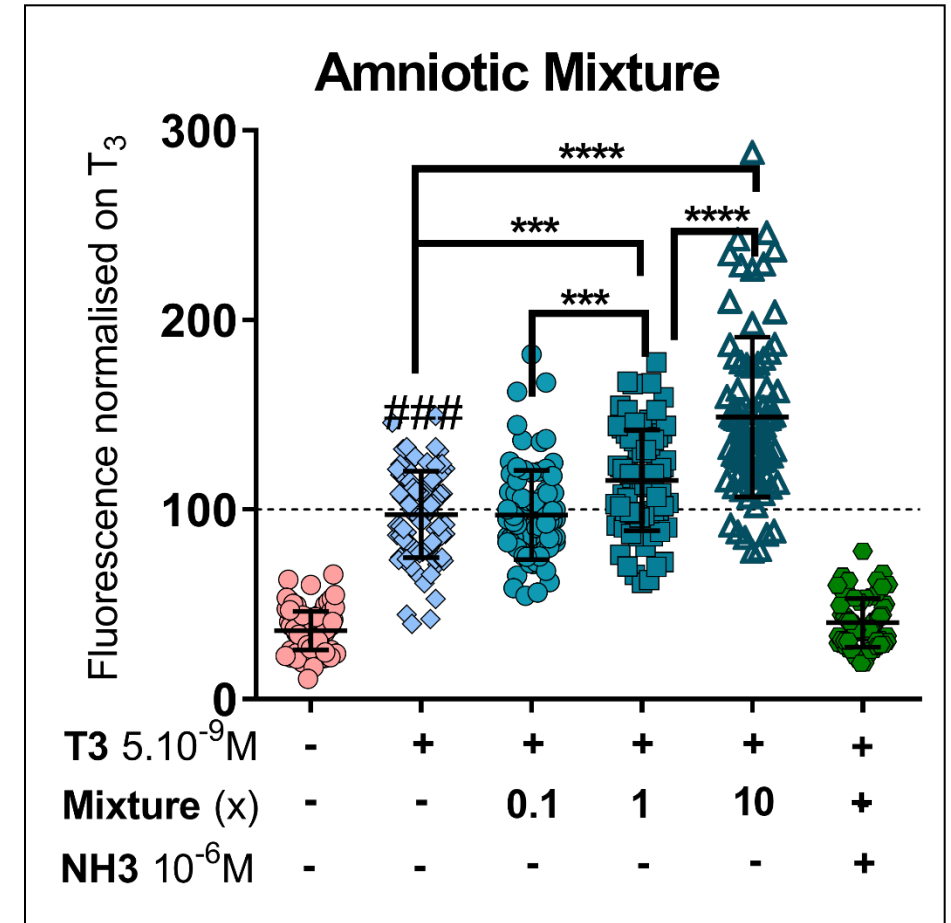
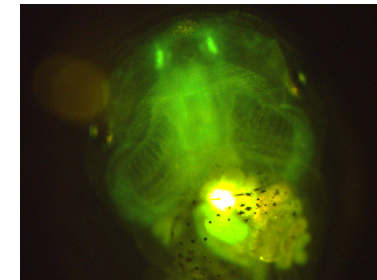
CTRL



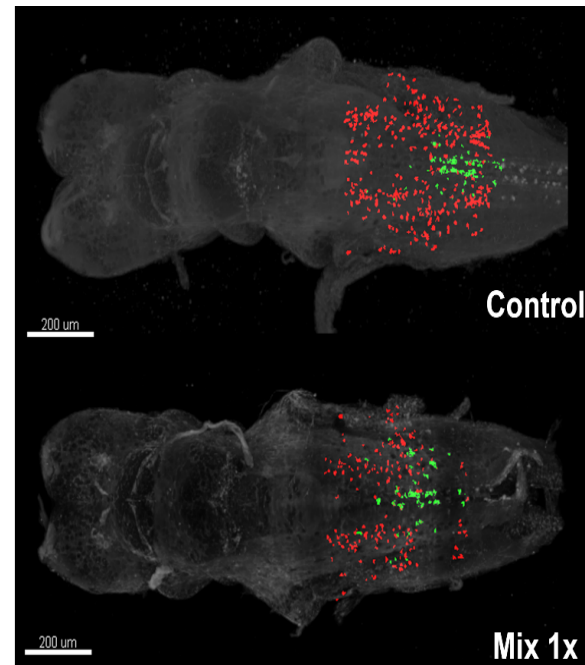
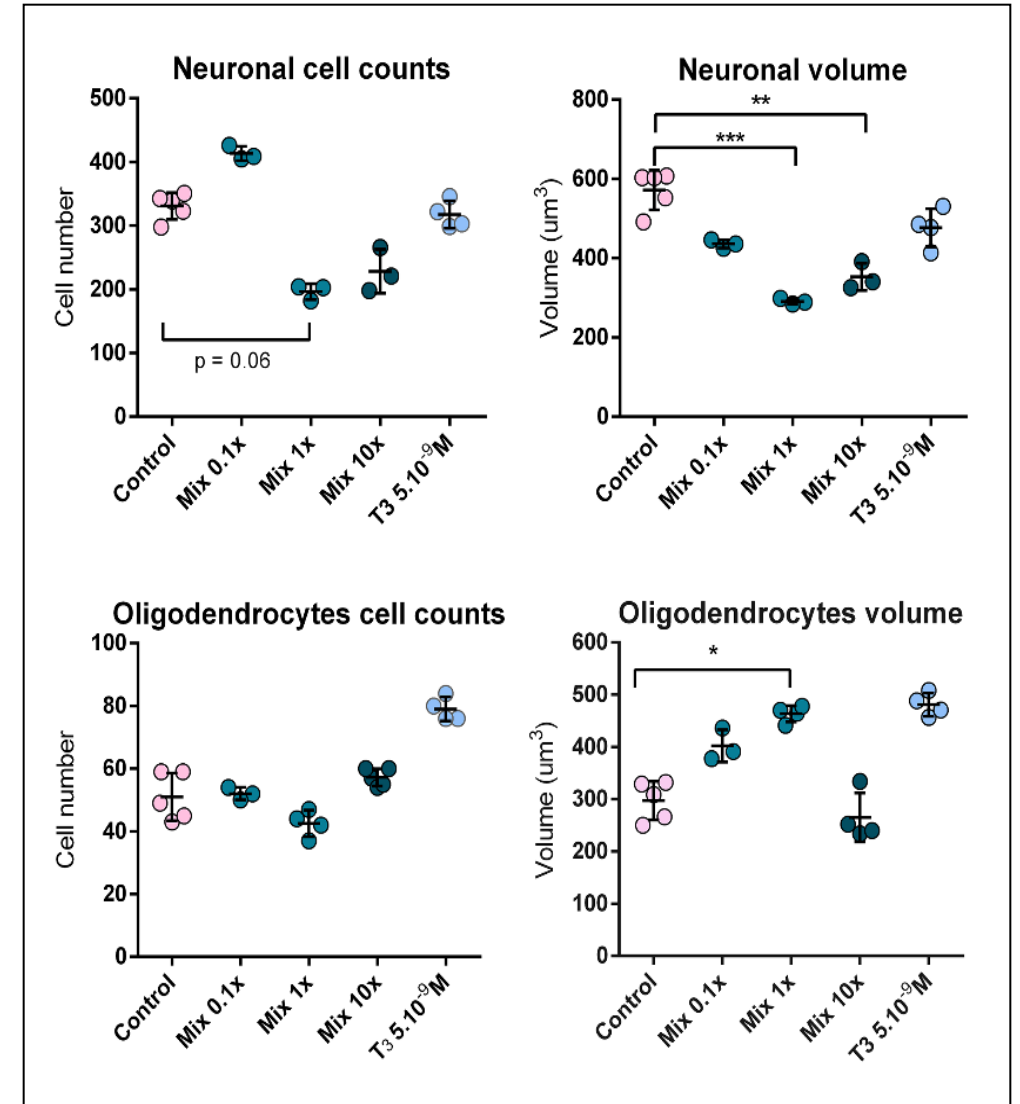
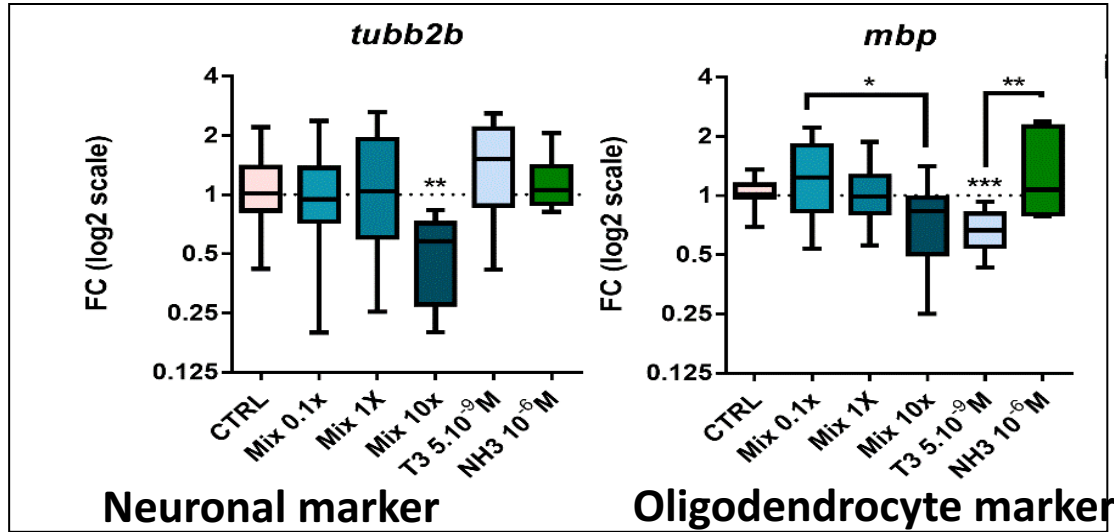
T3 5nM



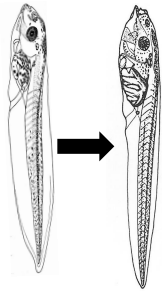
T3+ M 10X



Mixture modifies neural cell populations



Thyroid hormone disruption in the brain: Affected behavior

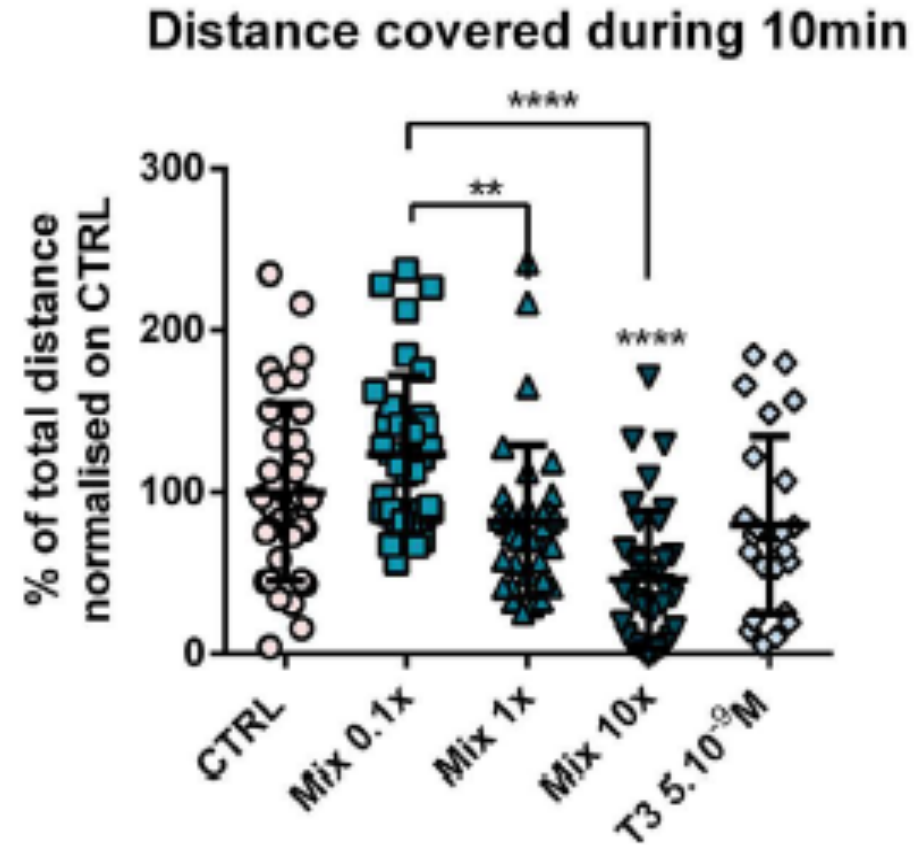
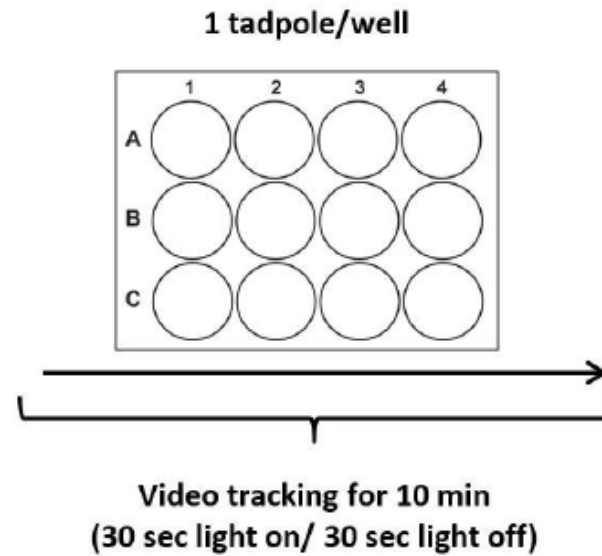


Mobility
assessment

NF45 NF48
3d treatment



DanioVision
Noldus tracking
system



Conclusions 1

- Mixture of chemicals is thyroid hormone disruptive
- All halogenated compounds tested alone disrupt TH
- Mixture affects brain gene expression, cell volume and organisation
- Mixture affects mobility (behaviour) at human exposure levels

B.Mughal

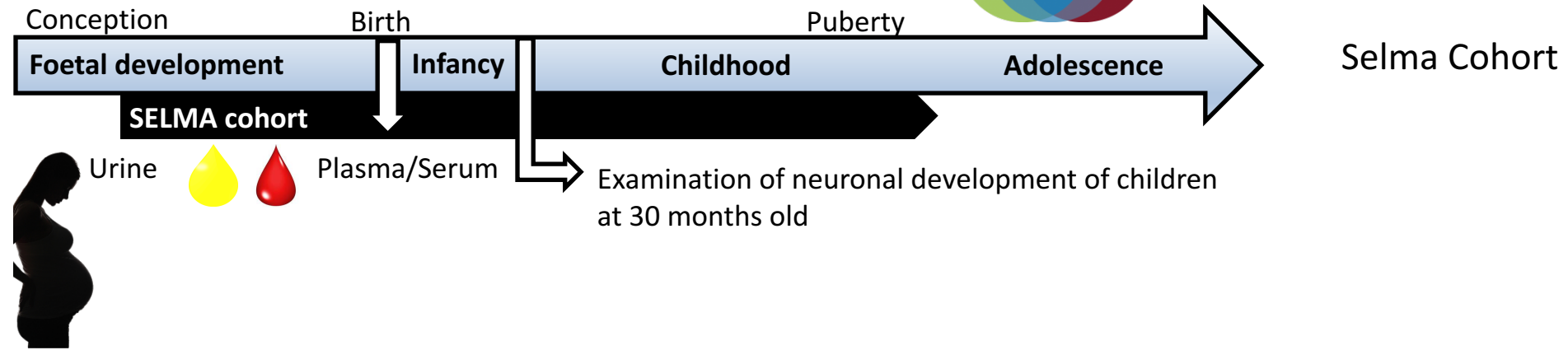
Testing thyroid hormone disrupting potency ?

- Mixture « Amniotic » vs single compounds
- Mixtures « Neurodevelopment » _ EDC MIX RISK

Strategy #2: Starting from epidemiology and adverse outcomes

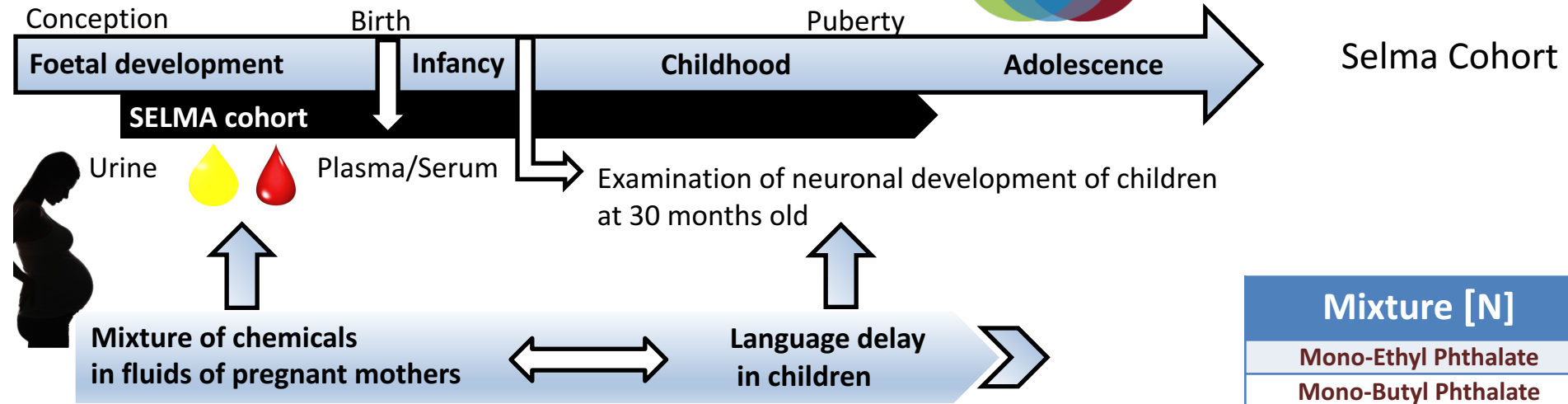


EDC-MixRisk
safe chemicals for future generations

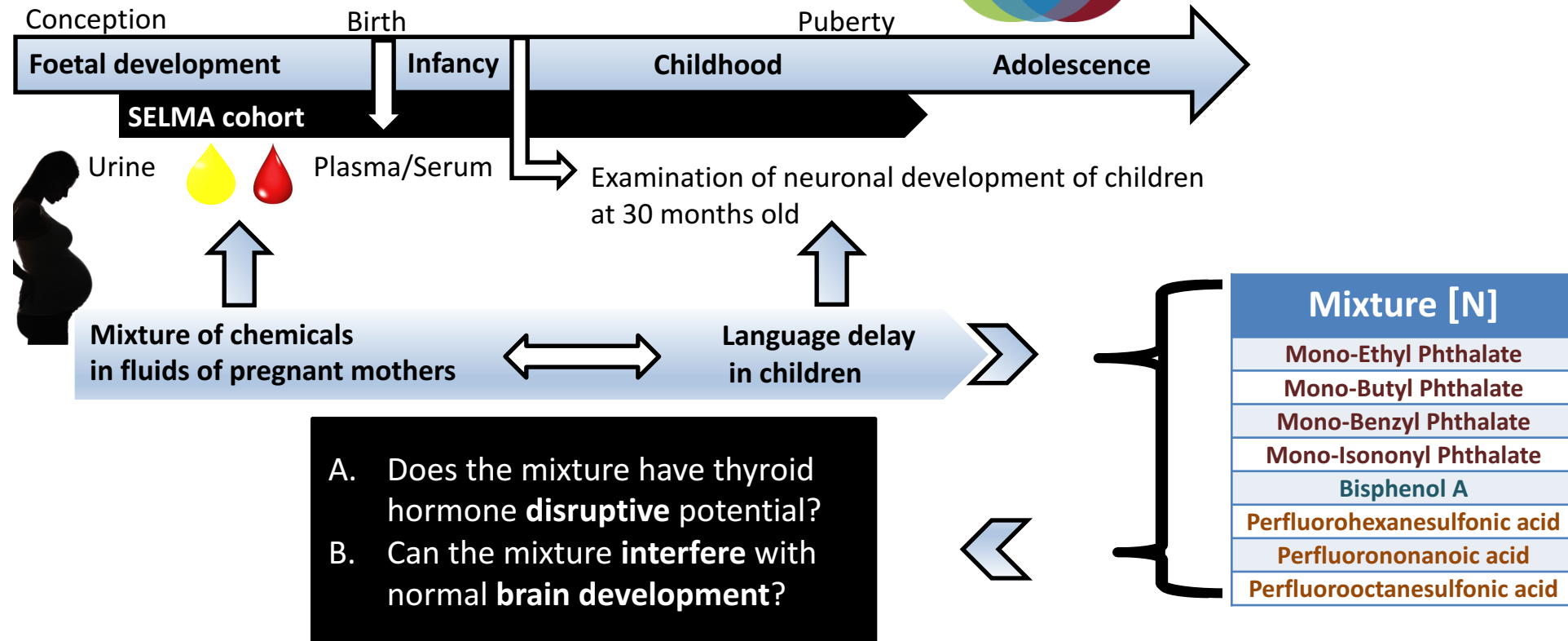


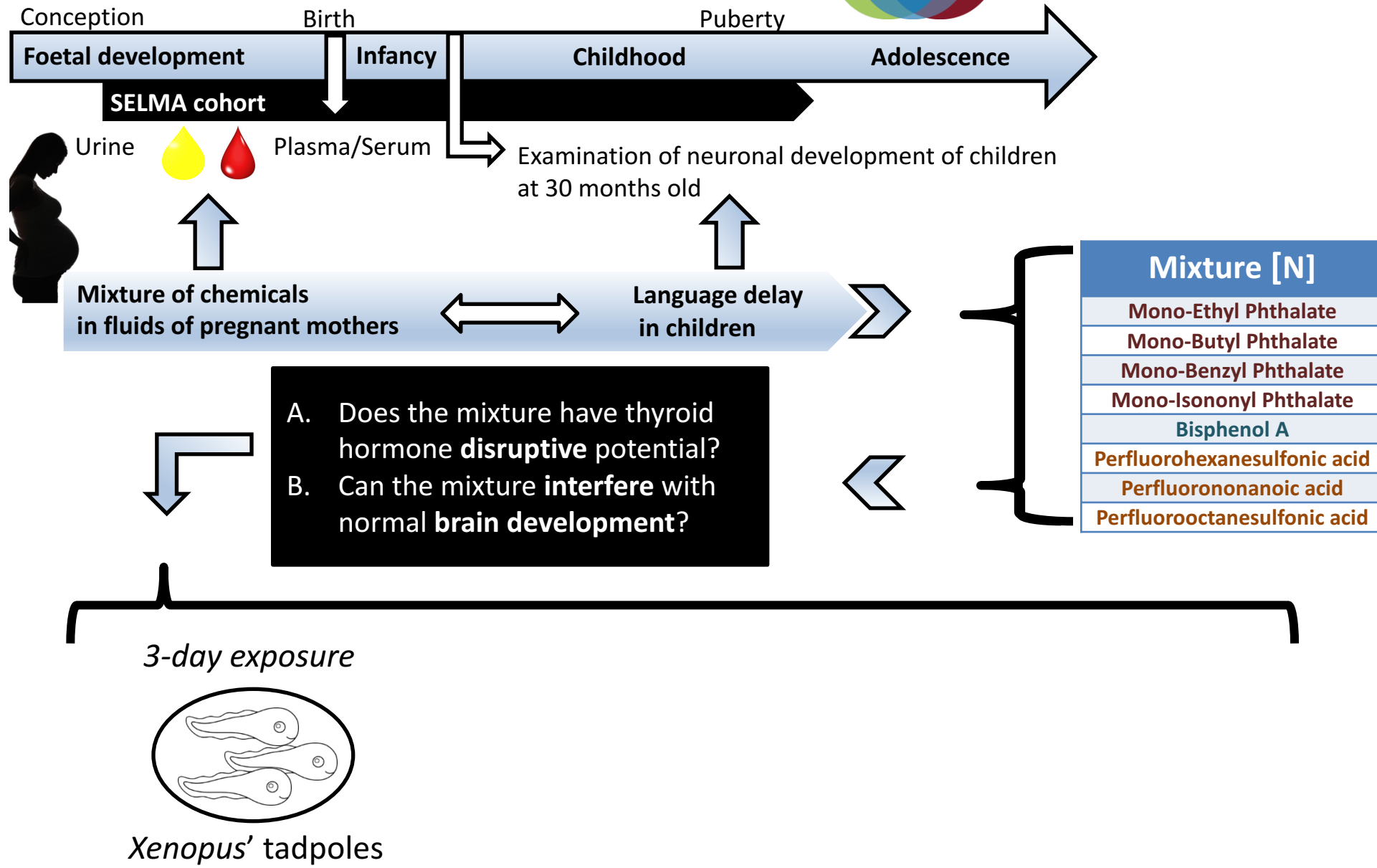


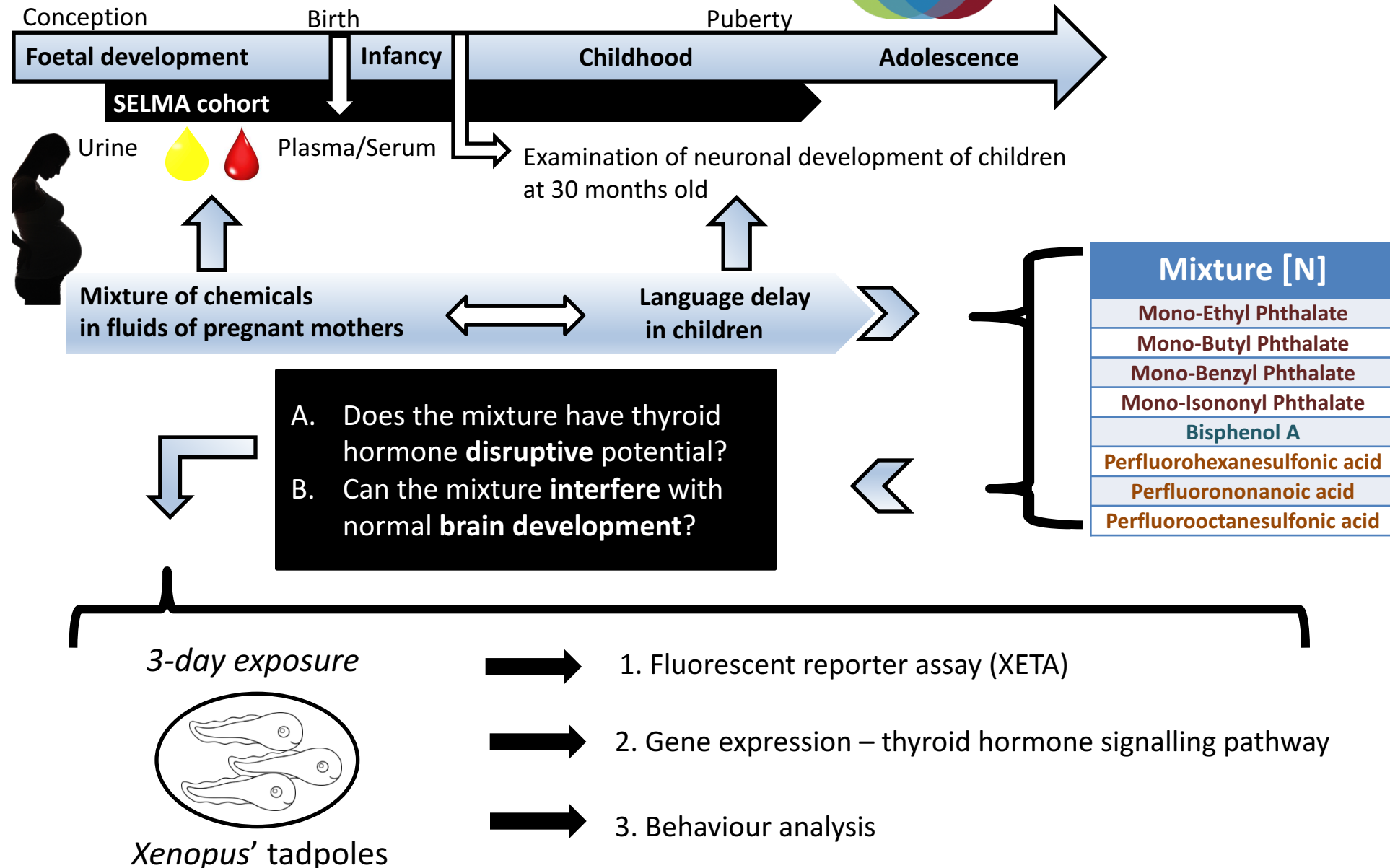
EDC-MixRisk
safe chemicals for future generations



Mixture [N]
Mono-Ethyl Phthalate
Mono-Butyl Phthalate
Mono-Benzyl Phthalate
Mono-Isononyl Phthalate
Bisphenol A
Perfluorohexanesulfonic acid
Perfluorononanoic acid
Perfluorooctanesulfonic acid



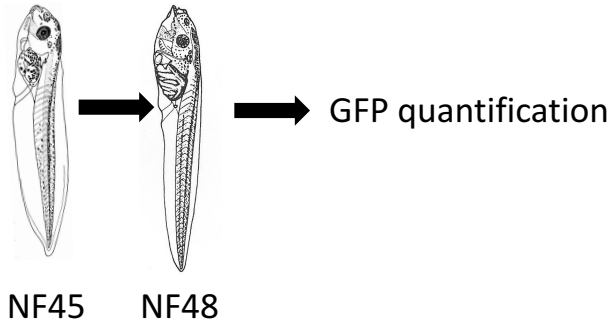




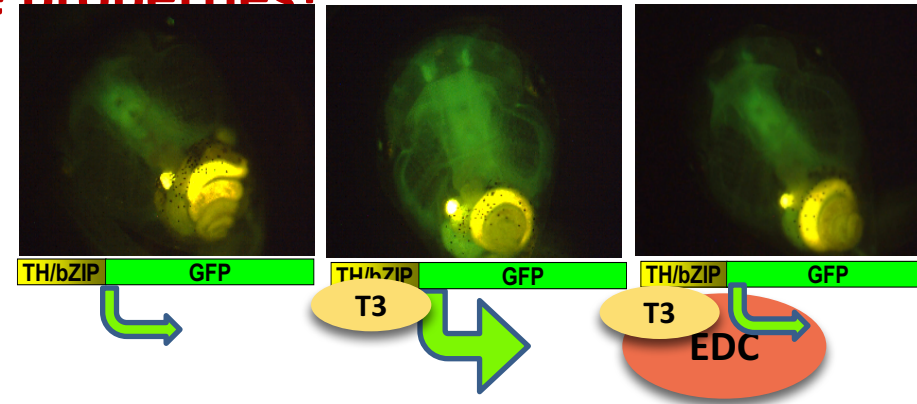
1. Can mixture of chemicals found in fluids of pregnant women have TH-disruptive properties?

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3 d treatment/24h renewal

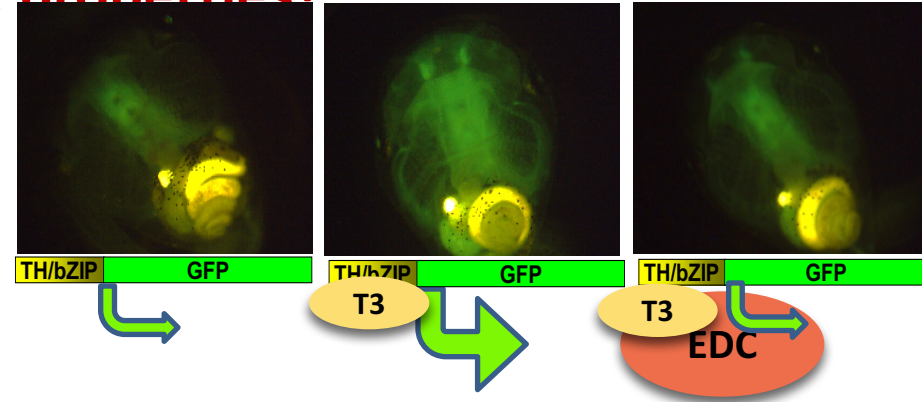
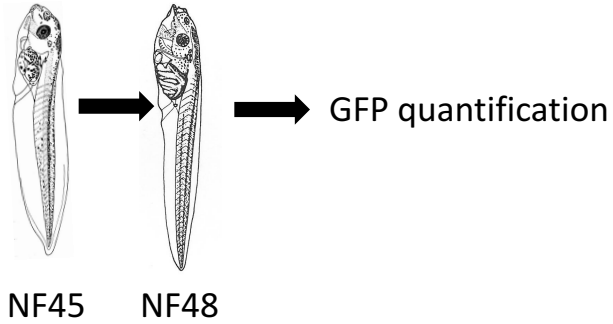


	1x N (mol/L)	10x N (mol/L)	100x N (mol/L)	1000x N (mol/L)
MEP	2.7E-08	2.7E-07	2.7E-06	2.7E-05
MBP	2.26E-08	2.26E-07	2.26E-06	2.26E-05
MBzP	1.05E-08	1.05E-07	1.05E-06	1.05E-05
MINP	2.06E-08	2.06E-07	2.06E-06	2.06E-05
BPA	4.0E-09	4.0E-08	4.0E-07	4.0E-06
PFHxS	3.20E-09	3.20E-08	3.20E-07	3.20E-06
PFNA	1.10E-09	1.10E-08	1.10E-07	1.10E-06
PFOS	1.03E-08	1.03E-07	1.03E-06	1.03E-05

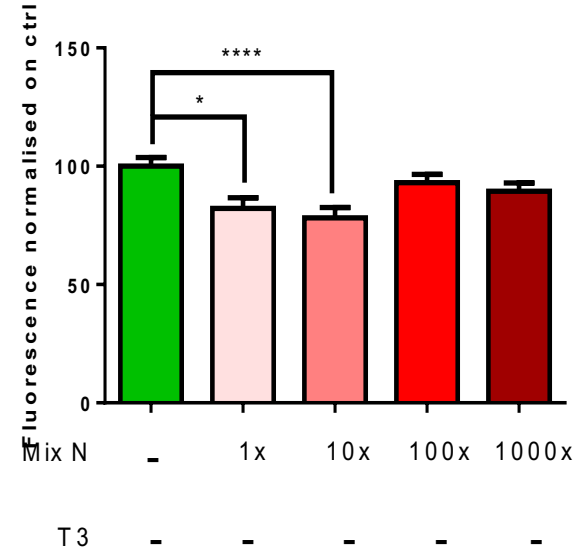


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3 d treatment/24h renewal



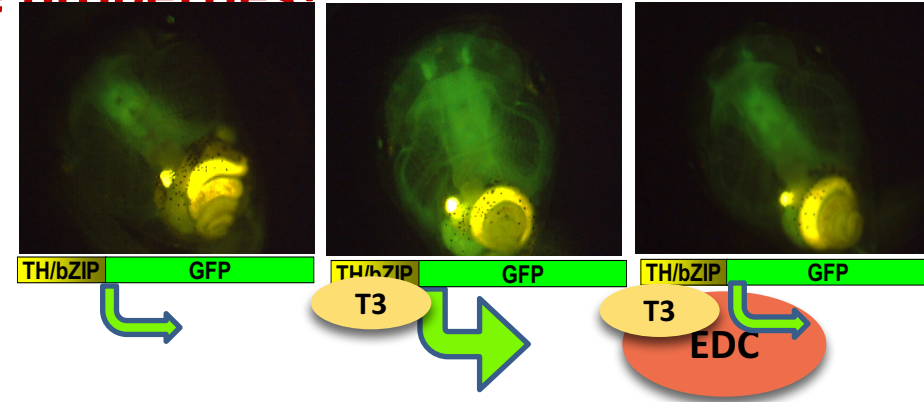
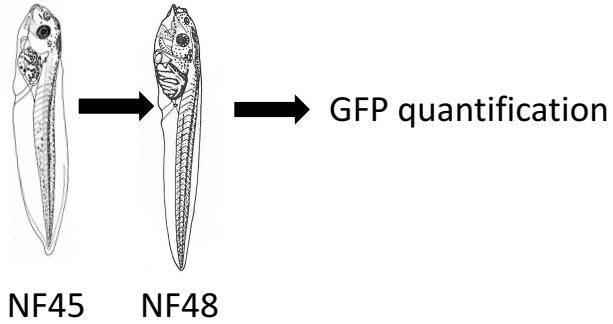
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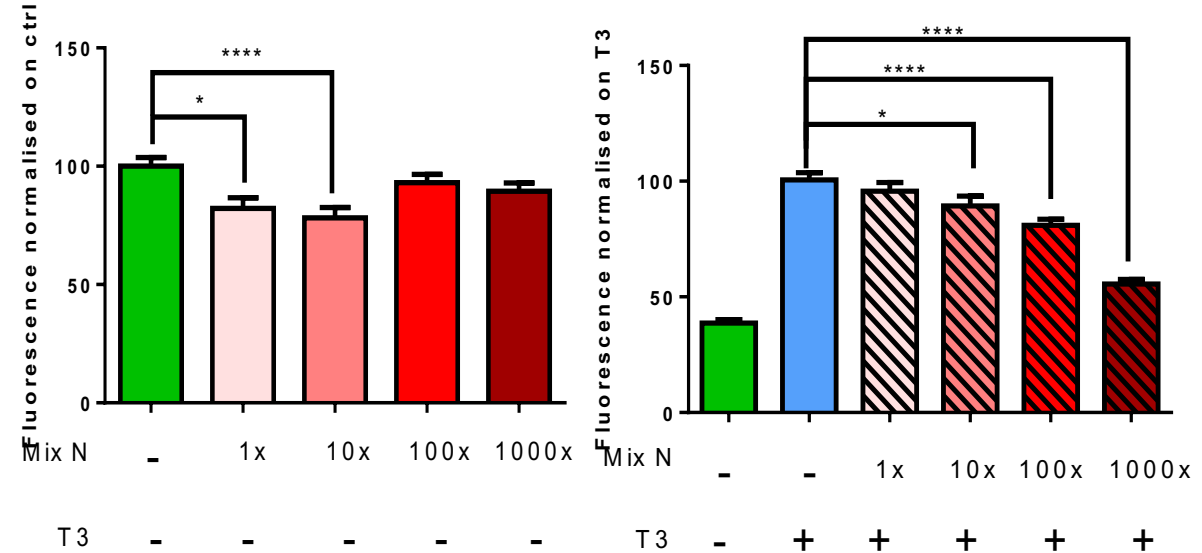
- Parametric one-way ANOVA
- Pool 4 independent experiments with each n=15

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3 d treatment/24h renewal



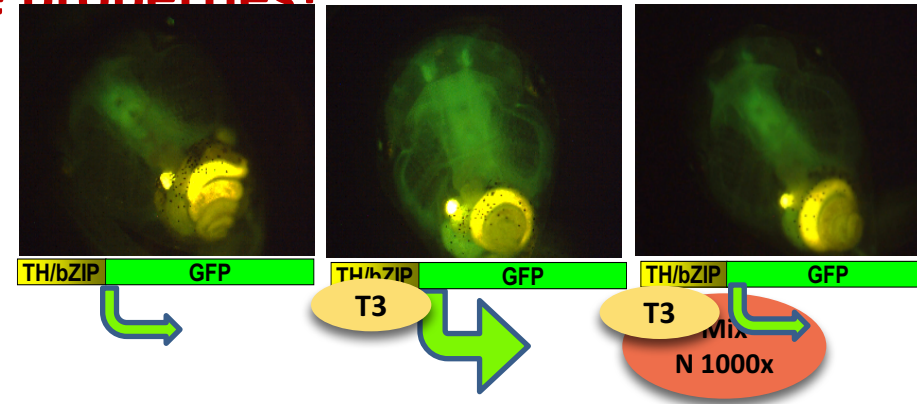
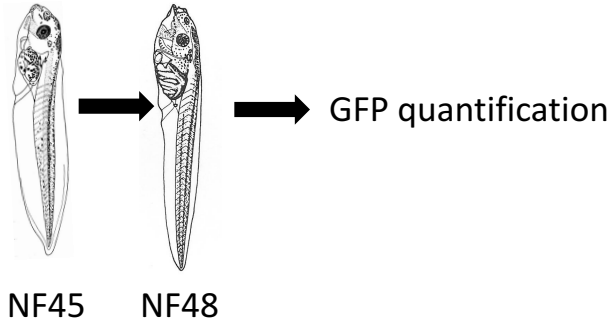
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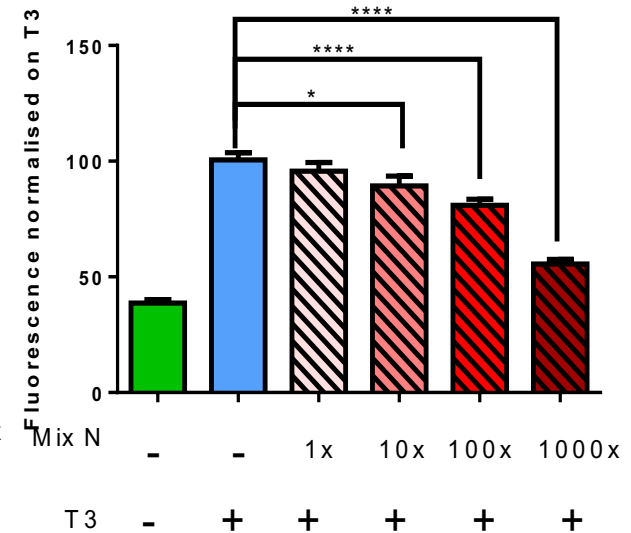
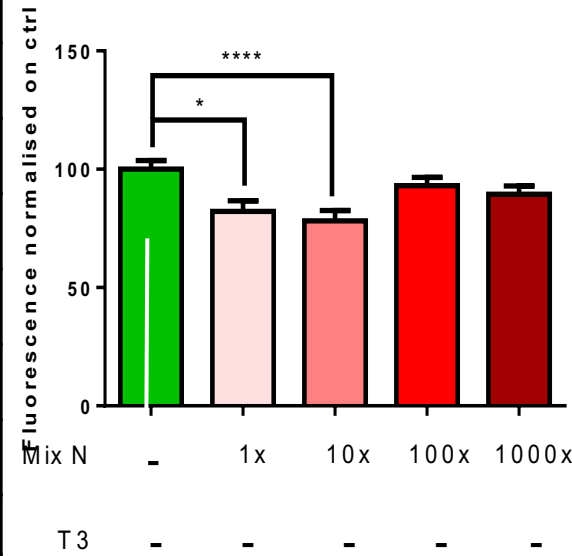
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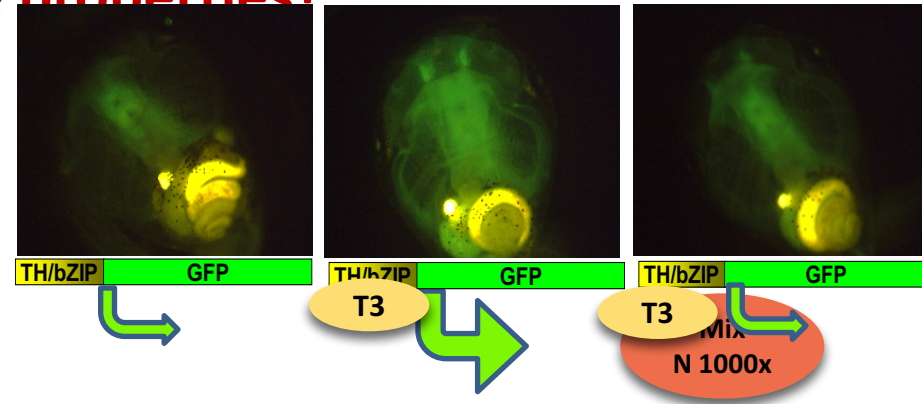
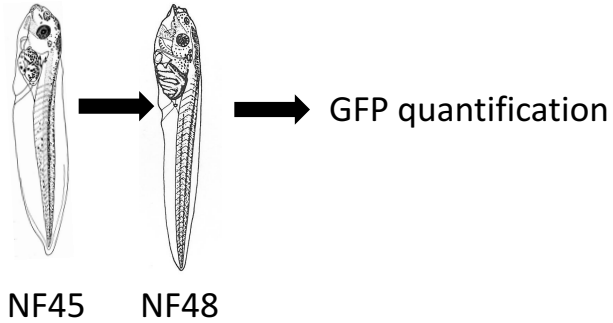


- Parametric one-way ANOVA
- Pool 4 independent experiments with each n=15

➔ **Mix N alters thyroid hormone availability at concentrations found in pregnant women only after 72 hours of exposure in *Xenopus***

1. Can mixture of chemicals found in fluids of pregnant women have TH-disruptive properties?

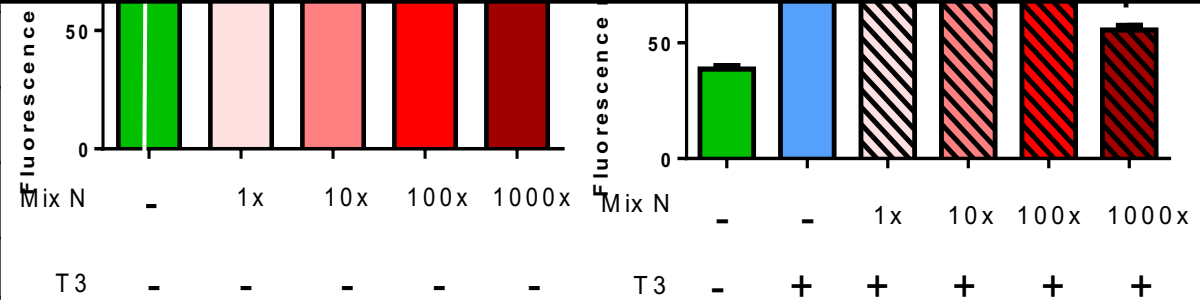
3 d treatment/24h renewal



	1x N (mol/L)	10x N (mol/L)	100x N (mol/L)	1000x N (mol/L)
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What are the consequences on TH related transcripts in brain with this mixture?

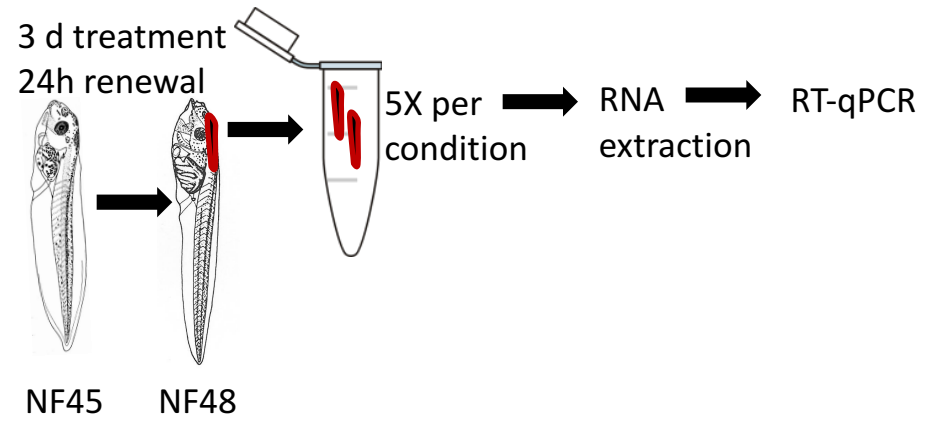
MINP	2.06E-08	2.06E-07	2.06E-06	2.06E-05
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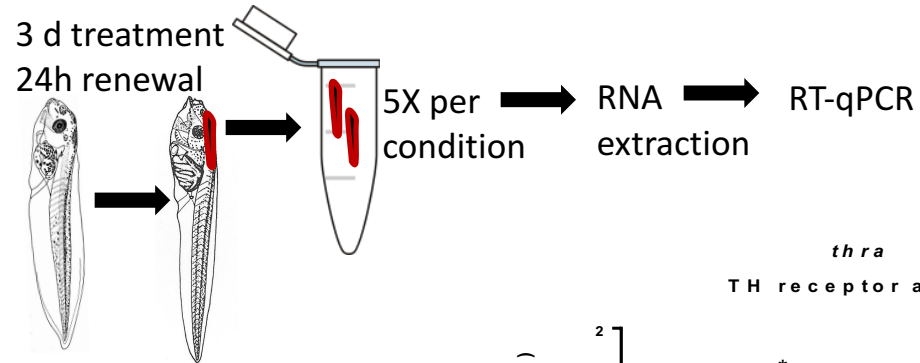
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➔ Mix N alters thyroid hormone availability at concentrations found in pregnant women only after 72 hours of exposure in *Xenopus*

2. Does the mixture affect brain gene expression in tadpoles?

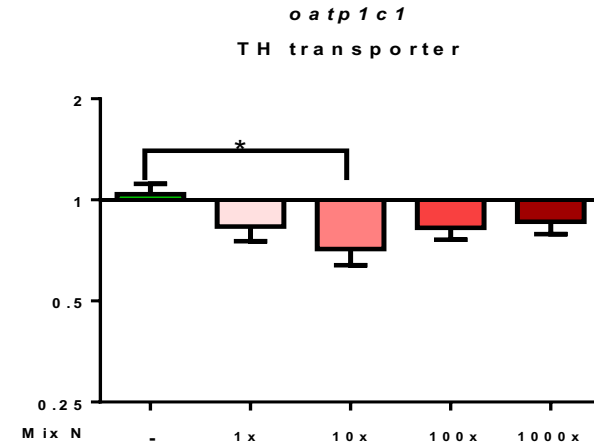
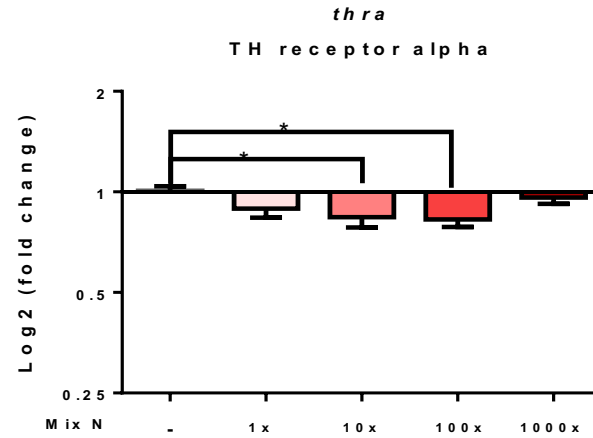


2. Does the mixture affect brain gene expression in tadpoles?

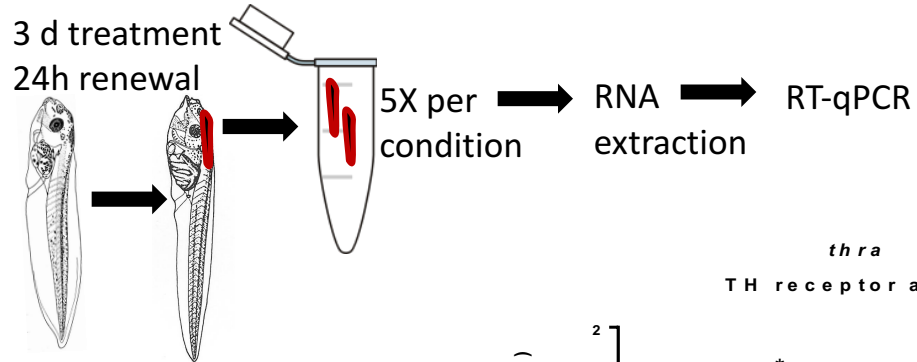


NF45 NF48

- Parametric one-way ANOVA
- 3 independent experiments with each exp n=5

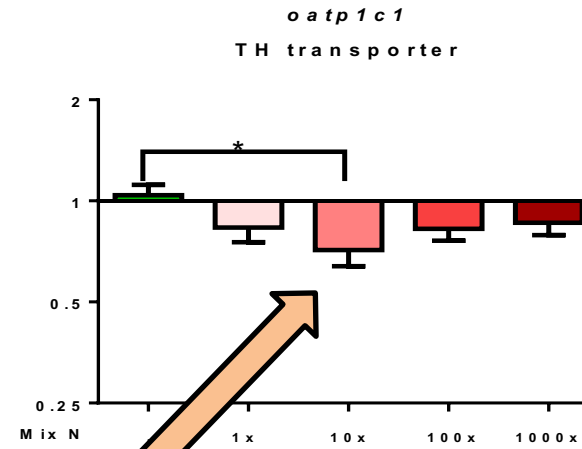
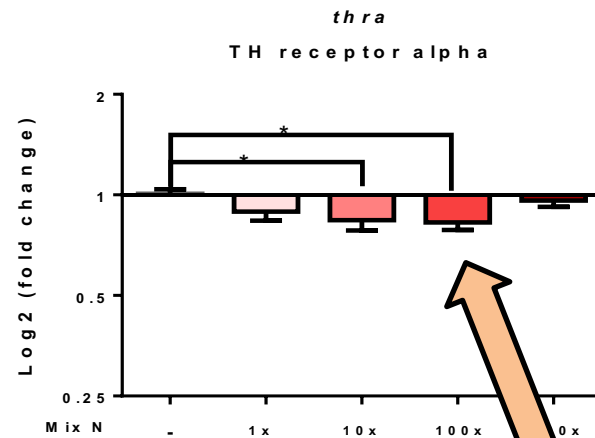


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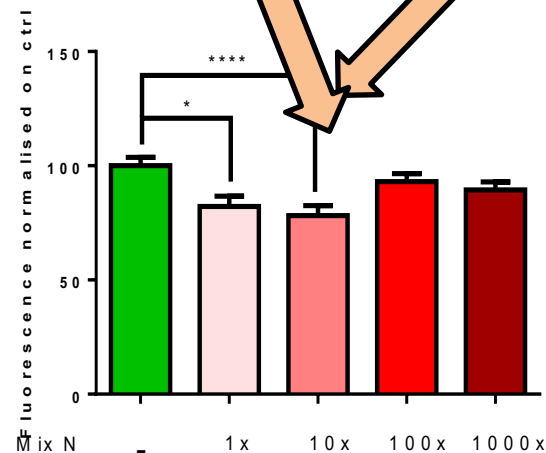
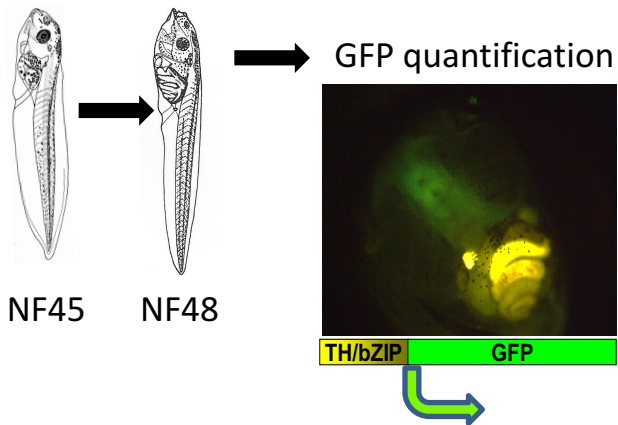


NF45 NF48

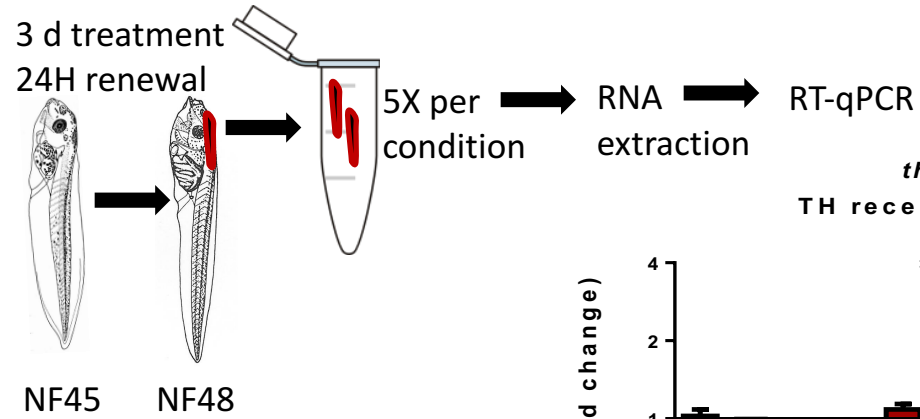
- Parametric one-way ANOVA
- 3 independent experiments with each exp n=5



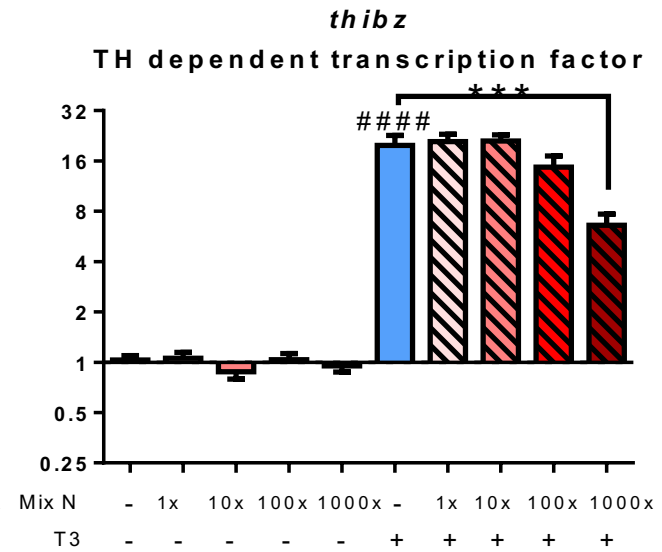
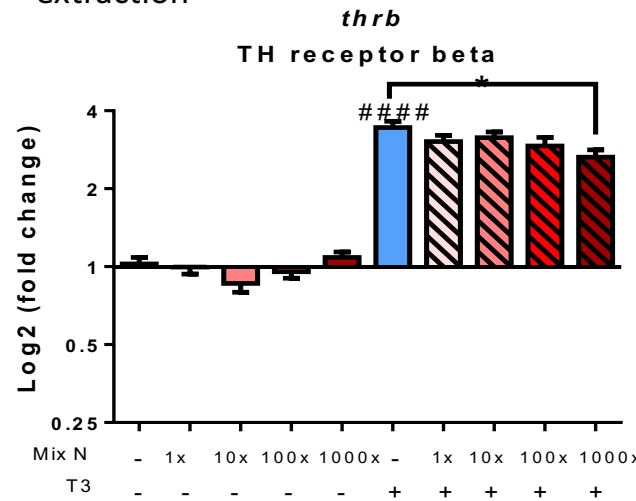
3 d treatment/24h renewal



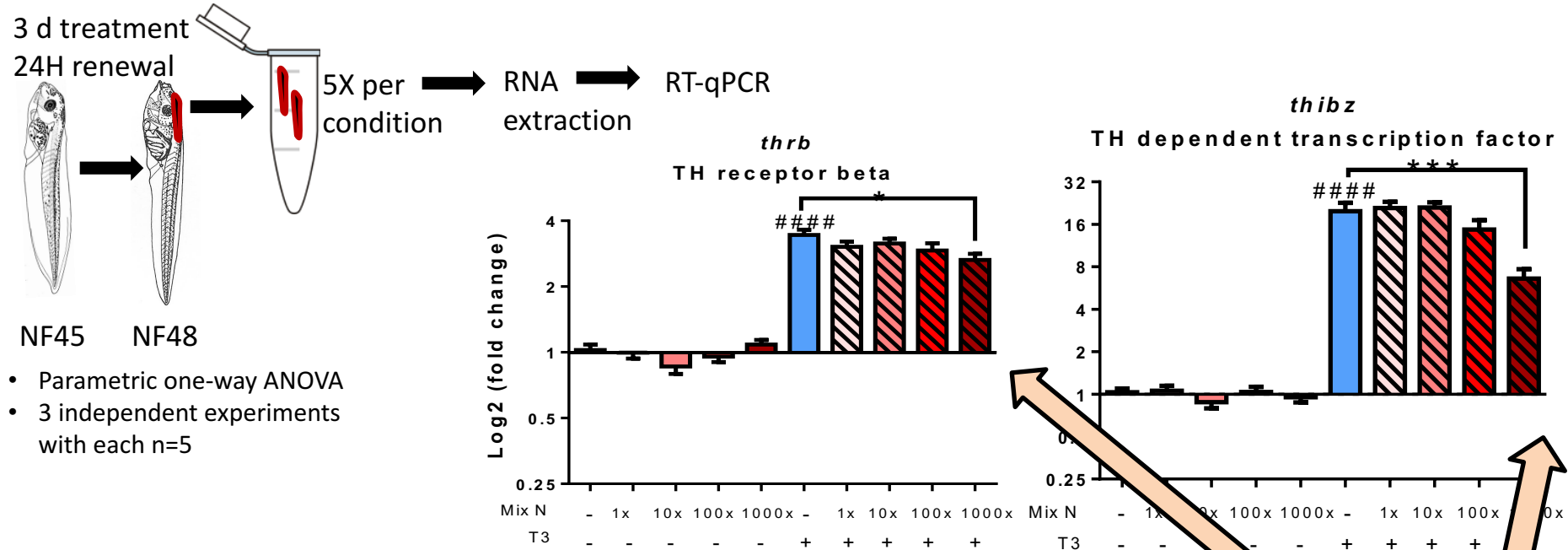
2. Does the mixture affect brain gene expression in tadpoles?



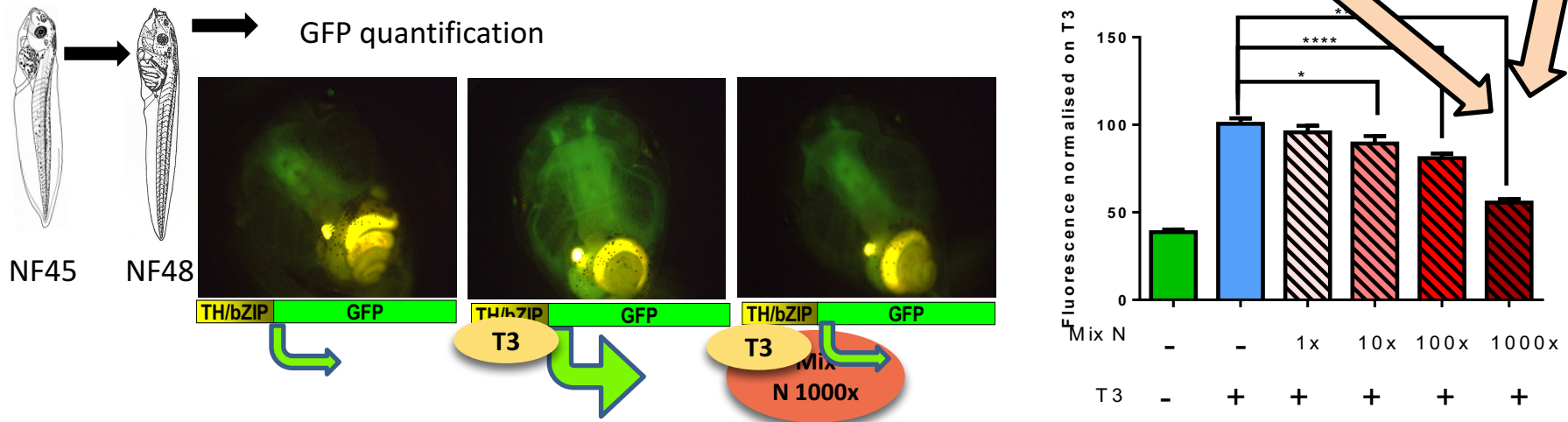
- Parametric one-way ANOVA
- 3 independent experiments with each n=5



2. Does the mixture affect brain gene expression in tadpoles?

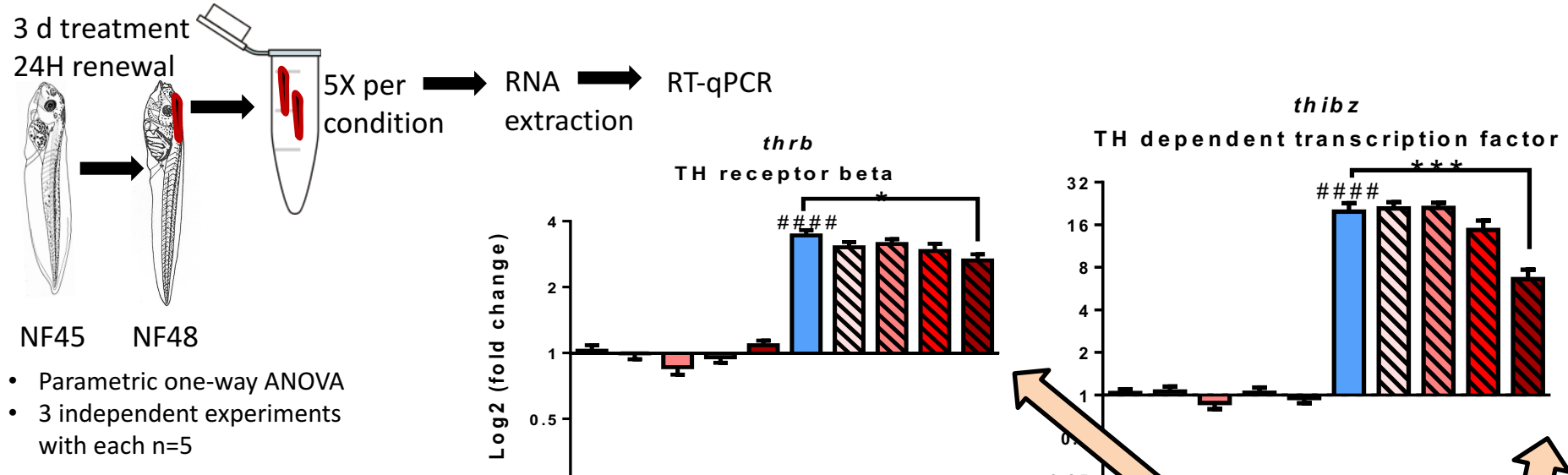


3 d treatment/24h renewal



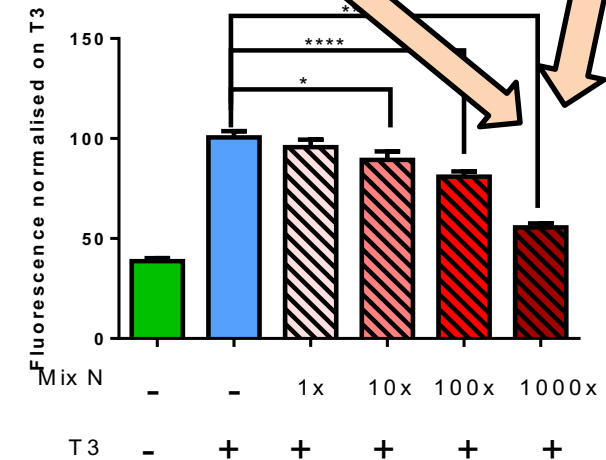
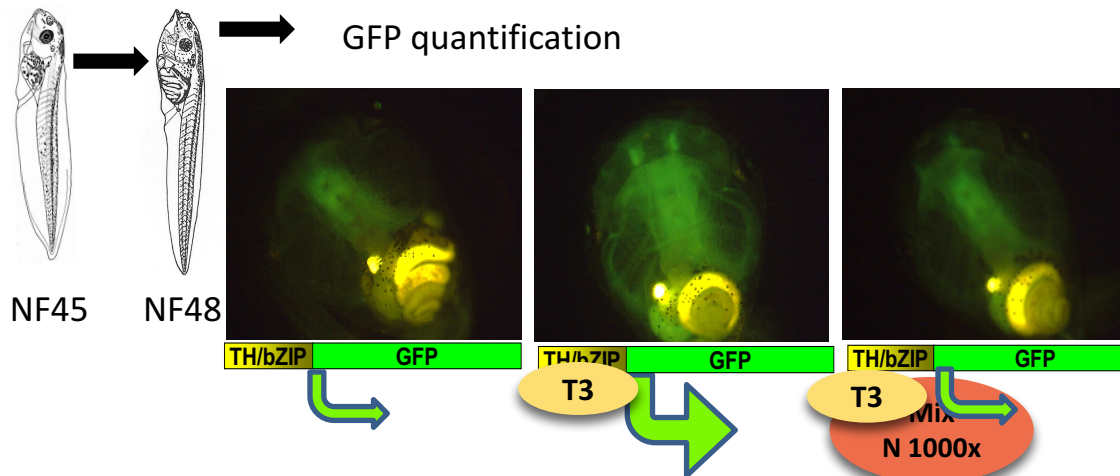
➔ Key genes of the TH signalling pathway are dysregulated in brains of exposed tadpoles to mixture N

2. Does the mixture affect brain gene expression in tadpoles?



Does this TH-disruptive mixture alter behaviour?

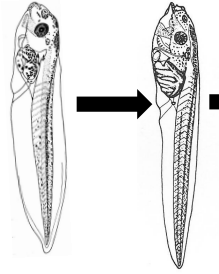
3 d treatment/24h renewal



➔ Key genes of the TH signalling pathway are dysregulated in brains of exposed tadpoles to mixture N

3. What are the consequences on behaviour of embryonic exposure with the identified mixture ?

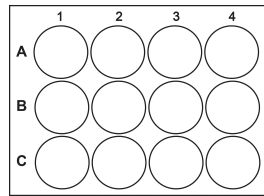
3 d treatment
24H renewal



NF45 NF48

Mobility
tracking

1tadpole/well



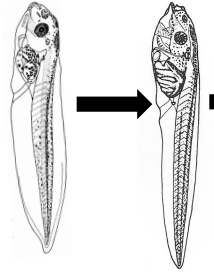
Video tracking for 10 min
(30 sec light on/ 30 sec light off)

- Non-parametric one-way ANOVA
- 3 independent experiments with each n=12



3. What are the consequences on behaviour of embryonic exposure with the identified mixture ?

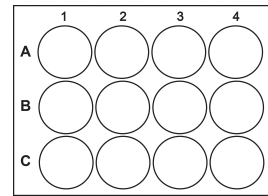
3 d treatment
24H renewal



NF45 NF48

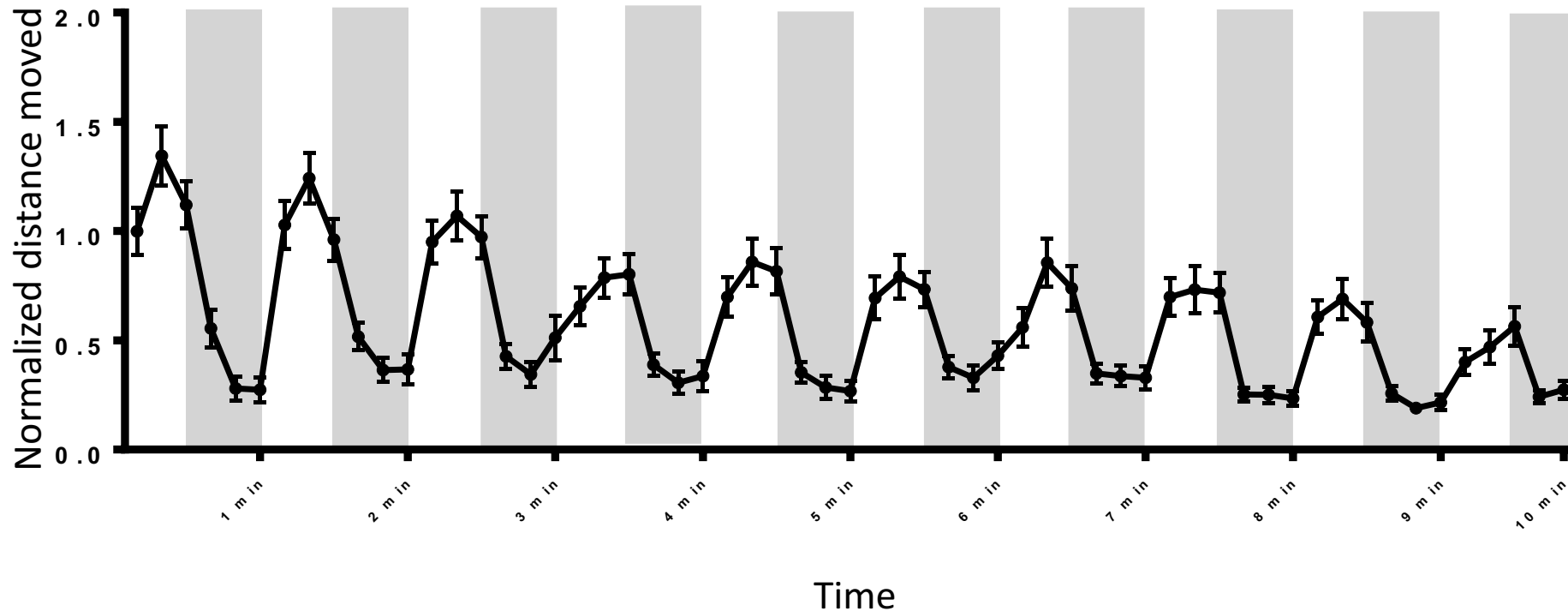
Mobility tracking

1 tadpole/well



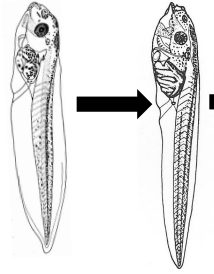
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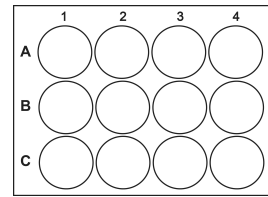
3 d treatment
24H renewal



NF45 NF48

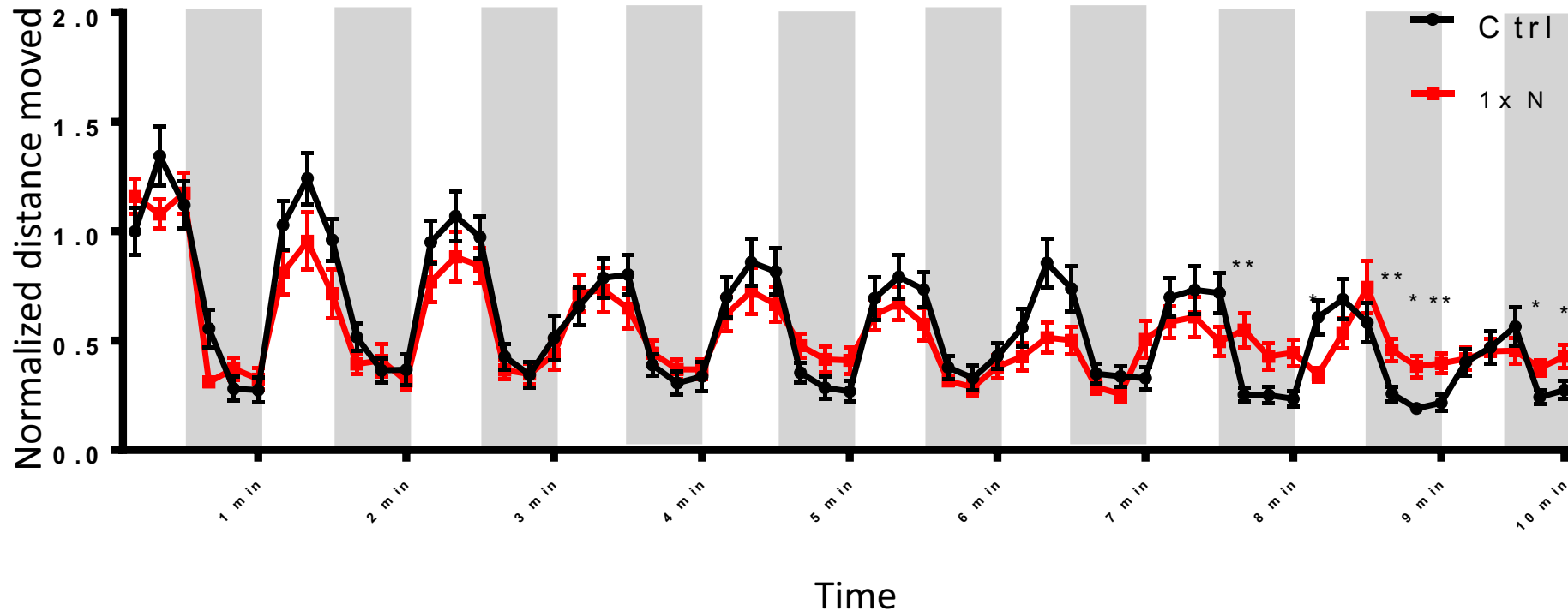
Mobility tracking

1 tadpole/well



Video tracking for 10 min
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3 d treatment
24H renewal



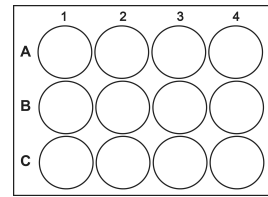
NF45



NF48

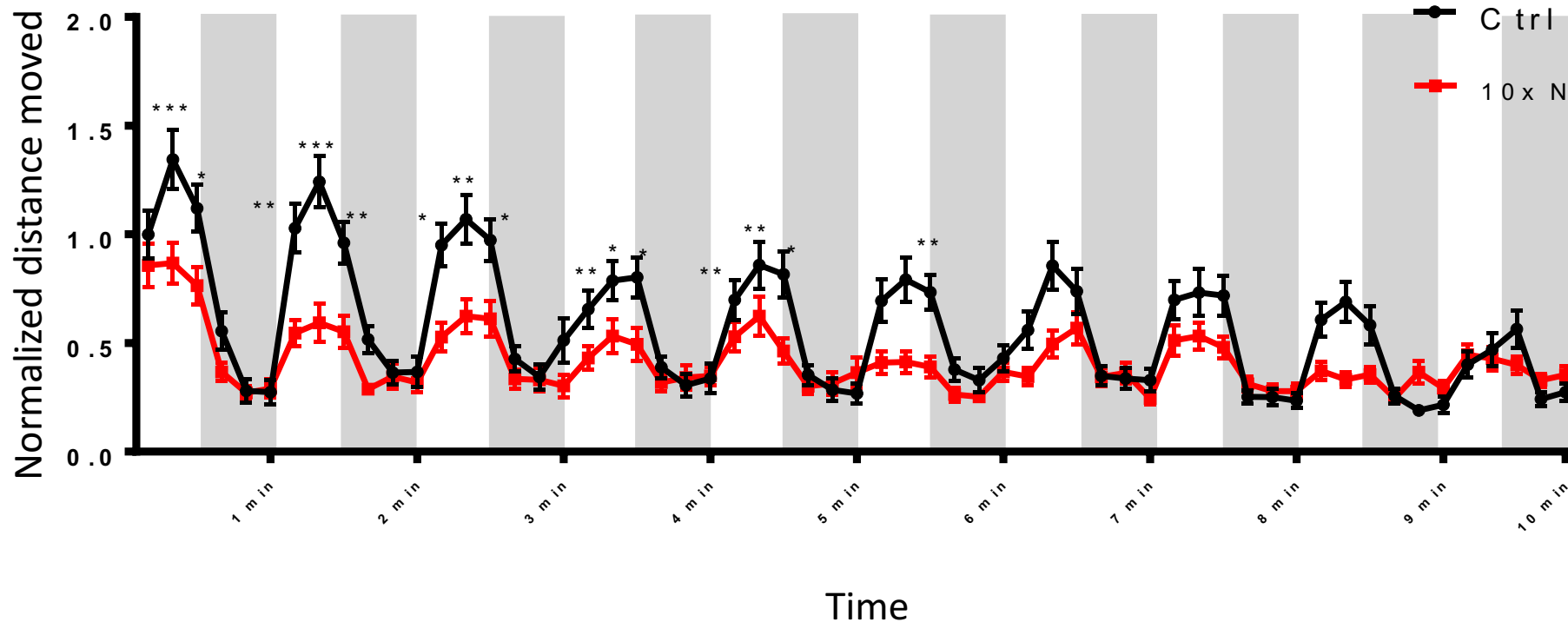
Mobility tracking

1 tadpole/well



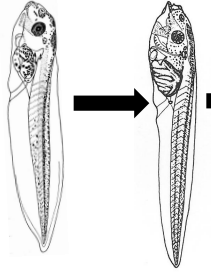
Video tracking for 10 min
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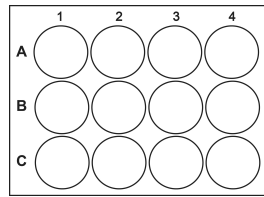
3 d treatment
24H renewal



NF45 NF48

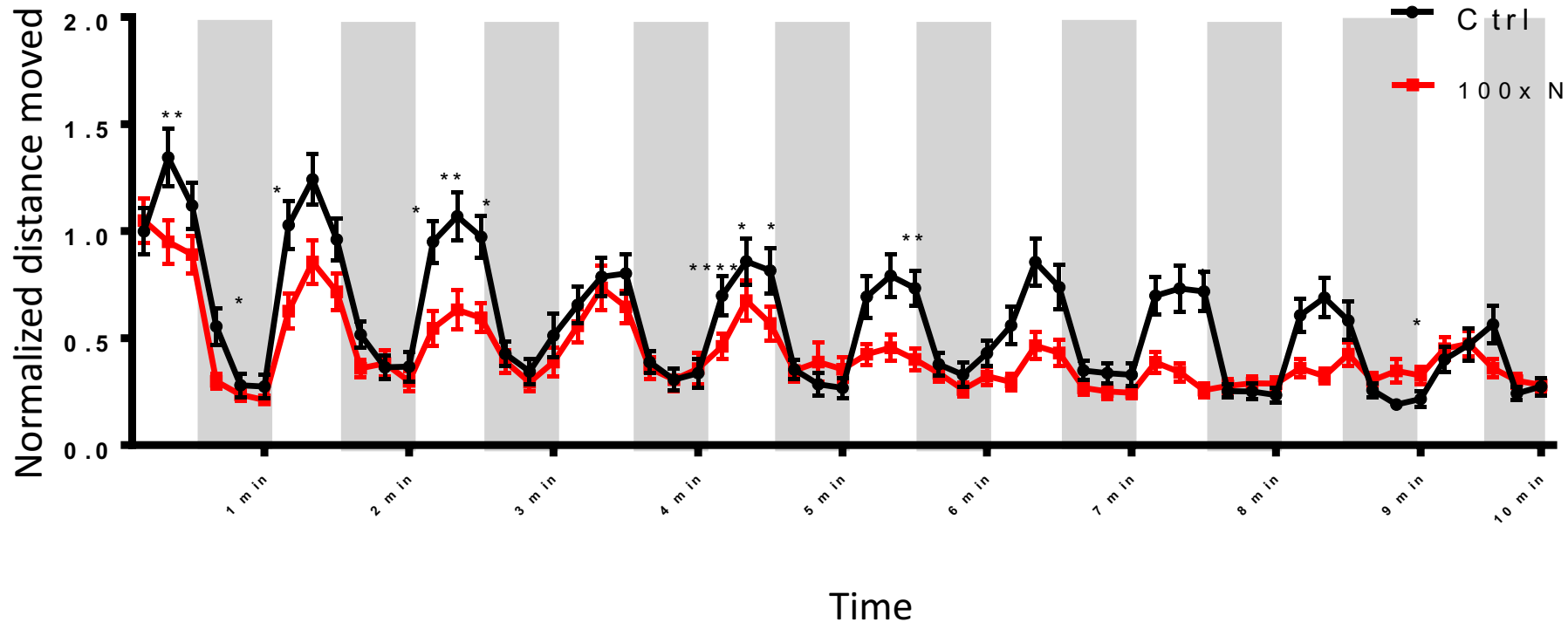
Mobility tracking

1 tadpole/well



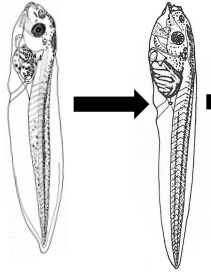
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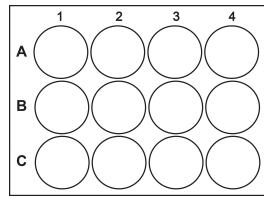
3 d treatment
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NF45 NF48

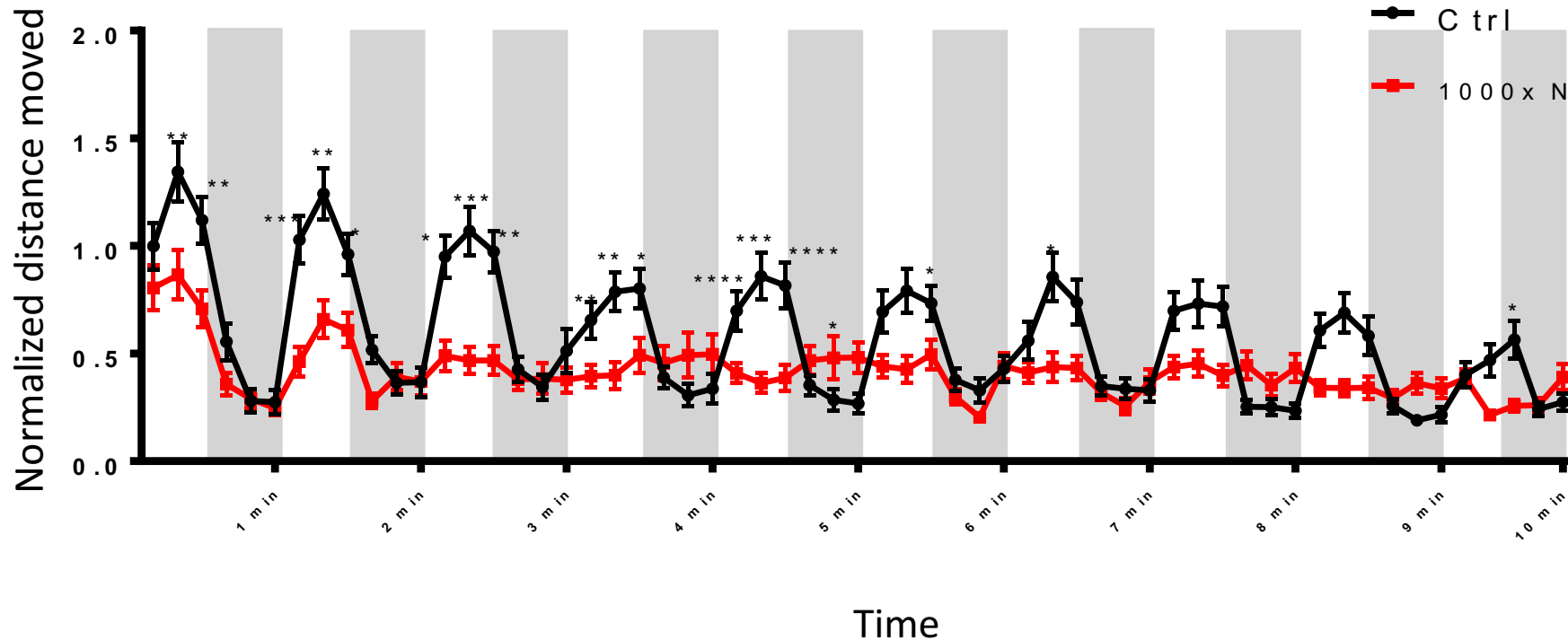
Mobility tracking

1 tadpole/well



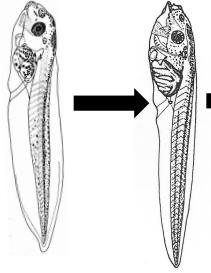
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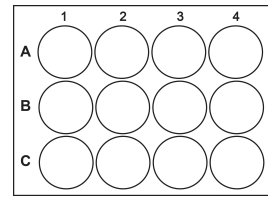
3 d treatment
24H renewal



NF45 NF48

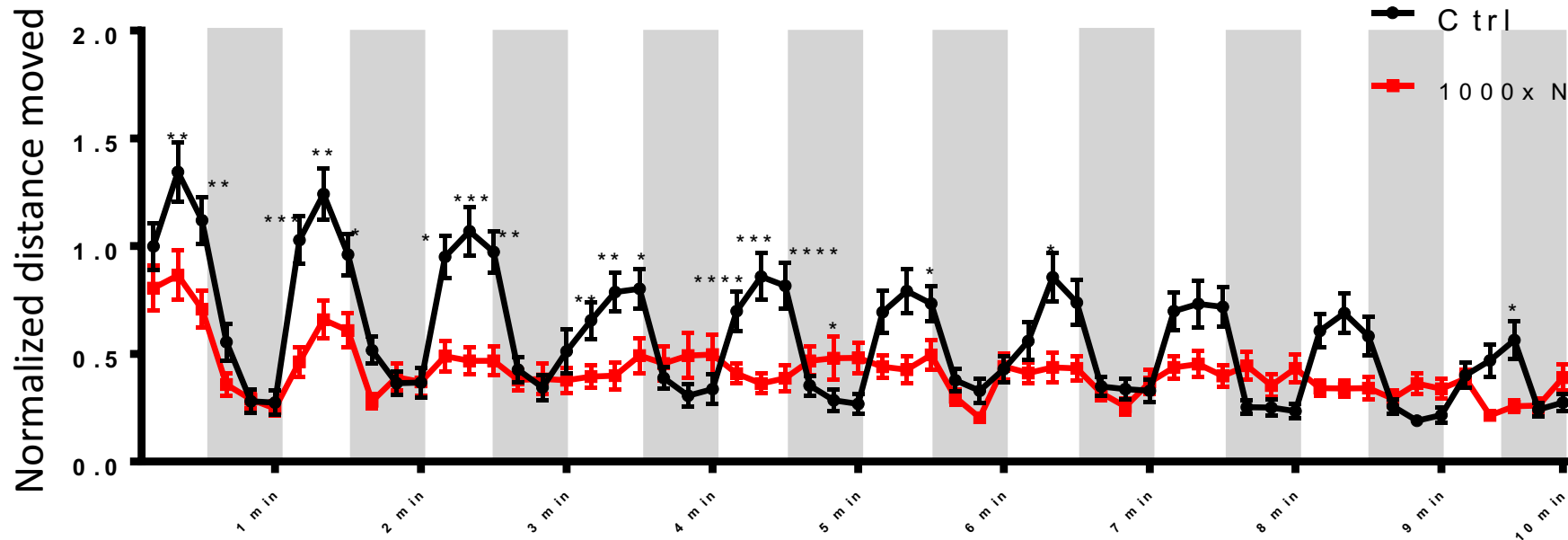
Mobility tracking

1 tadpole/well



Video tracking for 10 min
(30 sec light on/ 30 sec light off)

- Non-parametric one-way ANOVA
- 3 independent experiments with each n=12



➔ 10-,100-,1000-times more concentrated mixture N reduced mobility and reduced response to light stimuli after 8 minutes

Conclusions 2: on Neuronal related mixture

The mixture associated with neurodevelopmental delay in children:

1. Disrupts TH signalling
2. Affects brain gene expression
3. has potential to impede behaviour
= possible indication of aberrant brain development



M. Leemans



New Results

From Cohorts to Molecules: Adverse Impacts of Endocrine Disrupting Mixtures

Lina Birgersson, Gabor Borbely, Nicolo Caporale, Pierre-Luc Germain, Michelle Leemans, Filip Rendel, Giuseppe D'Agostino, Raul Bardini Bressan, Francesca Cavallo, Nadav Even Chorev, Vesna Munic Kos, Maddalena Lazzarin, Steven Pollard, Birgitta Sundstrom, Alejandro Lopez Tobon, Sebastiano Trattaro, Matteo Zanella, Ake Bergman, Pauliina Damdimopoulou, Maria Jonsson, Wieland Kiess, Efthymia Kittraki, Hannu Kiviranta, Mattias Oberg, Panu Rantakkoko, Christina Ruden, Olle Soder, Carl-Gustaf Bornehag, Barbara Demeneix, Jean-Baptiste Fini, Chris Gennings, Eewa Nanberg, Joelle Ruegg, Joachim Sturve, Giuseppe Testa

doi: <https://doi.org/10.1101/206664>

This article is a preprint and has not been peer-reviewed [what does this mean?].

In revision at Nature

Conclusions & thoughts

- Fine tuning of Thyroid hormone signaling is crucial for proper brain development
- Over two thirds of the chemicals tested disrupt thyroid hormone signalling
- These chemicals pass the placenta and are found in fetal compartment

Our chemical environment could interfere with thyroid hormone

- 1) A chimeric mixture made from « exposure data » showed impaired TH regulation, brain development and behavior
- 2) Mixture identified from language delay endpoint in human was validated in experimental models especially in disrupting TH signaling in vivo

Chemical may have direct or indirect effects and act in a mixture way which make the legislation difficult

Early chemical exposure disrupt harmonized development and could make vulnerable to disease development



MUSÉUM
NATIONAL D'HISTOIRE NATURELLE

Acknowledgements

Pr Barbara DEMENEIX

Sébastien LE MEVEL

Michelle LEEMANS

Melodie LETTMANN

Dr Bilal MUGHAL

Dr Petra ŠPIRHANZLOVÁ

Stephan COUDERQ



EDC-MixRisk
safe chemicals for future generations

CG BORNEHAG
Chris GENNINGS
Christian LINDH
Ake BERGMAN
Joelle RUEGG



Jean-Stéph JOLY
Pierre AFFATICATI
Laurie RIVIERE
Elodie De JOB



science and policy
for a healthy future

MIXTURE has T3 like effect and increases cell proliferation in hindbrain

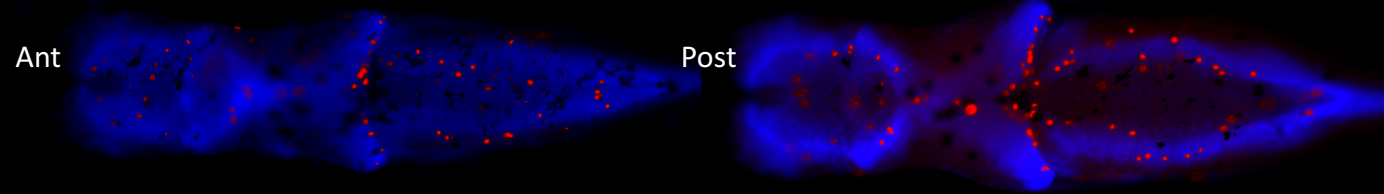
Immunos IN TOTO

X. Laevis brain
NF48 (3 d treated)

Dorsal view
Whole brain
Anti PH3 (red dots)
DAPI (blue)

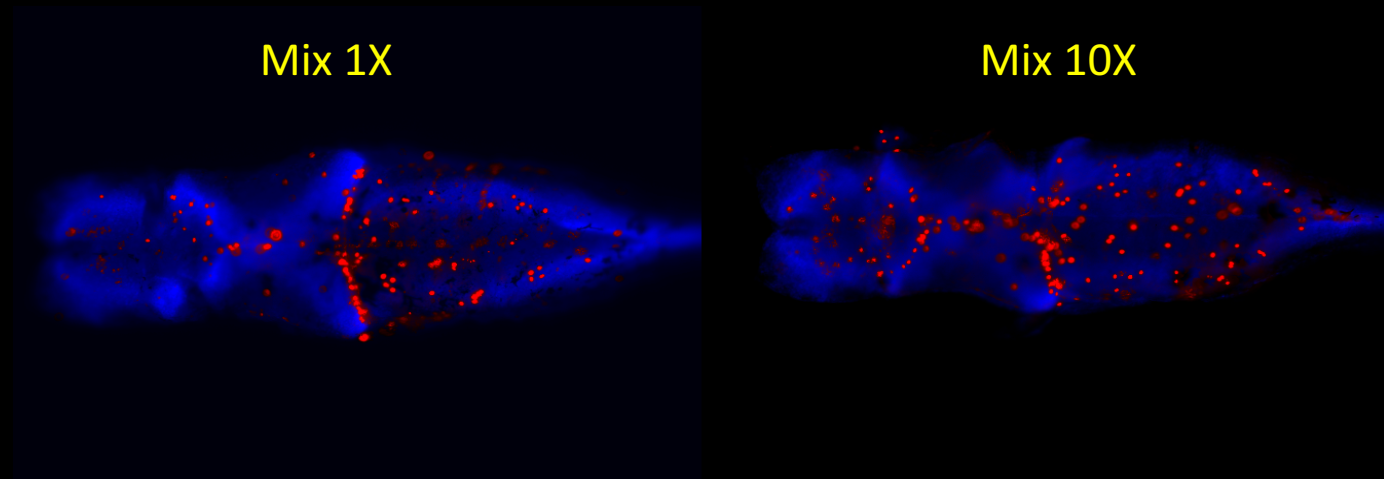
CT

Mix 0,1X



Mix 1X

Mix 10X



T3

T3 + Mix 10X

