

Centre National de Recherche Scientifique,  
Natural History Museum  
Paris

Which testing methods are needed to  
identify pesticides with Endocrine  
Disrupting Properties:  
of fish, frogs and men



Centre National de Recherche Scientifique,  
Natural History Museum  
Paris

Professor

Head, Evolution of Endocrine Regulations

Which testing methods are needed to  
identify pesticides with Endocrine  
Disrupting Properties:  
of fish, frogs and men



# None of the current test methods can address

- Effects on foetuses,
- Development of the reproductive axis
- Thyroid signalling determining brain development
- Need rapid, alternative test methods to animal experimentation: reduce, replace, refine (3R).

# 2012/2066(INI) - 14/03/2013 Text adopted by European Parliament, single reading

- **Parliament highlights that hormone-related disorders and illnesses** in humans have increased over the last 20 years, including **impaired sperm quality, early onset of puberty**, increased incidence of deformed sexual organs, increased incidence of certain forms of cancer, and metabolic diseases. Certain neurological disorders and neurodegenerative diseases, **impacts on neurodevelopmental functions**, the immune system or epigenetics, might be linked to exposure to **chemical substances with endocrine-disrupting properties**.
- **Protecting pregnant women and babies:** Members consider that protecting women from potential risks of endocrine disruptors for their reproductive health is of utmost importance and call on the Commission to support long-term studies monitoring women's health over large spans of their lives.

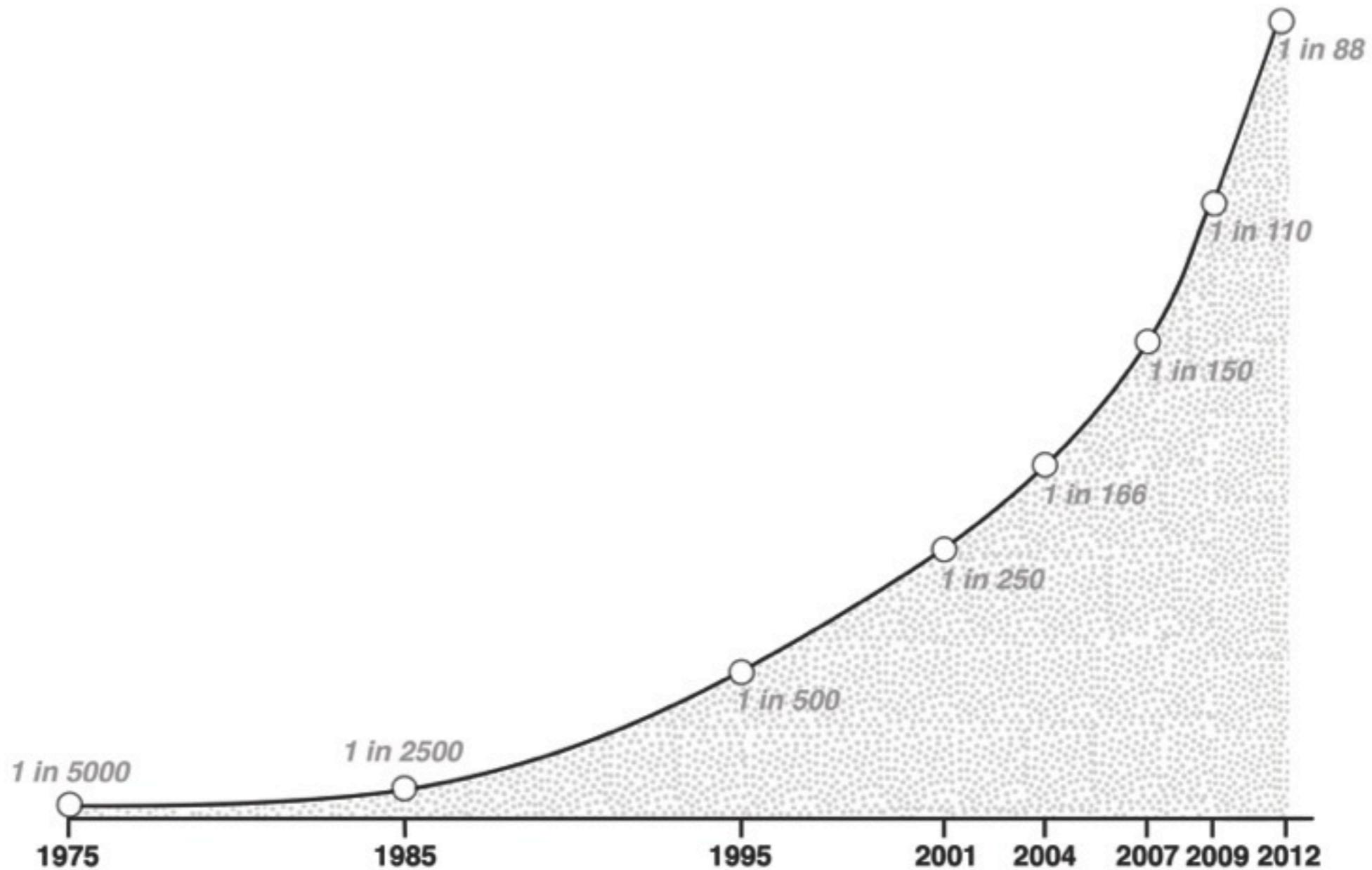
# 2012/2066(INI) - 14/03/2013 Text adopted by European Parliament, single reading

- **Parliament highlights that hormone-related disorders and illnesses** in humans have increased over the last 20 years, including **impaired sperm quality, early onset of puberty**, increased incidence of deformed sexual organs, increased incidence of certain forms of cancer, and metabolic diseases. Certain neurological disorders and neurodegenerative diseases, **impacts on neurodevelopmental functions**, the immune system or epigenetics, might be linked to exposure to **chemical substances with endocrine-disrupting properties**.
- **Protecting pregnant women and babies:** Members consider that protecting women from potential risks of endocrine disruptors for their reproductive health is of utmost importance and call on the Commission to support long-term studies monitoring women's health over large spans of their lives.

# 2012/2066(INI) - 14/03/2013 Text adopted by European Parliament, single reading

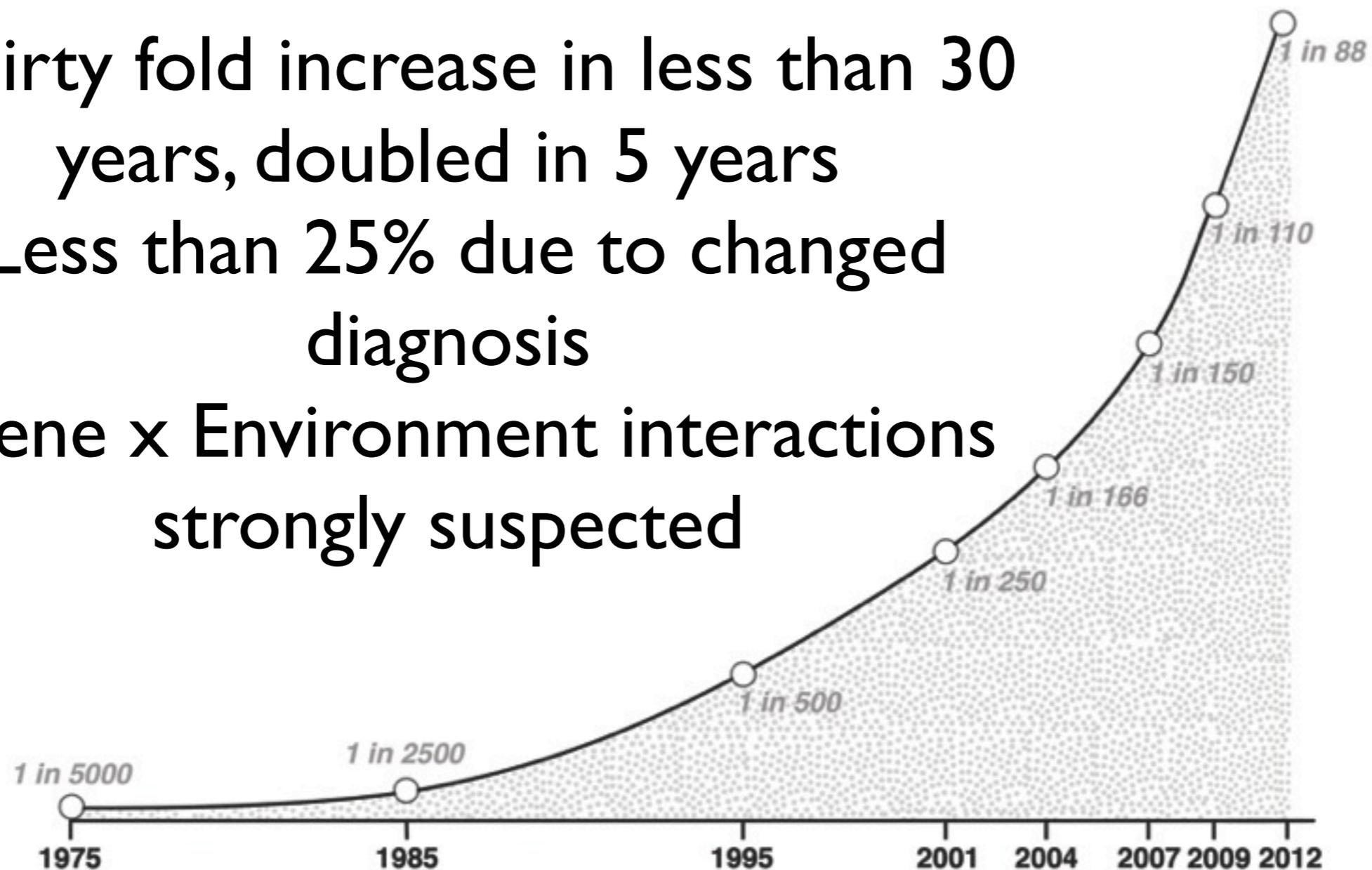
- **Parliament highlights that hormone-related disorders and illnesses** in humans have increased over the last 20 years, including **impaired sperm quality, early onset of puberty,** increased incidence of deformed sexual organs, increased incidence of certain forms of cancer, and metabolic diseases. Certain neurological disorders and neurodegenerative diseases, **impacts on neurodevelopmental functions,** the immune system or epigenetics, might be linked to exposure to **chemical substances with endocrine-disrupting properties.**
- **Protecting pregnant women and babies:** Members consider that protecting women from potential risks of endocrine disruptors for their reproductive health is of utmost importance and call on the Commission to support long-term studies monitoring women's health over large spans of their lives.

# Example: increased incidence of Autism Spectrum Disorders



# Example: increased incidence of Autism Spectrum Disorders

- Thirty fold increase in less than 30 years, doubled in 5 years
- Less than 25% due to changed diagnosis
- Gene x Environment interactions strongly suspected



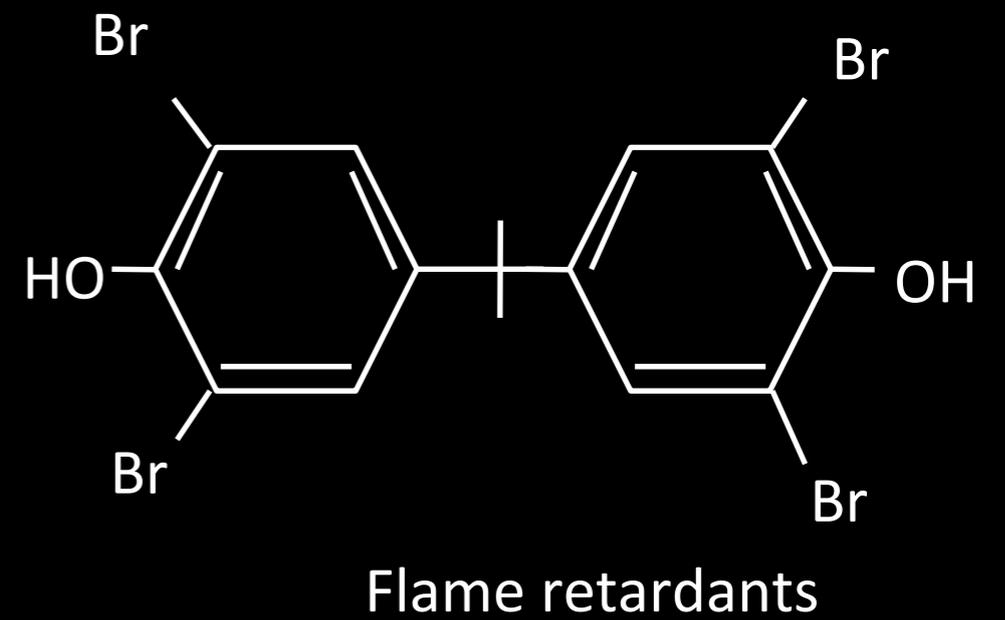
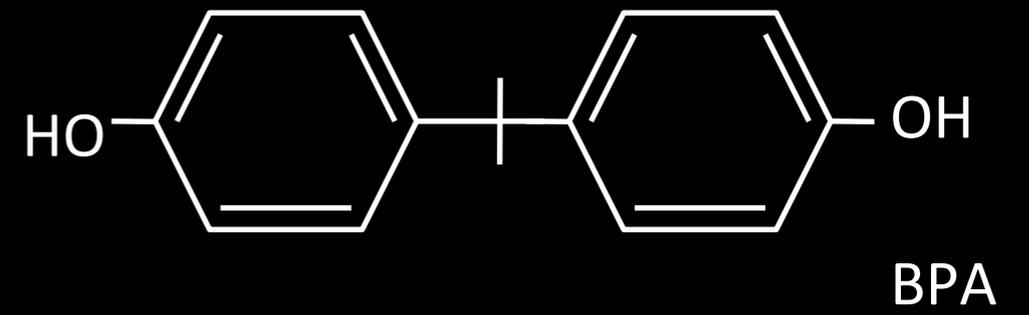
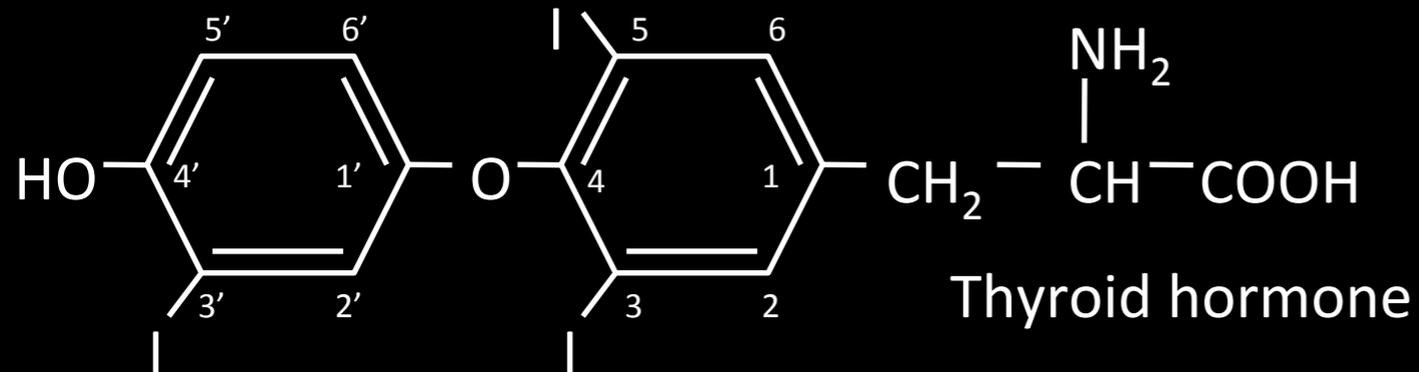
# Association of increased incidence of Autism with: thyroid hormone disruption and brain development

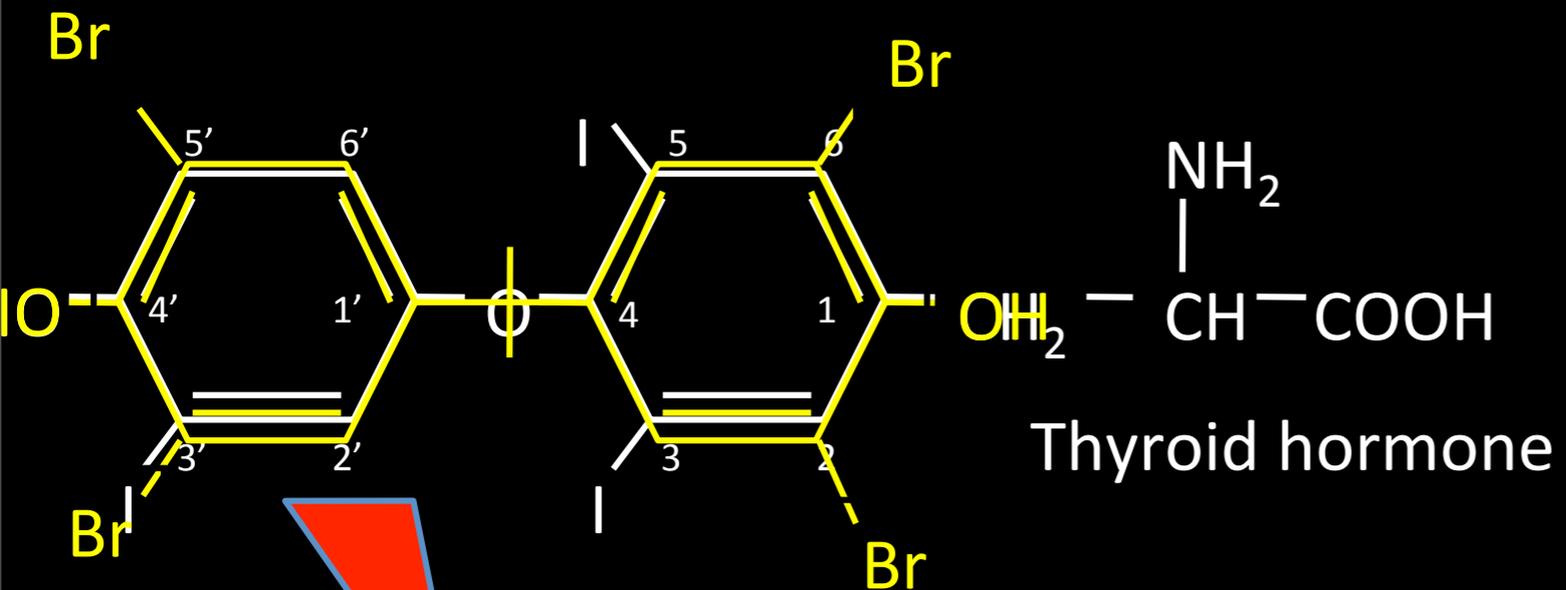
- Thyroid hormone is required for brain development in all vertebrates - humans to frogs and fish .. cretinism is a thing of the past in Europe - **but other neurodevelopmental disorders are increasing**
- A recent EFSA study showed that 103 of 287 pesticides screened were identified as affecting the thyroid or thyroid hormone systems!!
- Cumulative or cocktail effects - pesticides, flame retardants, plasticizers etc..
- Other endocrine systems affected : notably reproductive systems : all signal through nuclear receptors

# Association of increased incidence of Autism with: thyroid hormone disruption and brain development

- Thyroid hormone is required for brain development in all vertebrates - humans to frogs and fish .. cretinism is a thing of the past in Europe - **but other neurodevelopmental disorders are increasing**
- A recent EFSA study showed that 103 of 287 pesticides screened were identified as affecting the thyroid or thyroid hormone systems!!
- Cumulative or cocktail effects - pesticides, flame retardants, plasticizers etc..
- Other endocrine systems affected : notably reproductive systems : all signal through nuclear receptors

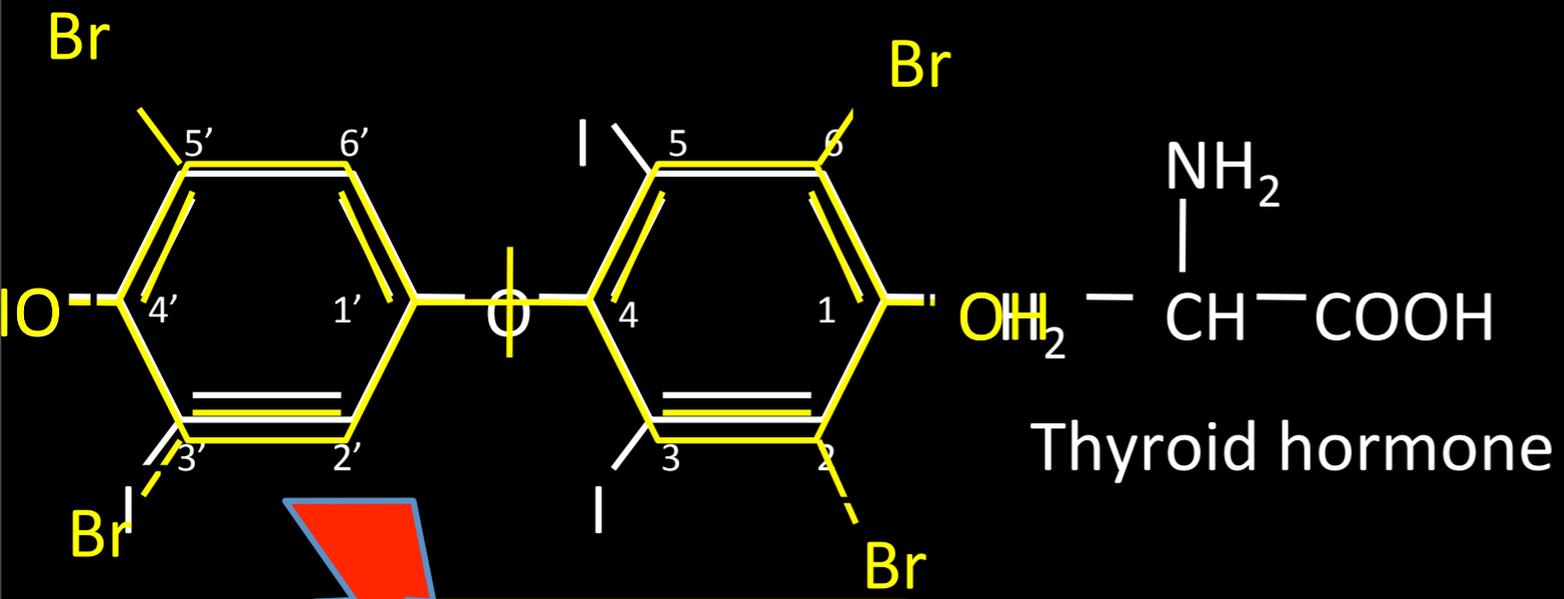
Thyroid hormone has exactly the same chemical structure and mode of action in all vertebrates from fish to frogs to humans..





Flame retardants





Flame retardants

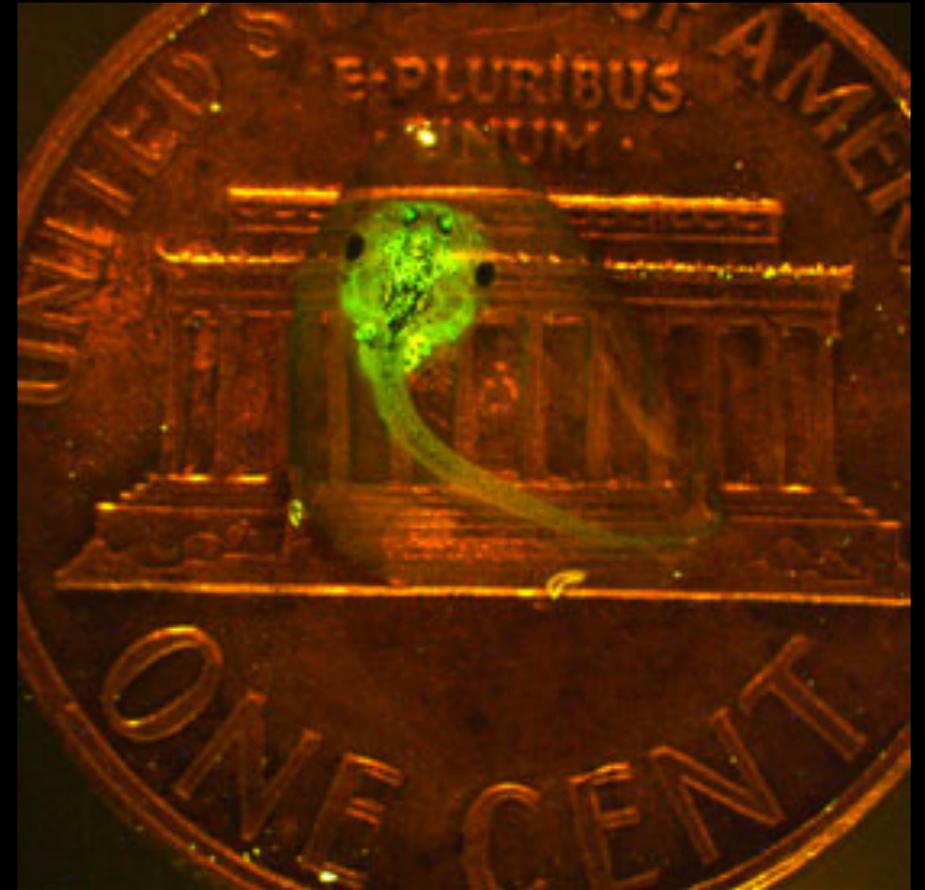


Thyroid hormone has exactly the same chemical structure and mode of action in all vertebrates from fish to frogs to humans..

# New testing methods at the in vivo/in vitro interface

interface

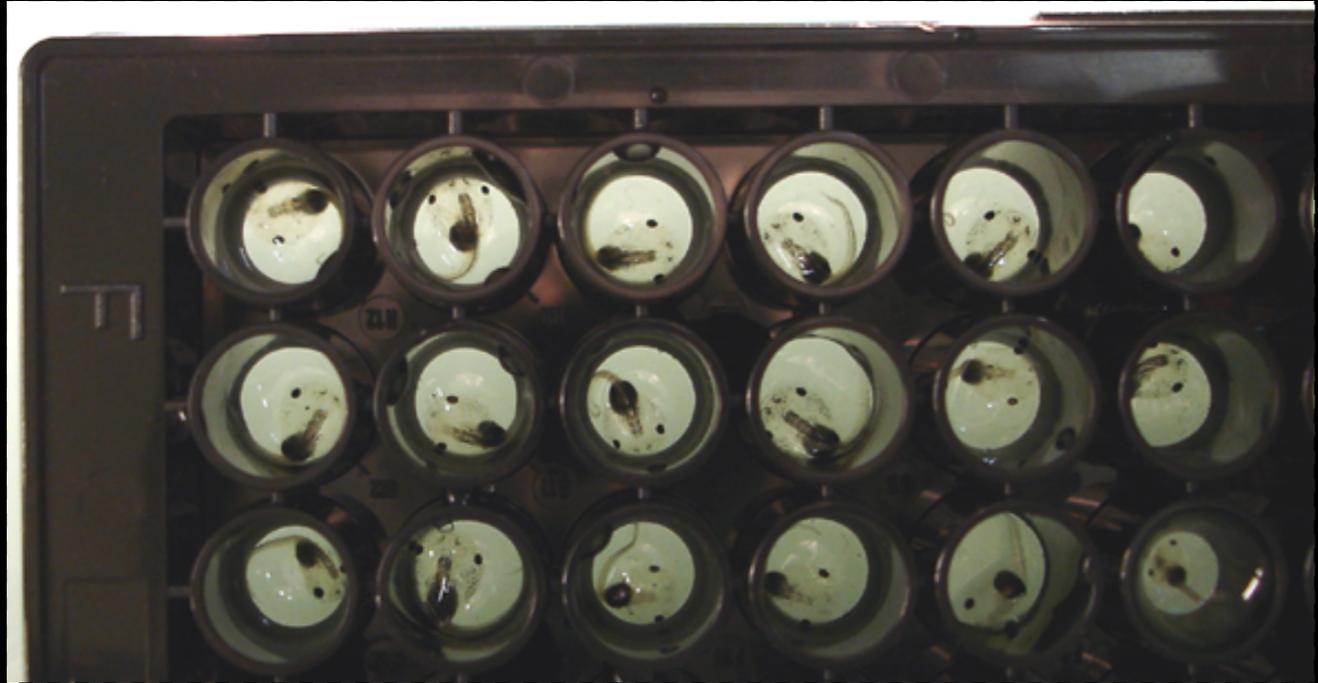
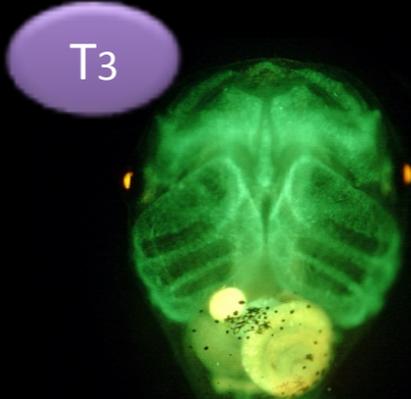
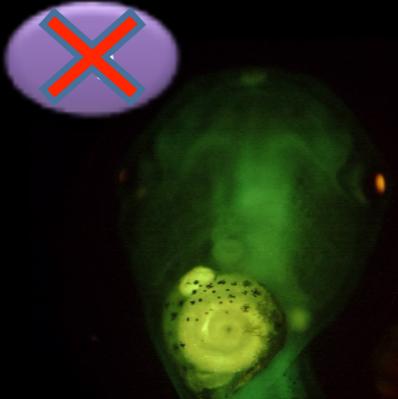
- Fish and Amphibian embryos
- Fluorescent : rapid readout
- Physiological parallels with vulnerable windows of foetal development in mammals



# Early stage embryos can be used for rapid, physiological screening



T3 10-8M

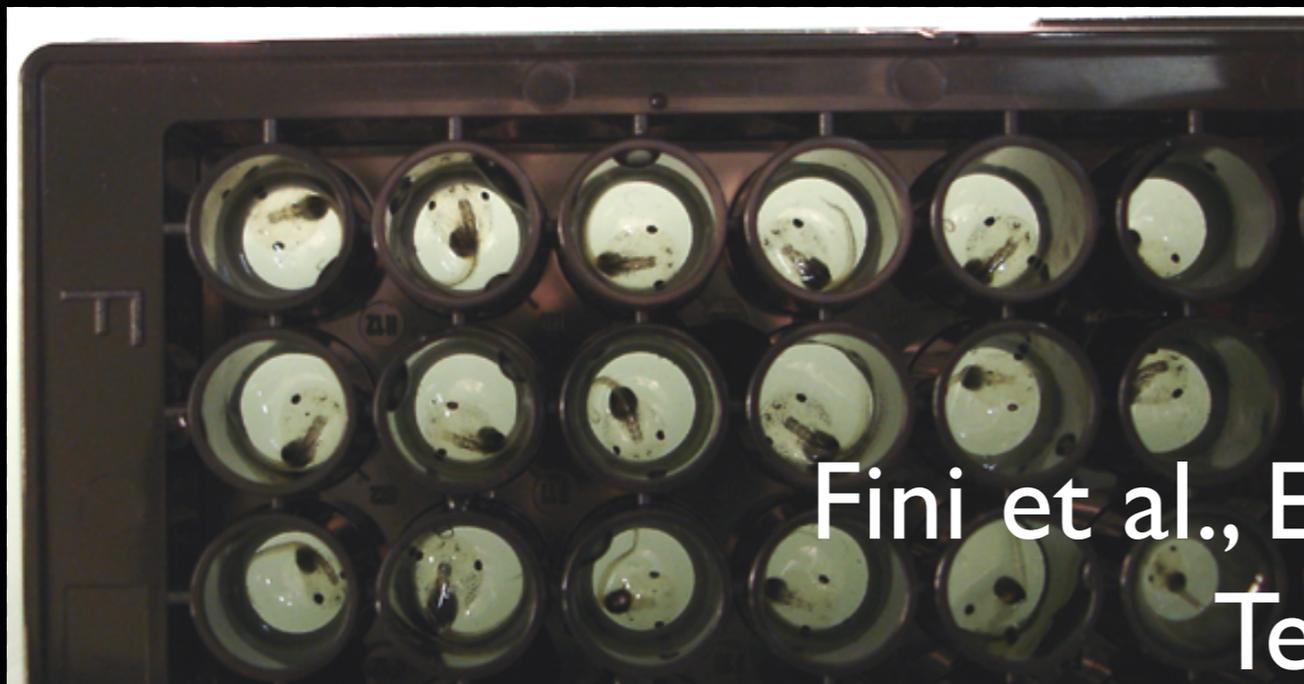
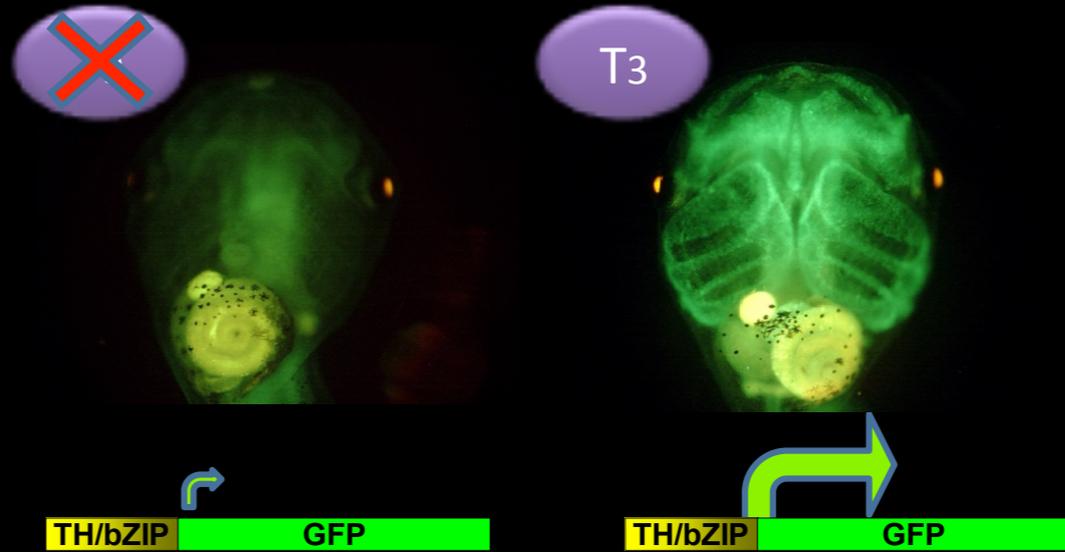


Automatic plate reading....

# Early stage embryos can be used for rapid, physiological screening

treatment  
n = 15 t      24h      48h      72h

T3 10<sup>-8</sup>M

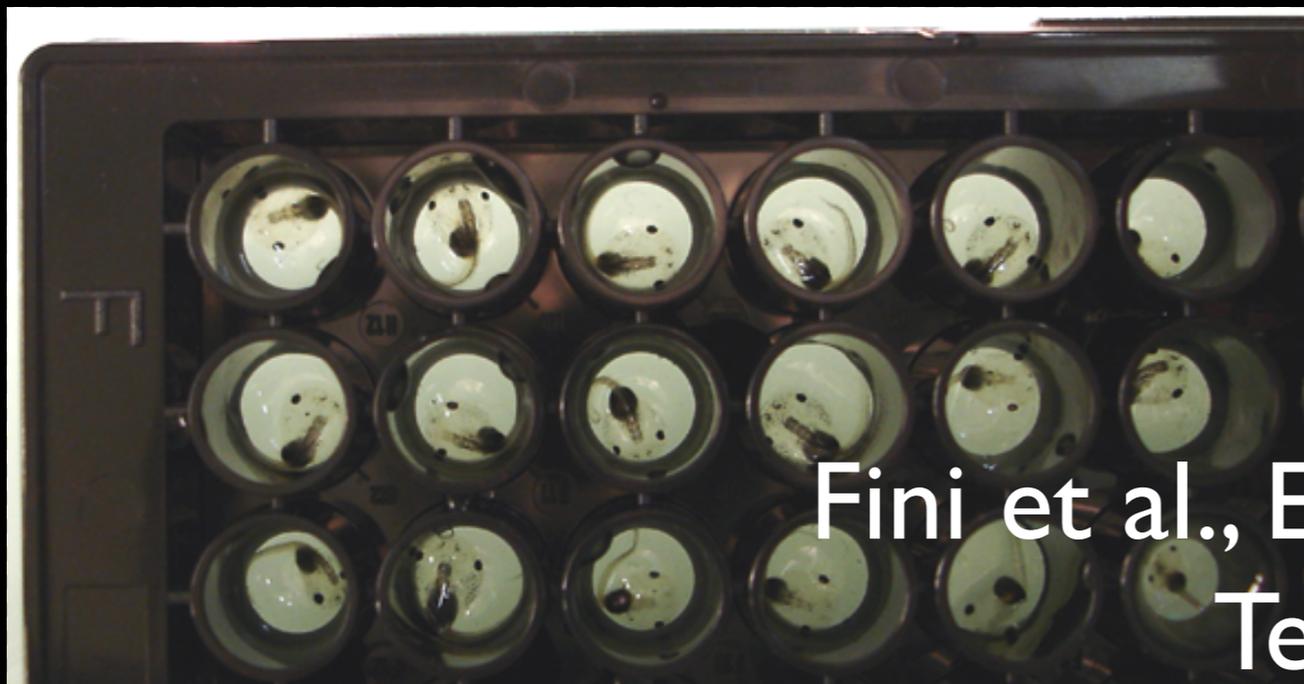
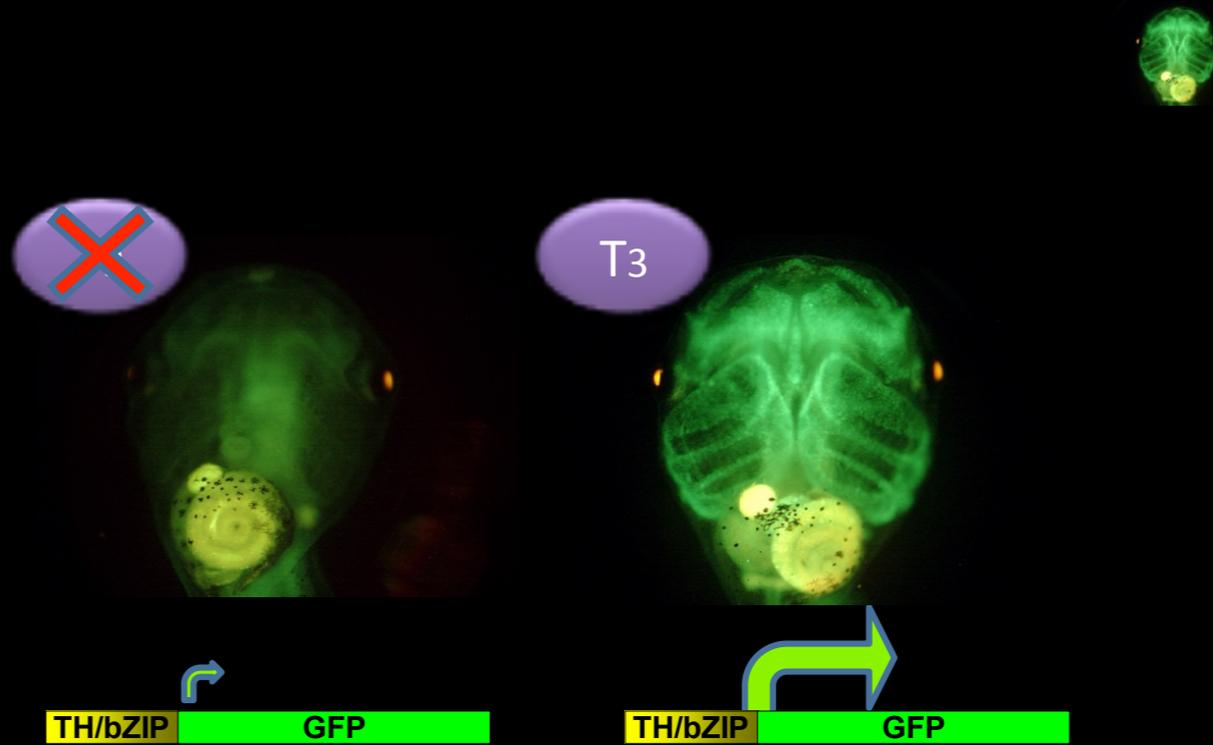
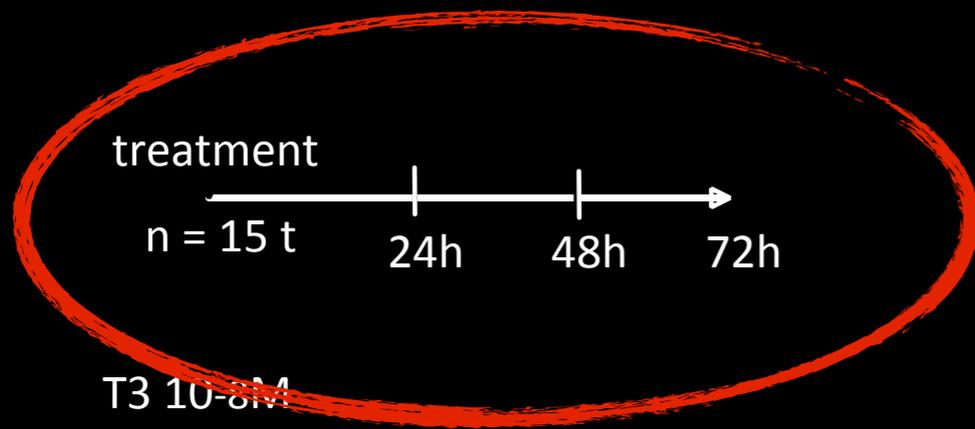


Automatic plate reading....

Fini et al., Environmental Science & Technology 2007

Currently being validated by the OECD in a ring test: France, US, Japan.

# Early stage embryos can be used for rapid, physiological screening



Automatic plate reading....

Fini et al., Environmental Science & Technology 2007

Currently being validated by the OECD in a ring test: France, US, Japan.

# SCIENTIFIC AMERICAN™

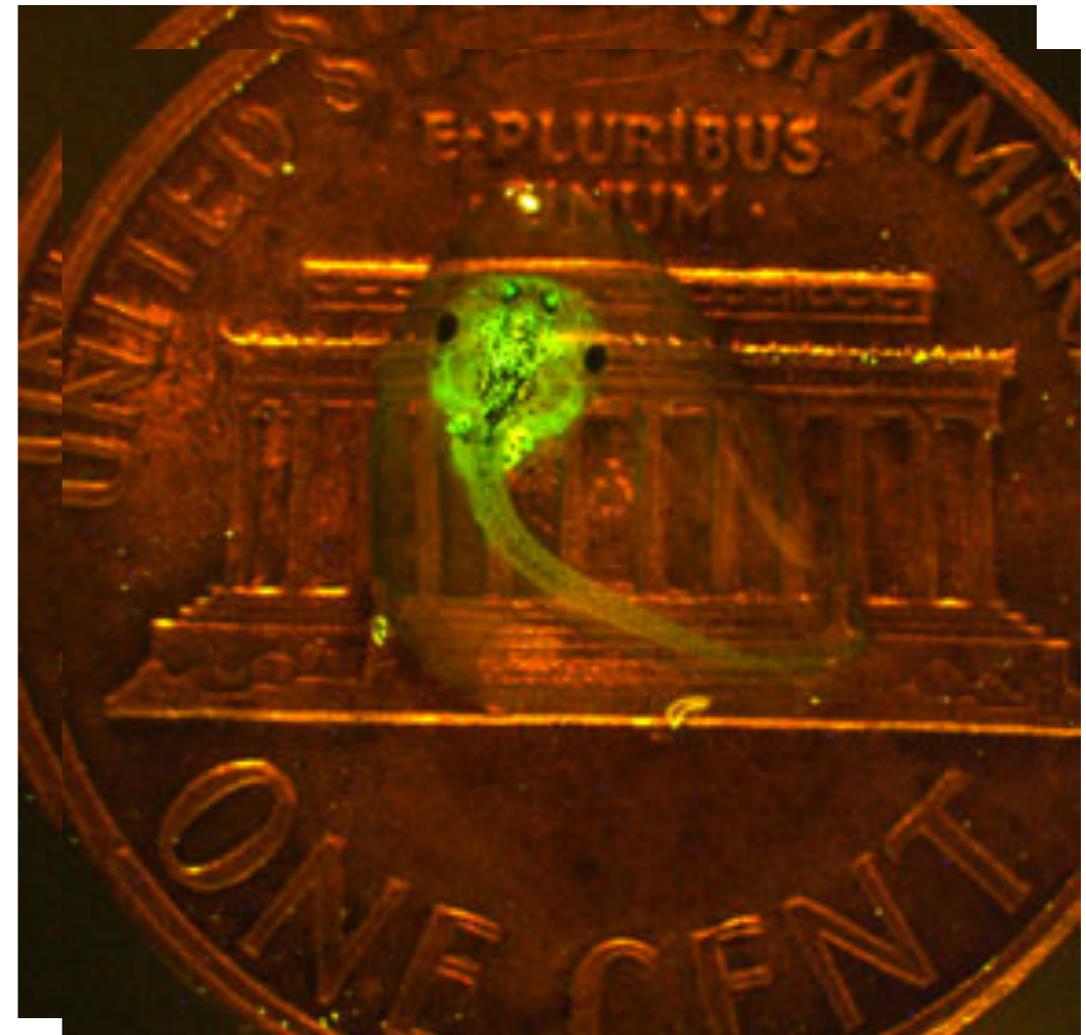
February 7, 2013

## **Unhealthy Glow: Fluorescent Tadpoles Expose Chemical Contamination**

Transgenic fish fry and larval frogs light up when exposed to hormone-disrupting compounds in water

By [Erica Gies](#)

A French biotechnology company has created a tadpole that fluoresces when it encounters chemical contaminants in water...The test promises to shed light on a class of endocrine disruptors.....



# Fish and Amphibian embryos for endocrine disruption testing

- Embryos are almost transparent can be combined with fluorescence signalling for rapid screening
- Many advantages of an in vitro test ( precision, low cost, high throughput screening)
- WITH ALL the advantages of an in vivo test ( physiological pertinence, tissue specificity , adsorption , metabolism, excretion)
- Non-feeding embryos (living on yolk): useful 3R alternatives to animal testing (Reduce, Replace, Refine)

# The case of Chlorpyrifos: selected for re-review by the European commission en 2012

- The review of chlorpyrifos is one of the first-ever cases in which an approved pesticide is being reviewed. Chlorpyrifos, an insecticide used on grapes and potatoes, was approved provisionally in 2006.
- In the US - 5 million tons used annually
- The EU review is particularly welcome...

# The case of Chlorpyrifos: selected for re-review by the European commission en 2012

- The review of chlorpyrifos is one of the first-ever cases in which an approved pesticide is being reviewed. Chlorpyrifos, an insecticide used on grapes and potatoes, was approved provisionally in 2006.
- In the US - 5 million tons used annually
- The EU review is particularly welcome...

Because

...

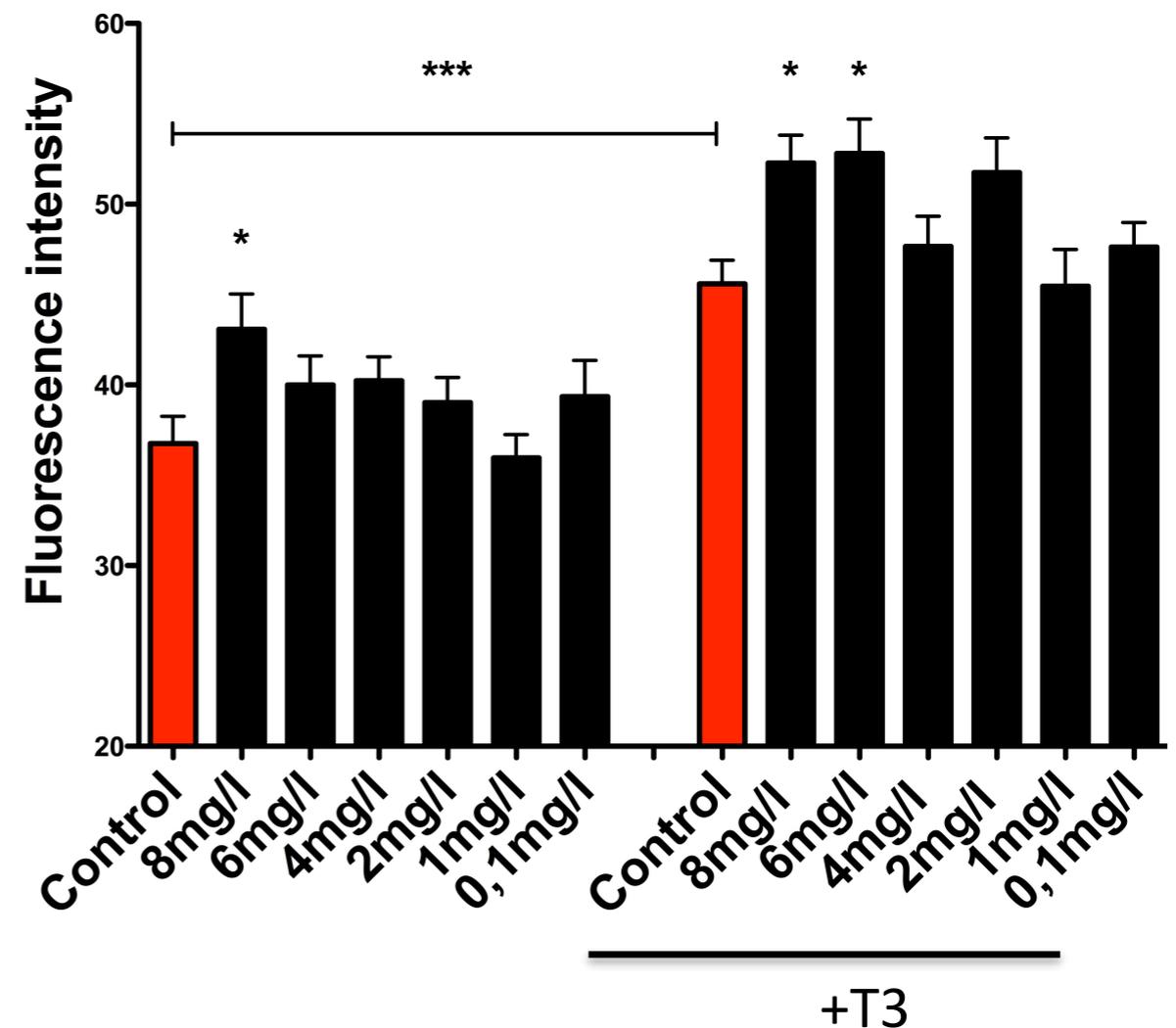
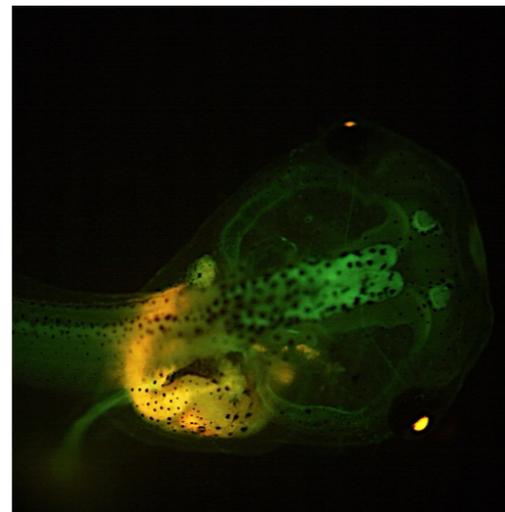
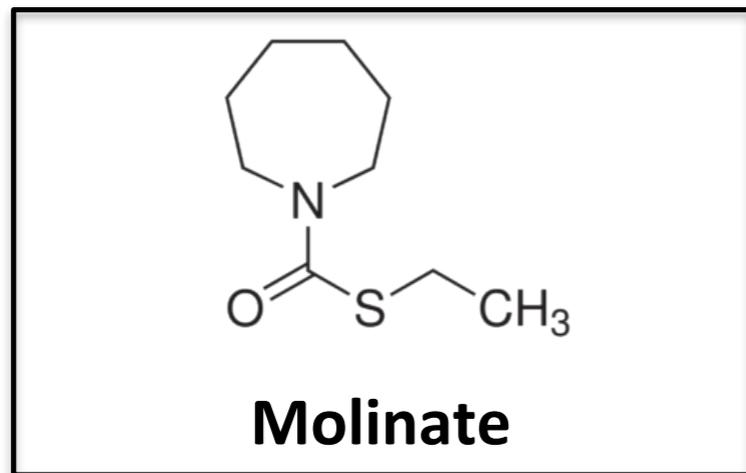
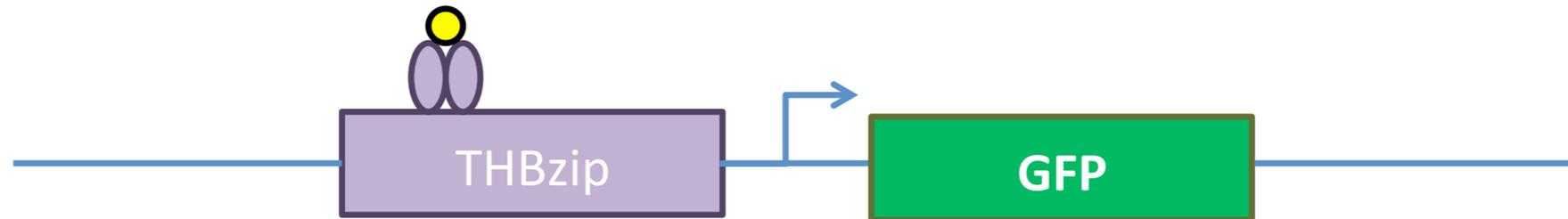
# UK CRD report on 70 pesticides...

## March 2013

- The human health assessment for chlorpyrifos , which is relevant to mammalian wildlife species, indicated that *“No adverse effects related to endocrine disruption have been identified in the range of regulatory toxicological tests. These indicate that the major toxicological effect is decreased cholinesterase activity. However, there are some recent but non-regulatory studies that indicate that chlorpyrifos has effects on both the thyroid and male reproductive systems. **There has been a study in mice showing perturbation of thyroid hormones in dams, but there is no information in this study on adverse effects manifested from these alterations.**”* ??????

•

# A fluorescence based screen for thyroid hormone disruption in *Xenopus* embryos: the case of Molinate



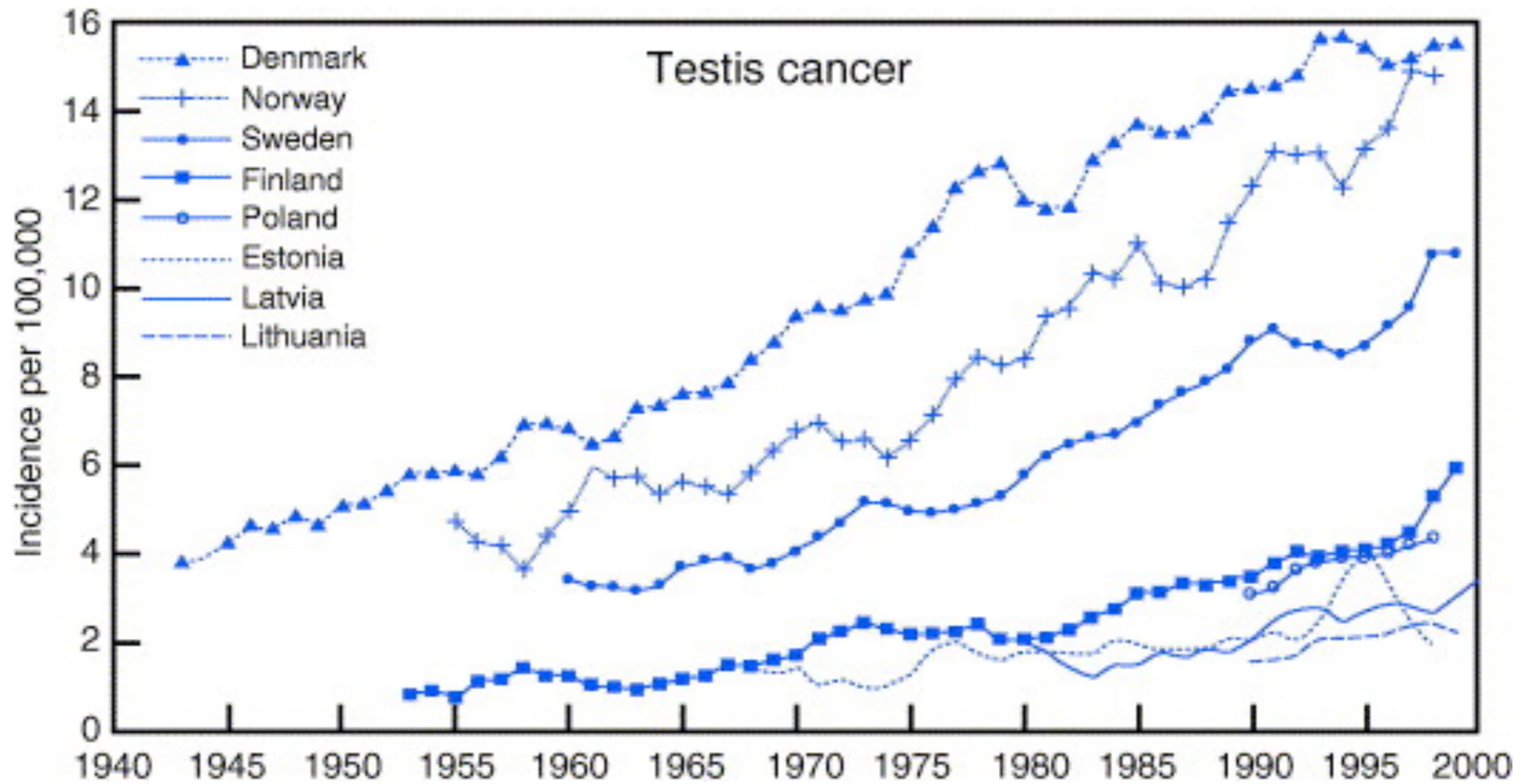
# Fluorescent embryo tests also applicable to

- Examples of estrogens and (anti) - androgens

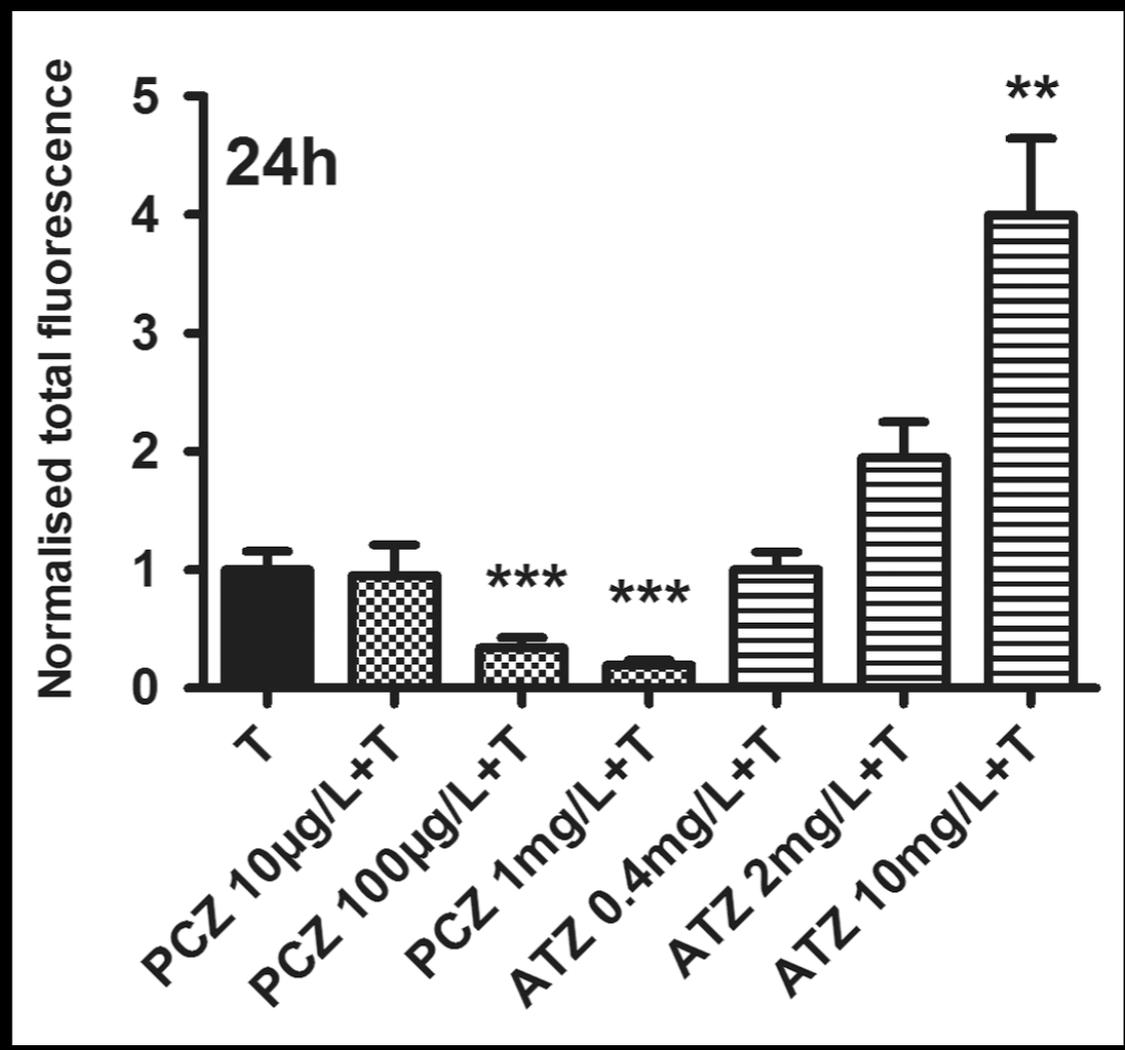
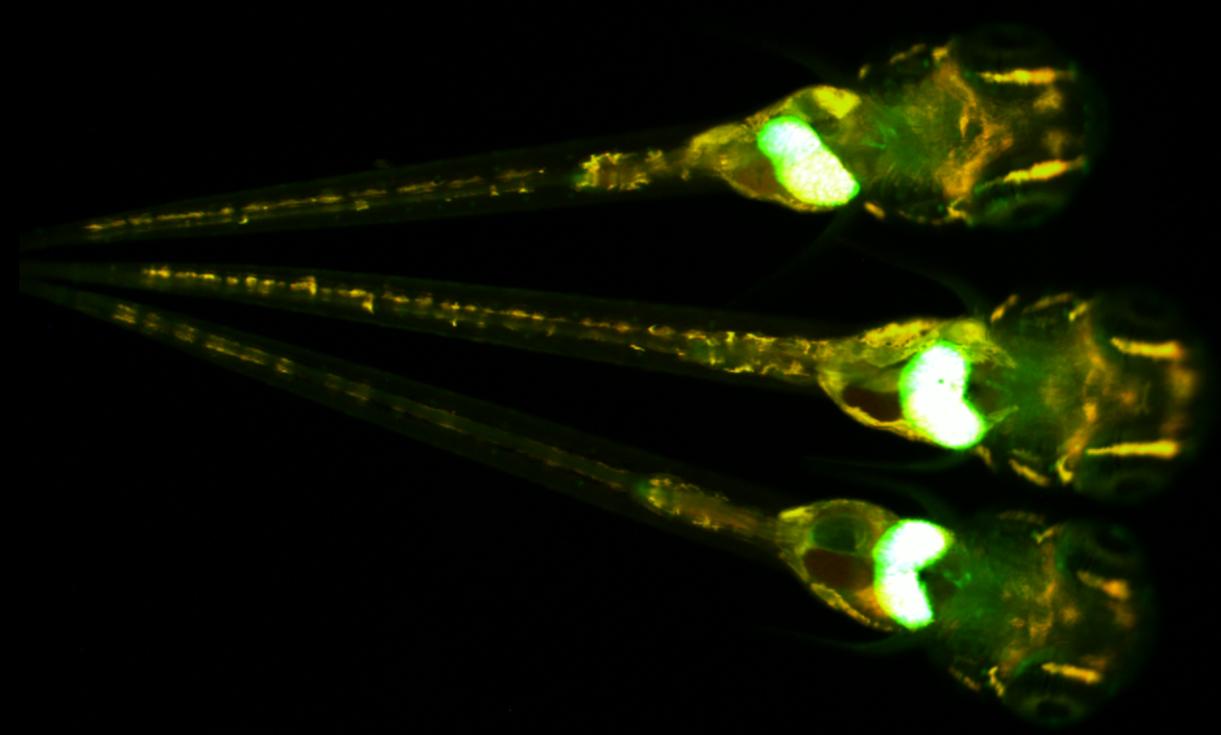
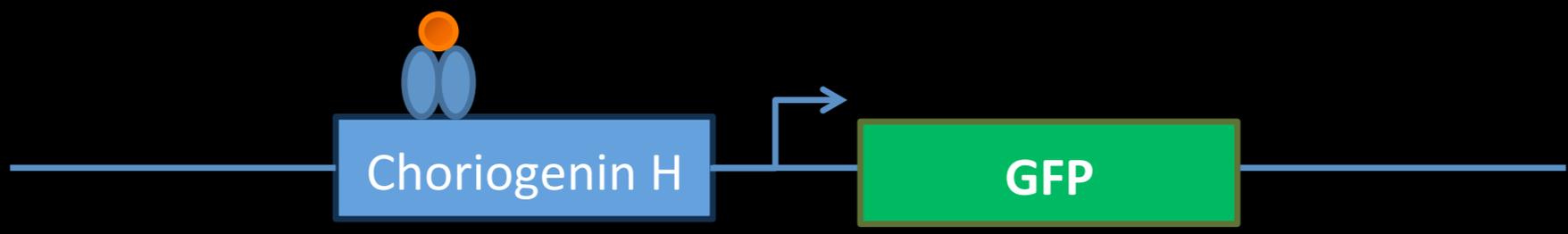
# Increasing incidence of interlinked reproductive disorders in men, women and in wildlife populations

- Precocious puberty, Infertility,
- From impaired spermatogenesis to testicular cancer..
- Breast, ovarian, uterine cancers,
- Endometriosis

# Increasing incidence of testicular cancer



# Rapid detection of steroid signalling modulation with transgenic medaka: examples of Atrazine (Roundup) and Perchloraz (fungicide)



# Fish and Amphibian embryo tests currently available or undergoing OECD validation

- Thyroid disruption
- Androgen disruption
- Estrogen disruption
- Using these tests will provide information on links to environmental factors affecting early development:
- Associations with neurodevelopmental disorders such as Autism and many reproductive disorders including cancer

# Amphibians as models of human development

---

The rapid amphibian development allows to anticipate physiopathological phenomena that act in the long term in humans

Similar specific responses between amphibian vertebrate and humans

48 hours of development:

Genes and proteins homologies

Complex nervous system  
(central / peripheral)

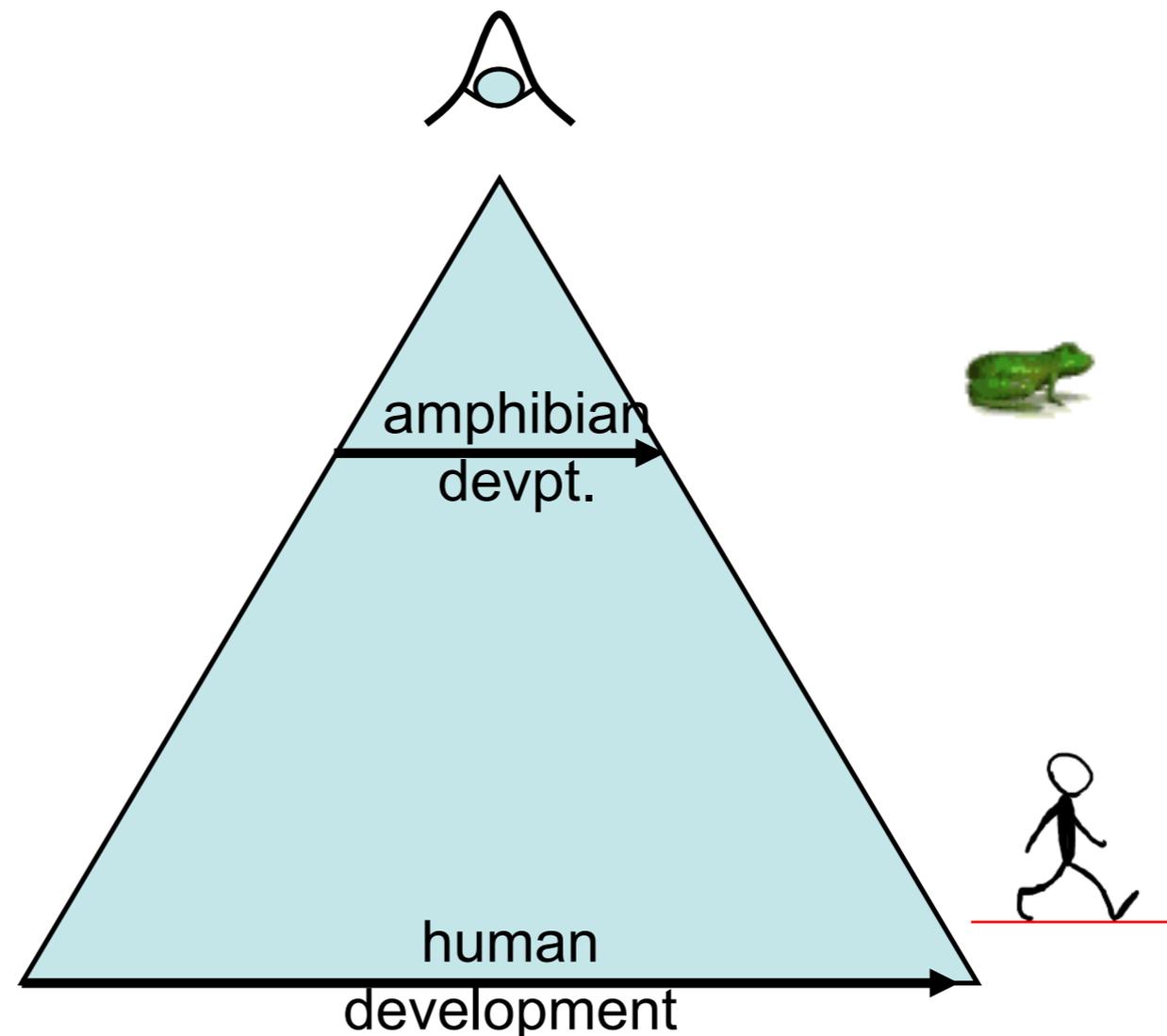
Cartilage (>bones)

Skin (Derma>Epiderma)

Eye

Cardiovascular

Immune system



# Conclusions: I

- Rapid, physiologically meaningful, new generation tests are available for thyroid, estrogen and androgen signalling - all of the main signalling pathways implicated in neurodevelopmental and reproductive disorders.
- Many of these tests are based on fluorescent read-out from fish and amphibian embryos that respect the 3R principle
- Evolutionary conservation of nuclear receptor signalling ensures that are relevant to human health
- Provide insight into effects of chemicals on particularly vulnerable periods of early, foetal, development
- Across the EU numerous academic and SME-based labs are developing and optimising these approaches

# Conclusions II

- These tests can be run in days - usually 3 days - versus weeks and months needed for current tests.
- They cost between 5 and 10 times less than their counterparts on mammalian systems.
- They directly incorporate metabolism into testing - not the case for the majority of cell based systems.
- Many of these tests are currently undergoing validation by the OECD

# Recommendations

- These new embryo-based methods can better represent effects on early development than classic toxicology
- These tests and the results obtained, must be incorporated into current pesticide evaluation procedures.

# Reminder: health costs of Endocrine Disruption outweigh those of testing

- KPMG 2006 (cited by Les Echos) study on chemical industry costs of applying REACH for 152 substances over 11 years: **2.3 Billion €** (0.05% of turnover)
- Health care costs saved over 30 years: **50 Billion €**
- Since then health costs have increased due to increased incidence of non-communicable disease
- Testing methods have improved with reduced costs
- Testing for better replacements reduces costs even further: e.g. green screen approaches

# Alternatives to in vivo tests to detect endocrine disrupting chemicals (EDCs) in fish and amphibians

*S. Scholz<sup>1</sup>, P. Renner<sup>1</sup>, L.S. Ortego<sup>2</sup>, S. Belanger<sup>3</sup>, F. Busquet<sup>4</sup>, R. Davi<sup>5</sup>, B. Demeneix<sup>6</sup>, J. Denny<sup>7</sup>, M. Léonard<sup>8</sup>, M. McMaster<sup>9</sup>, D. Villeneuve<sup>7</sup> and M. Embry<sup>10</sup>*

<sup>1</sup> Helmholtz Centre for Environmental Research, Leipzig, Germany

<sup>2</sup> Bayer Crop Science, Research Triangle Park, NC, USA

<sup>3</sup> Procter and Gamble, Cincinnati, OH, USA

<sup>4</sup> ECVAM, Ispra, Italy

<sup>5</sup> ExxonMobil Biomedical Sciences, Inc., Amandale, NJ, USA

<sup>6</sup> Musée Nationale d'Histoire Naturelle, Paris, France

<sup>7</sup> US EPA, Duluth, MN, USA

<sup>8</sup> L'Oreal, Aulnay Sous Bois, France

<sup>9</sup> Environment Canada, Burlington, ON, Canada

<sup>10</sup> ILSI HESI, Washington, DC, USA



# Alternatives to in vivo tests to detect endocrine disrupting chemicals (EDCs) in fish and amphibians

*S. Scholz<sup>1</sup>, P. Renner<sup>1</sup>, L.S. Ortego<sup>2</sup>, S. Belanger<sup>3</sup>, F. Busquet<sup>4</sup>, R. Davi<sup>5</sup>, B. Demeneix<sup>6</sup>, J. Denny<sup>7</sup>, M. Léonard<sup>8</sup>, M. McMaster<sup>9</sup>, D. Villermaux<sup>7</sup>, M. Embry<sup>10</sup>*

Supported by industry

<sup>1</sup> Helmholtz Centre for Environmental Research, Leipzig, Germany

<sup>2</sup> Bayer Crop Science, Research Triangle Park, NC, USA

<sup>3</sup> Procter and Gamble, Cincinnati, OH, USA

<sup>4</sup> ECVAM, Ispra, Italy

<sup>5</sup> ExxonMobil Biomedical Sciences, Inc., Amandale, NJ, USA

<sup>6</sup> Musée Nationale d'Histoire Naturelle, Paris, France

<sup>7</sup> US EPA, Duluth, MN, USA

<sup>8</sup> L'Oreal, Aulnay Sous Bois, France

<sup>9</sup> Environment Canada, Burlington, ON, Canada

<sup>10</sup> ILSI HESI, Washington, DC, USA



HESI

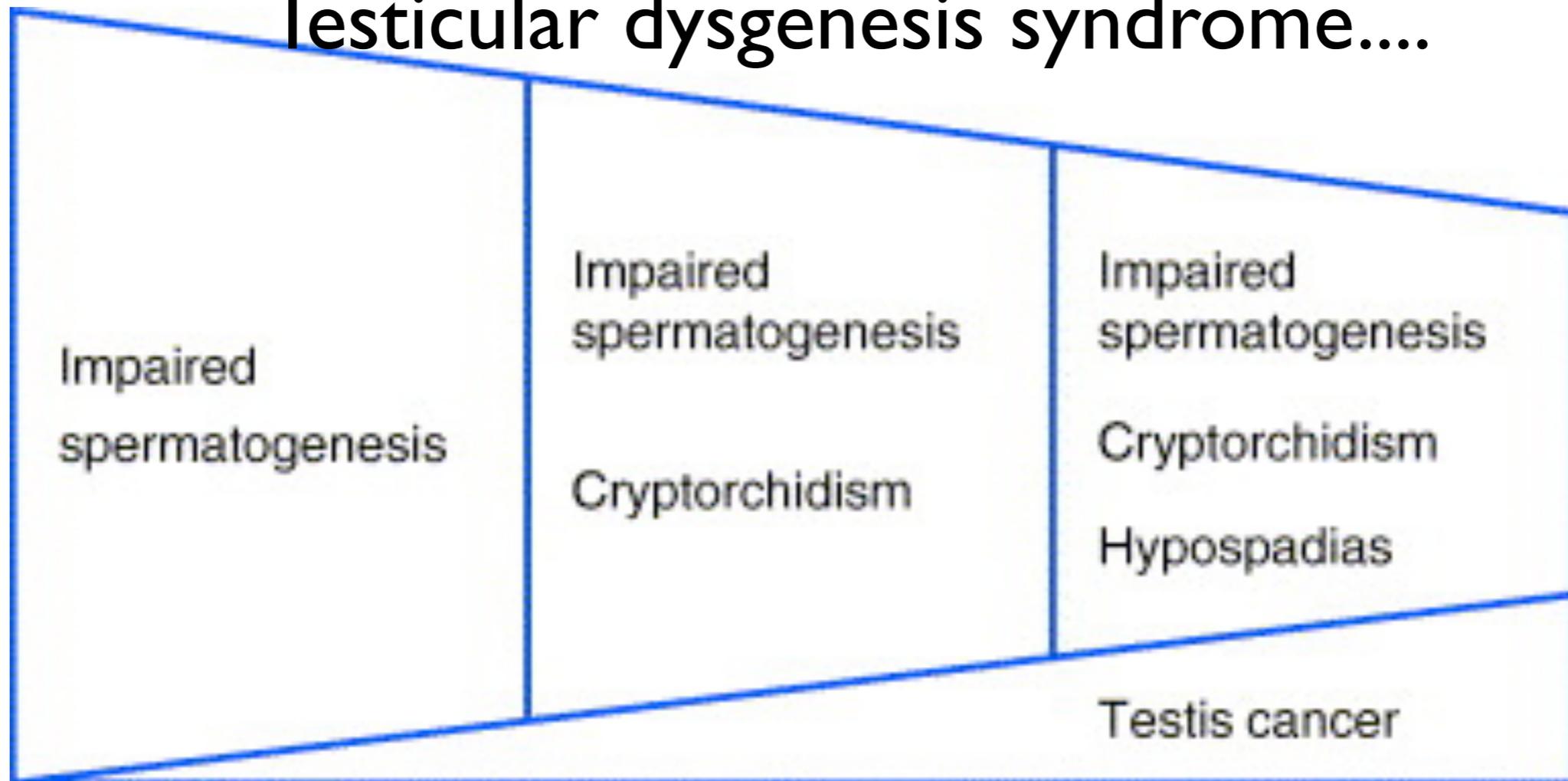


HELMHOLTZ

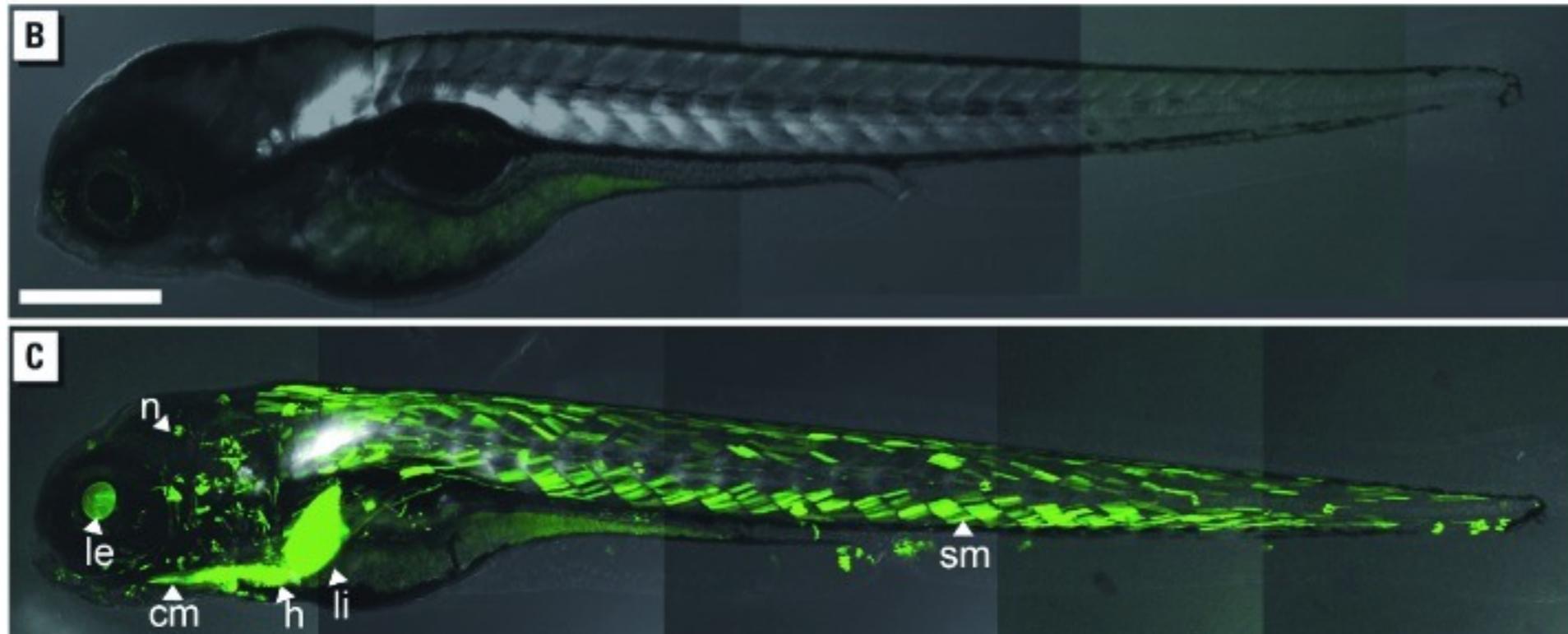
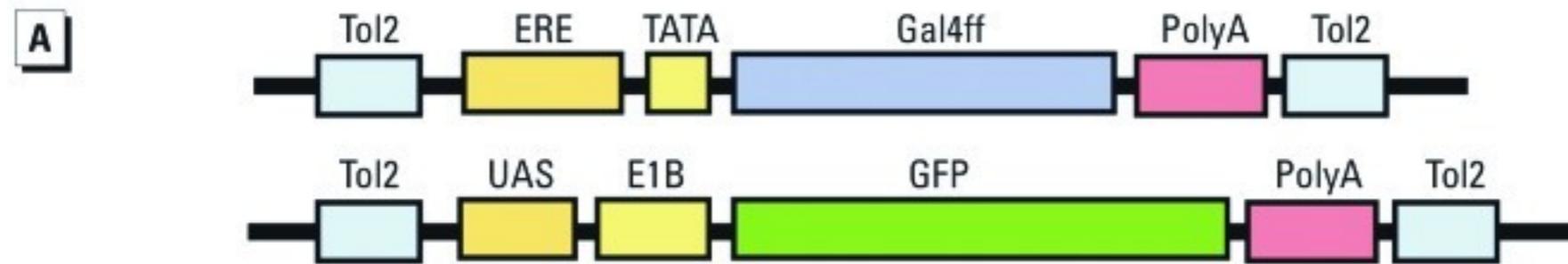
CENTRE FOR  
ENVIRONMENTAL  
RESEARCH - UFZ

# Increasing incidence of interlinked reproductive disorders in men and women and in wildlife populations

## Testicular dysgenesis syndrome...



# Example of a Zebrafish embryos as an estrogen readout



Lee O, Takesono A, Tada M, Tyler CR, Kudoh T.  
Environ Health Perspect. 2012 Jul; 120(7):990-6

# An anti-androgen screen in fluorescent reporter medaka

