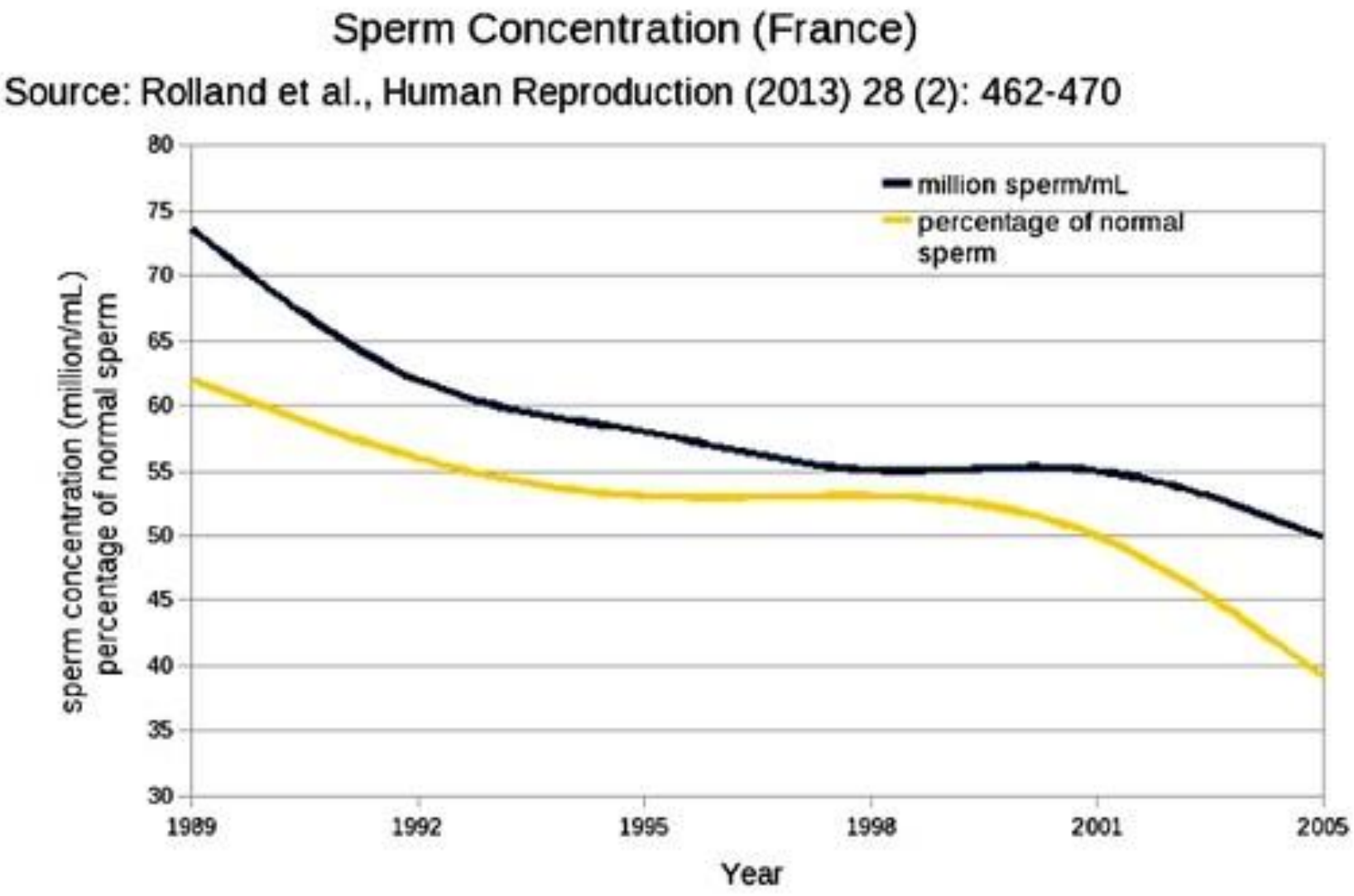
양서류 변태독성 시험법을 이용한 갑상선호르몬 교란성 연구

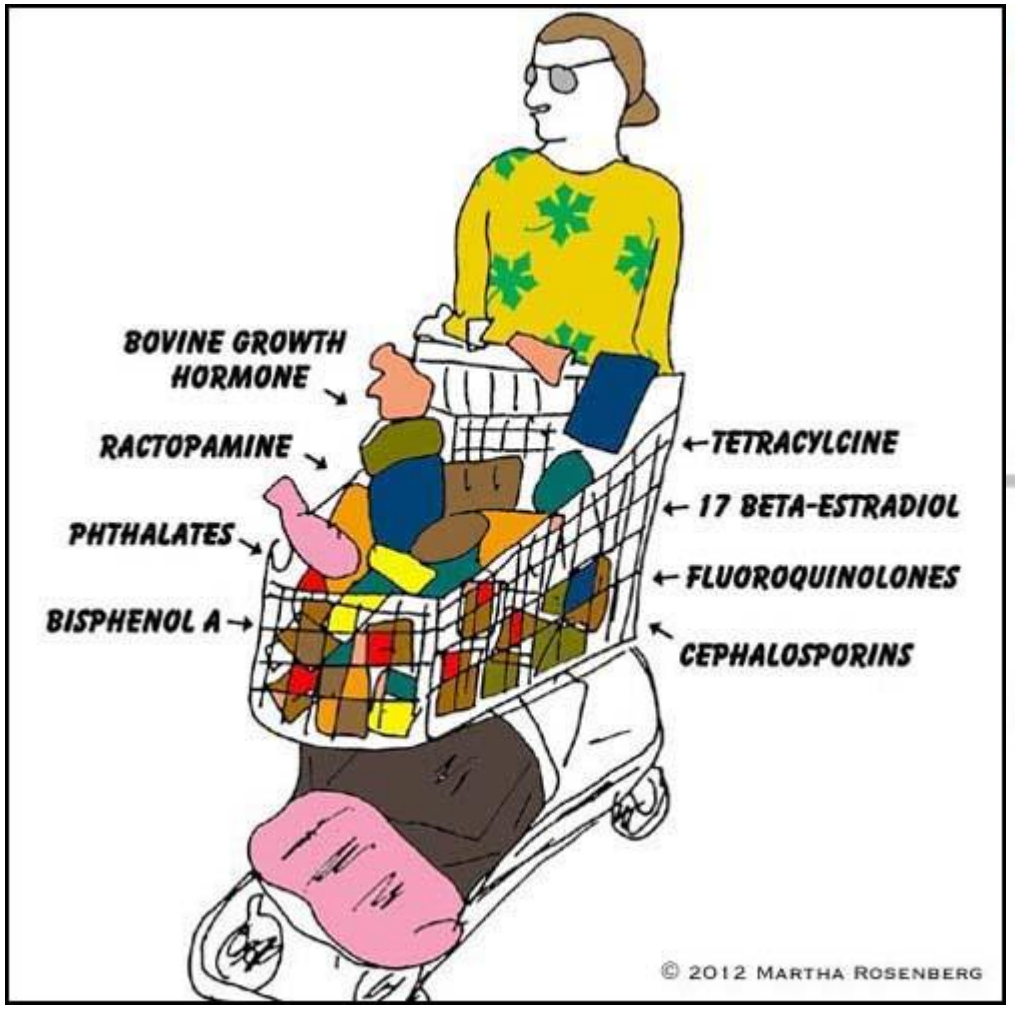
박 찬 진

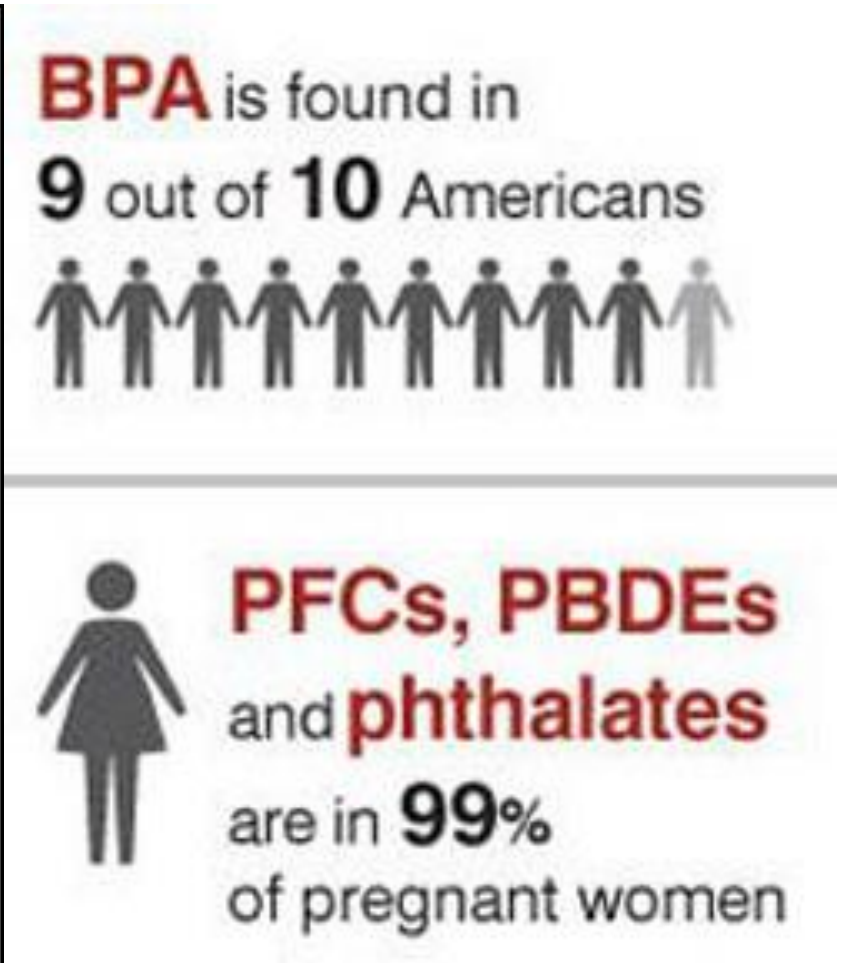
한양대학교 생명과학과

Endocrine disruption

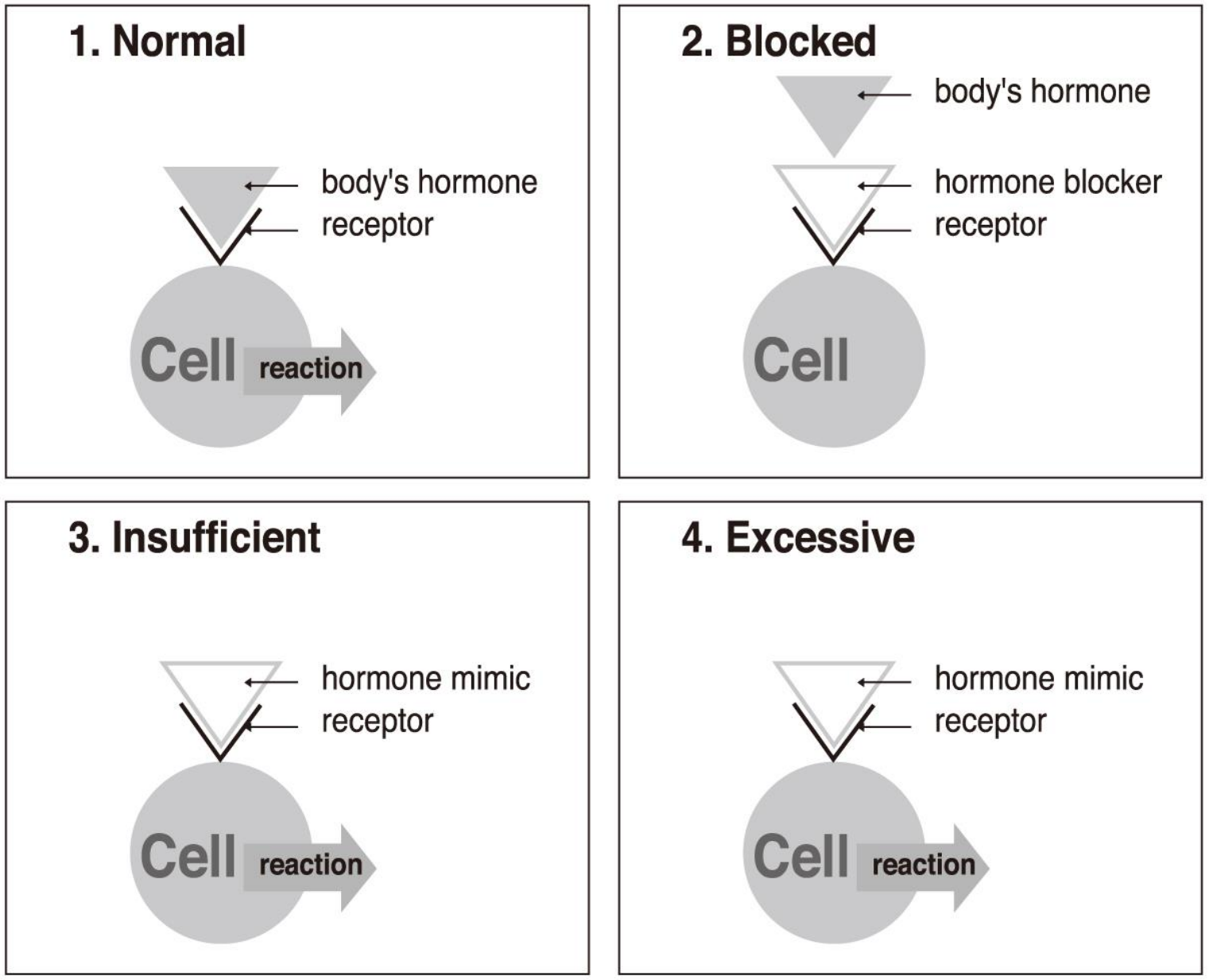


Endocrine disruptors

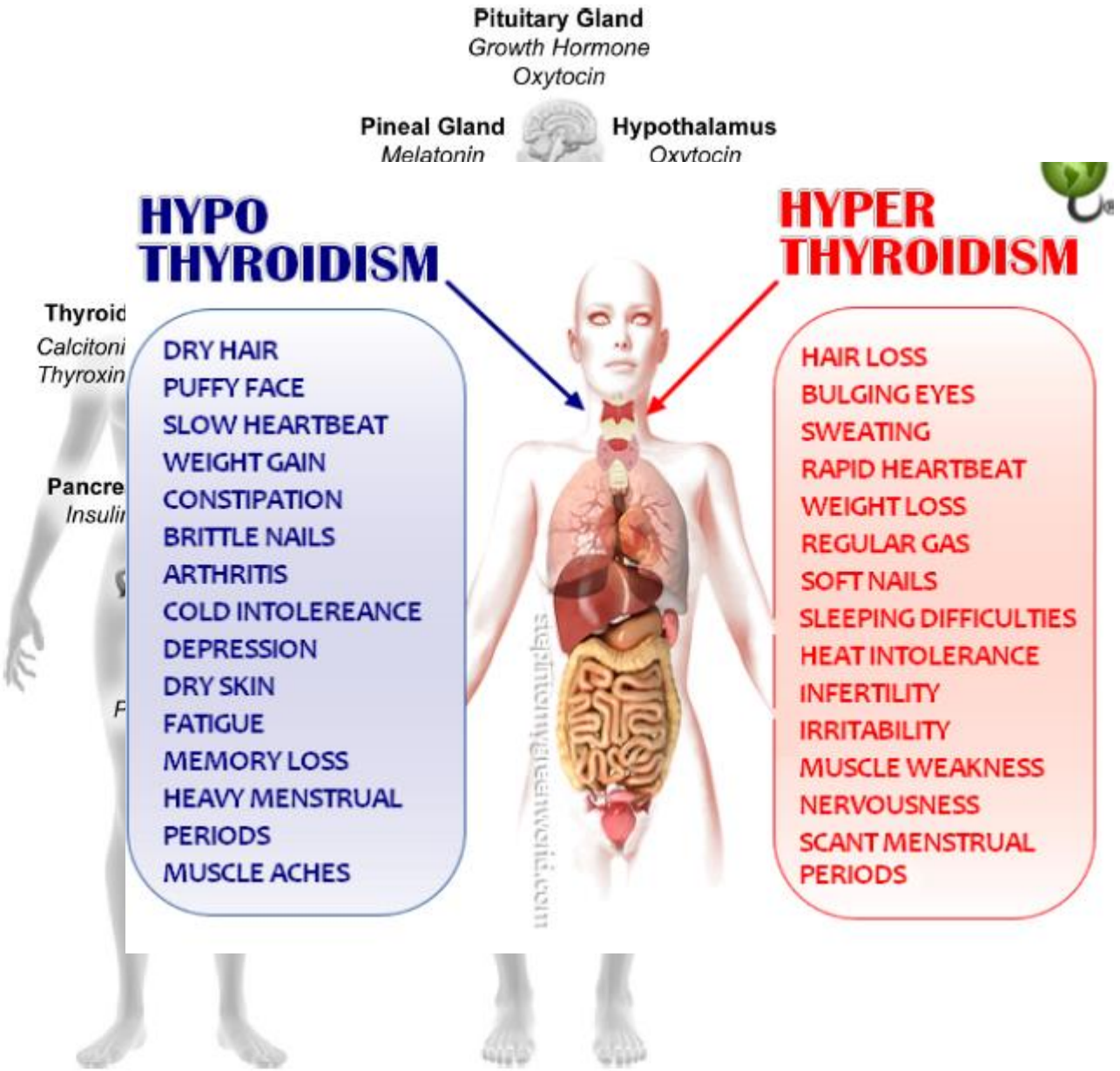




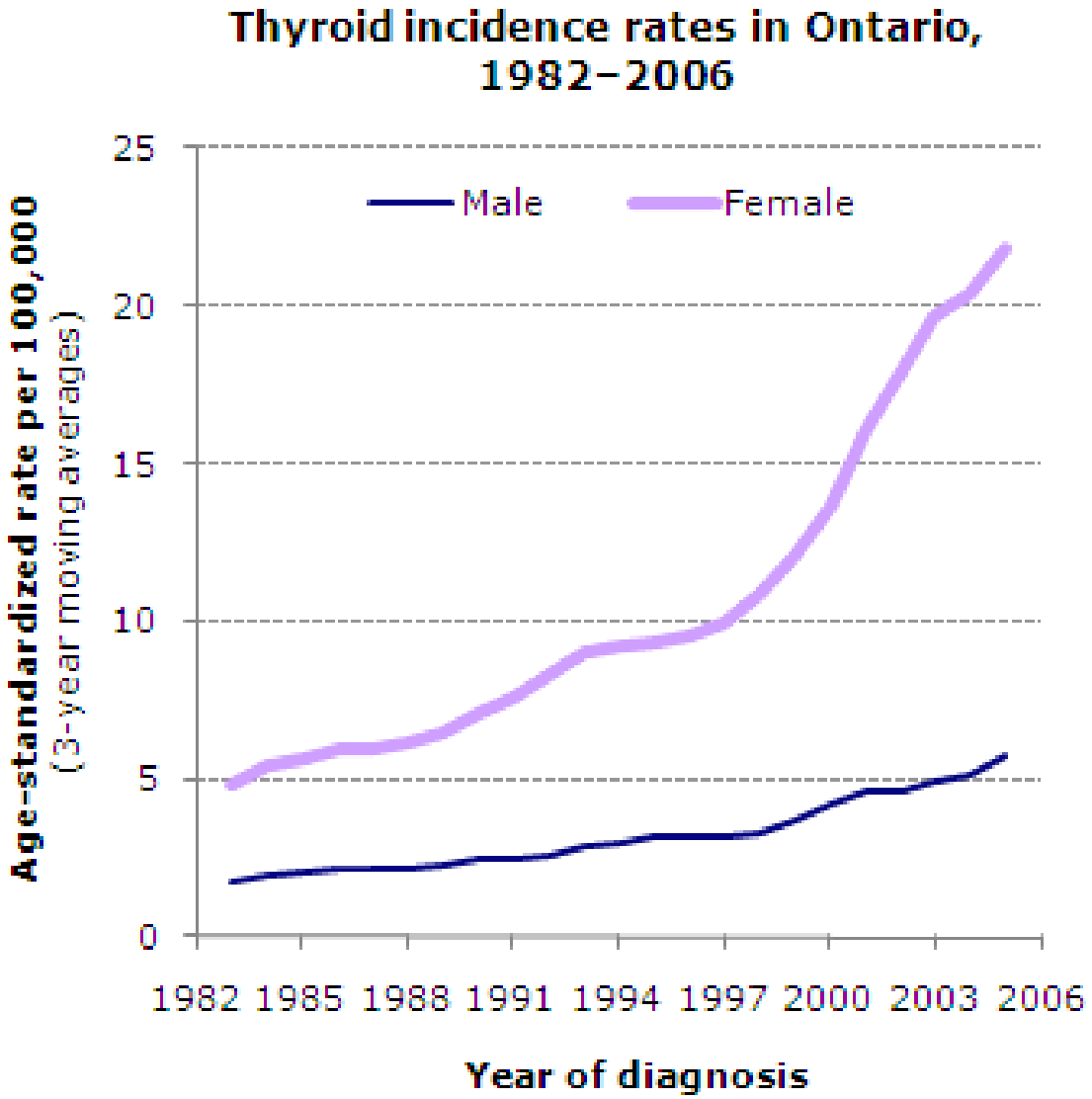
Action of endocrine disruptors



Endocrine system

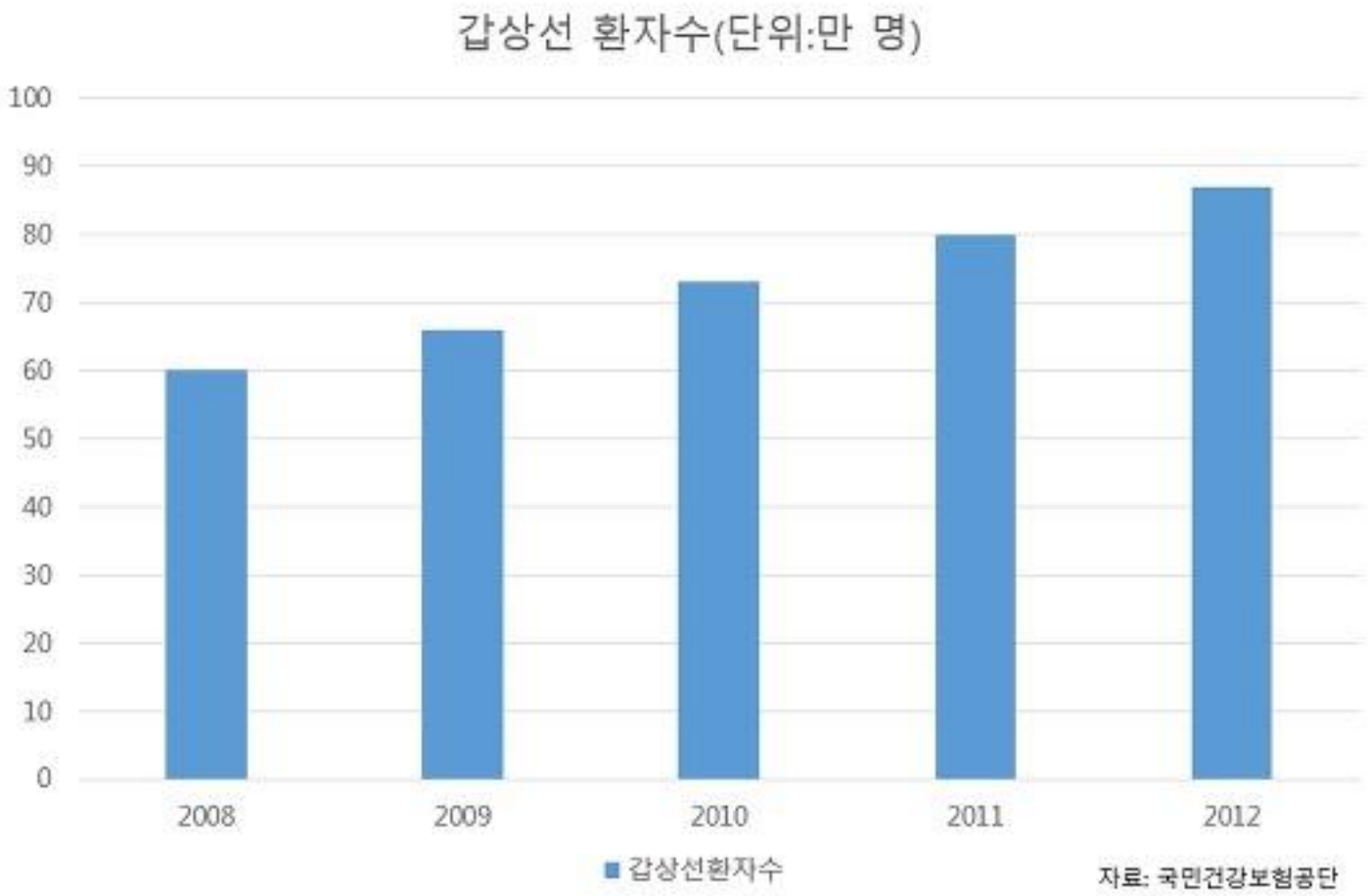


Increase of thyroid disease

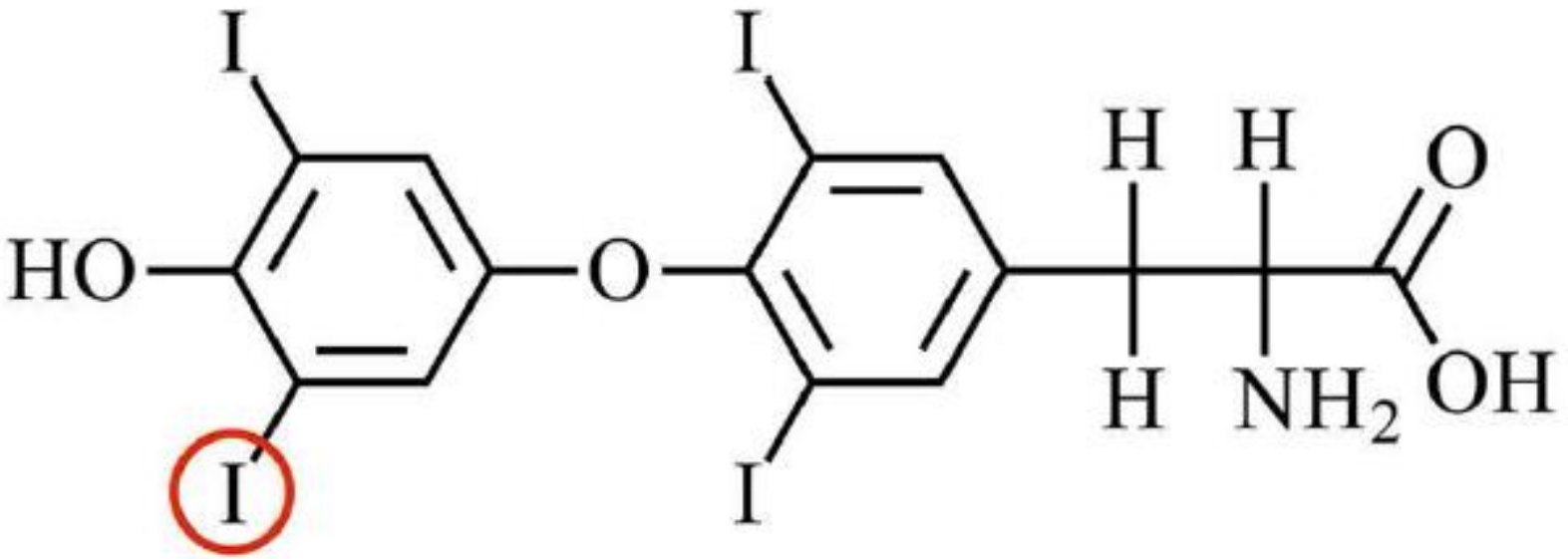


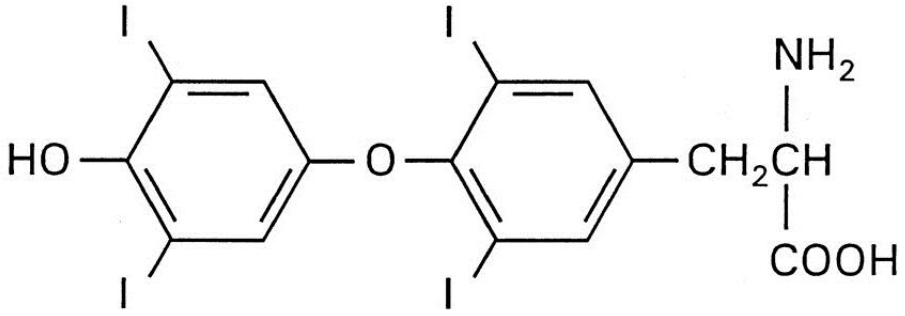
Source: Cancer Care Ontario (Ontario Cancer Registry, 2009)

Increase of thyroid disease

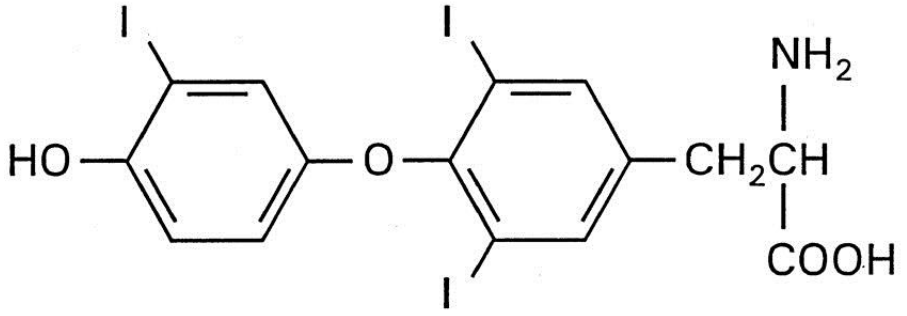


Thyroid hormones



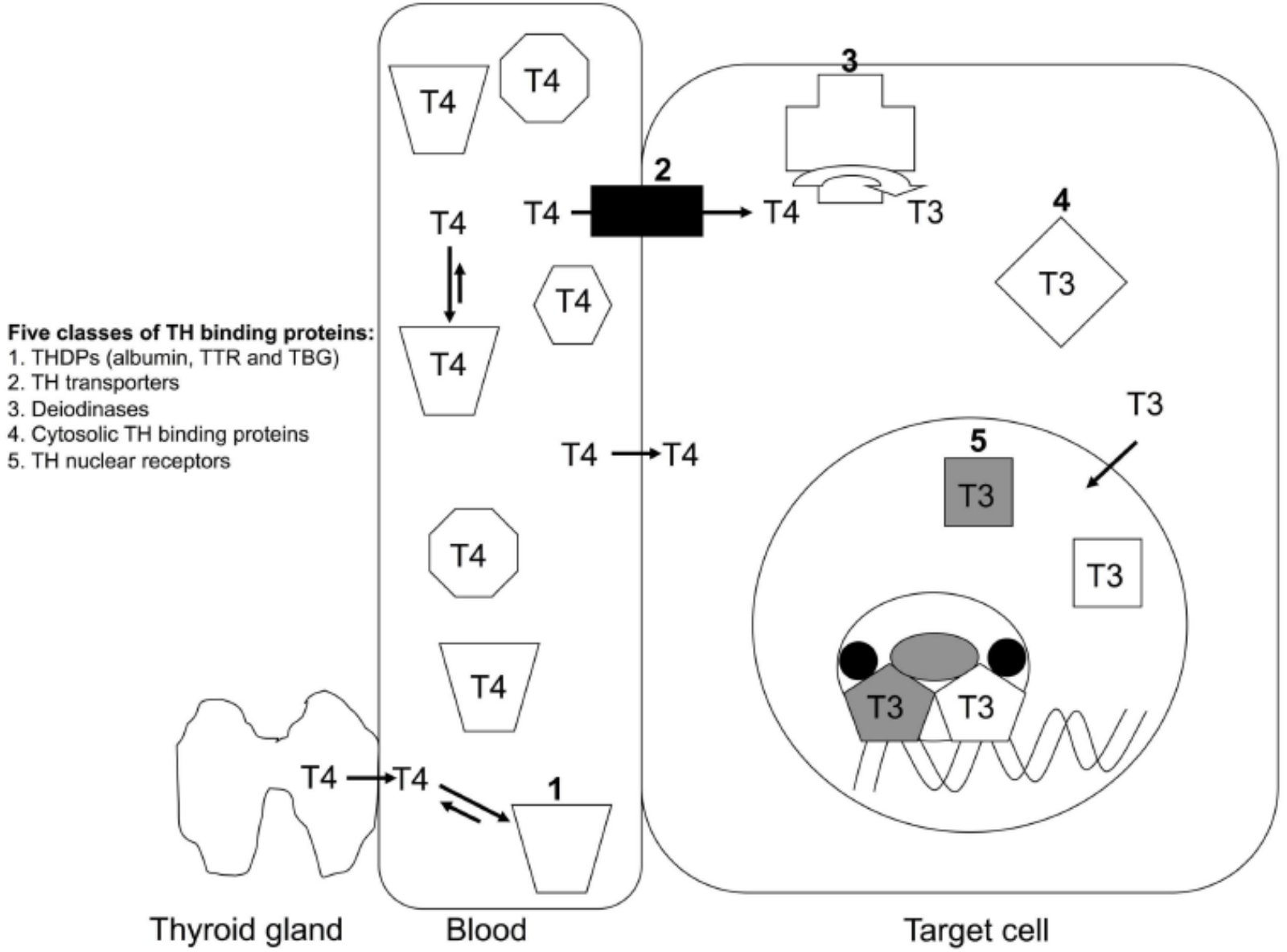


Thyroxine
(T_4)

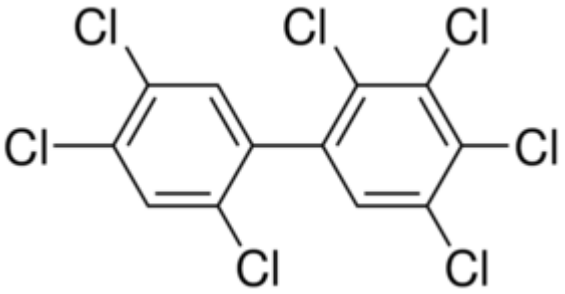


3,3′5-Triiodo-L-Thyronine
(T_3)

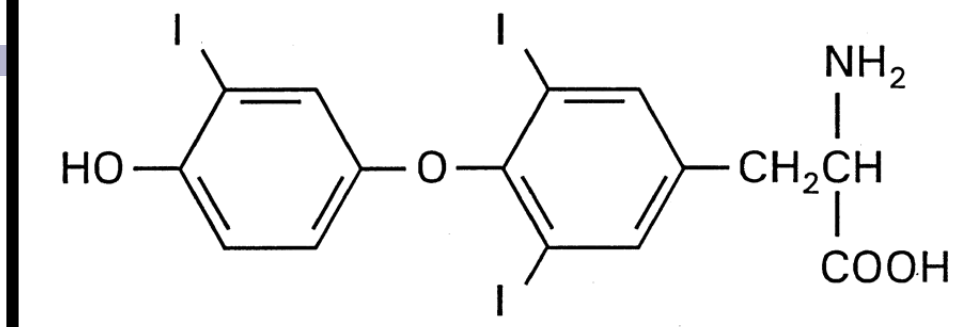
Thyroid hormones



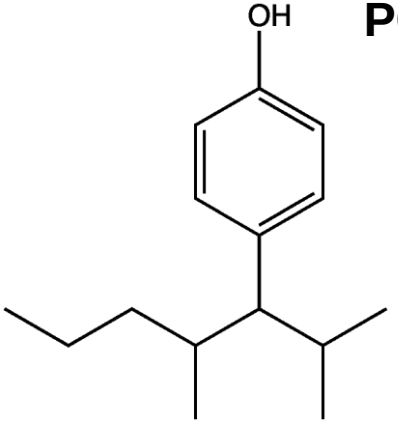
Thyroid system disruptors



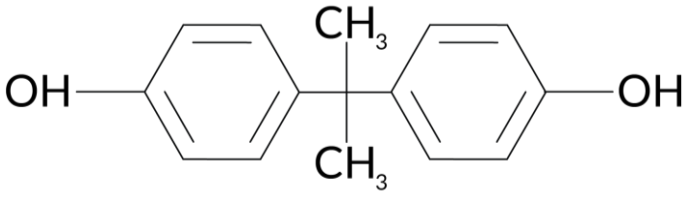
PCB



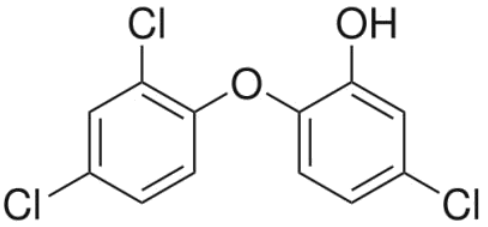
3,3'5-Triiodo-L-Thyronine
(T_3)



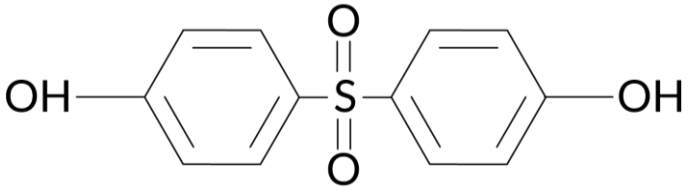
Nonylphenol



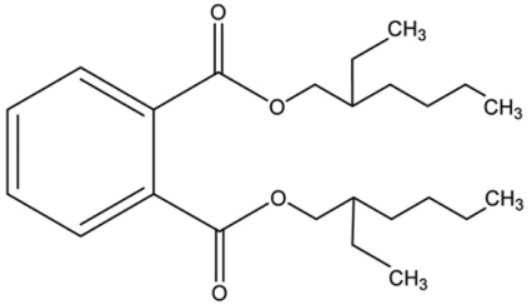
Bisphenol-A



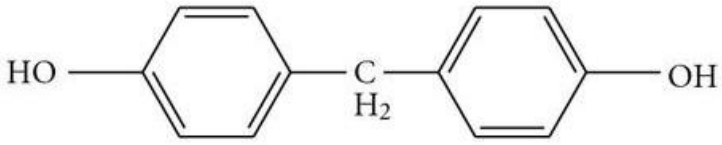
Triclosan



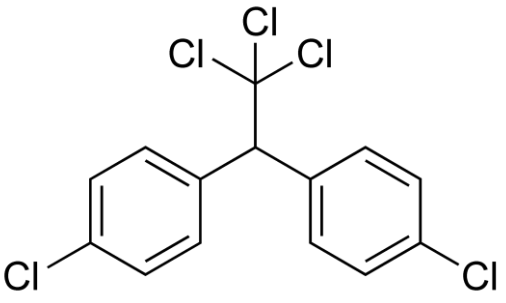
Bisphenol-S



DEHP

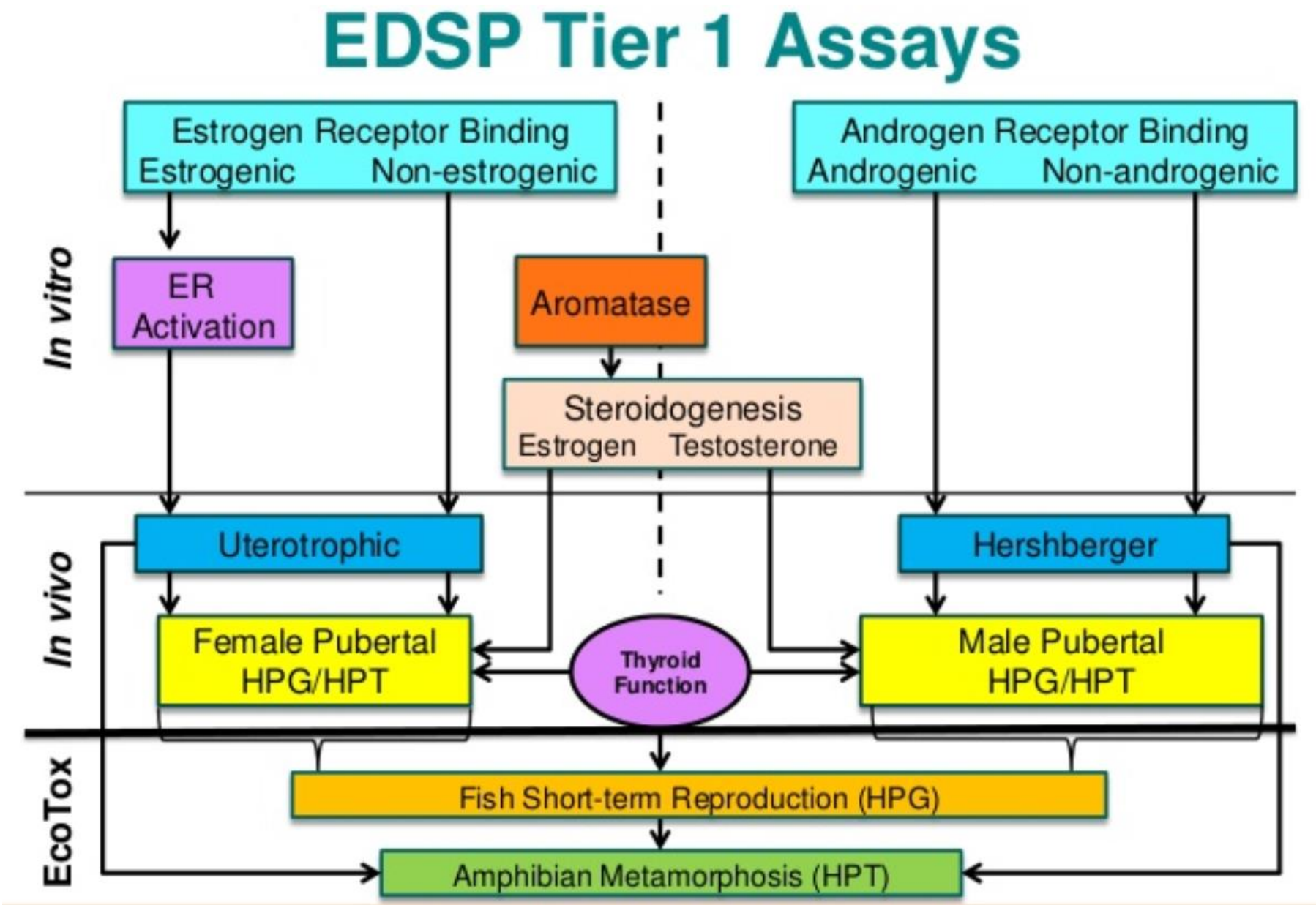


Bisphenol-F



DDT

Endocrine Disruptor Screening Program (EDSP)



Endocrine disruption in thyroid system

Thyroid Disruptors	Mechanism	Effect
Perchlorates, thiocyanate, nitrate, bromates, phthalates	Blocking uptake of iodide into thyroid cell	Decreased synthesis of T3 and T4
Methimazole, amitrole, soy isoflavones, benzophenone 2	Blocking production of TPO in thyroid follicles	Decreased synthesis of T3 and T4
PCBs, pentachlorophenol, flame retardants, phthalates	Competitive binding to thyroid transport protein (TTR)	Possible effect on fetal brain T4 production
Dioxin, PBDE, chlordane	Altering transport across cell membrane	Increased biliary elimination of T3 and T4
Acetochlor (herbicide), PCBs	Enhanced hepatic metabolism	Increased biliary metabolism of T3 and T4
PCBs, triclosan, pentachlorophenol, dioxin, difuran	Inhibition of sulfation	Decreased sulfation of thyroid hormones leading to possible decrease of peripheral T3 synthesis
FD&C red dye #3, PCBs, octyl-methoxycinnamate	Inhibition of deiodinase activity	Decreased peripheral T3 synthesis
PCBs, bisphenol A, hexachlorobenzene, flame retardants	Altering binding to thyroid receptor	Altered thyroid hormone directed gene transcription
DDT, PCBs	Inhibiting TSH receptor	Decreased production of T3 and T4

Endocrine Disruptor Screening Program (EDSP)

OECD conceptual framework

- Level 1 = Existing data and non-test information
- Level 2 = In vitro assays providing data about selected endocrine mechanism(s)/pathway(s)
- Level 3 = In vivo assays providing data about selected endocrine mechanism(s)/pathway(s) => AMA TG 231
- Level 4 = In vivo assays providing data on adverse effects on endocrine relevant endpoint
- Level 5 = In vivo assays providing more comprehensive data on adverse effects on endocrine relevant endpoints over more extensive parts of the life cycle of the organisms

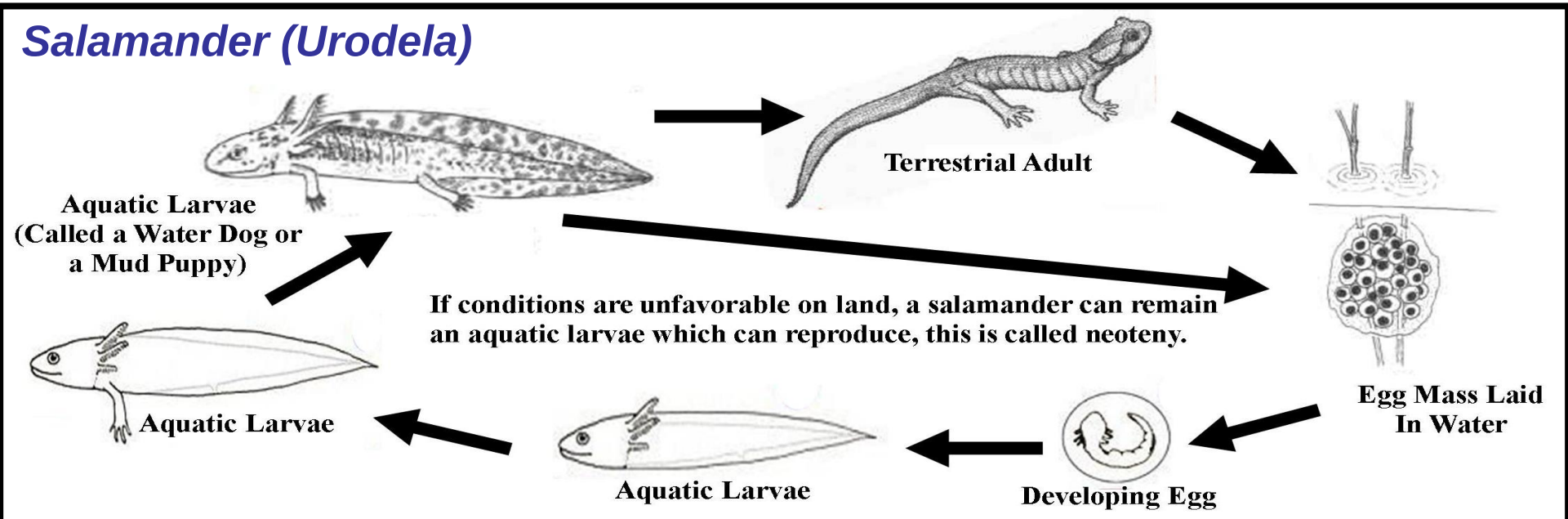
EDSP level3 non-mammalian assay

	Non mammalian toxicology
Level 3 *In vivo* assays providing data about selected endocrine mechanism(s)/ pathway(s)1	Xenopus embryo thyroid signalling assay (when/if TG is available) **Amphibian Metamorphosis assay (OECD TG 231) – (anti-)Thyroid** Fish Reproductive Screening Assay (OECD TG 229) – estrogens, androgens, aromatose inhibitors, Fish Screening Assay (OECD TG 230) -– estrogens, androgens, anit-androgens, aromatose inhibitors, Androgenized female stickleback screen (GD 140)

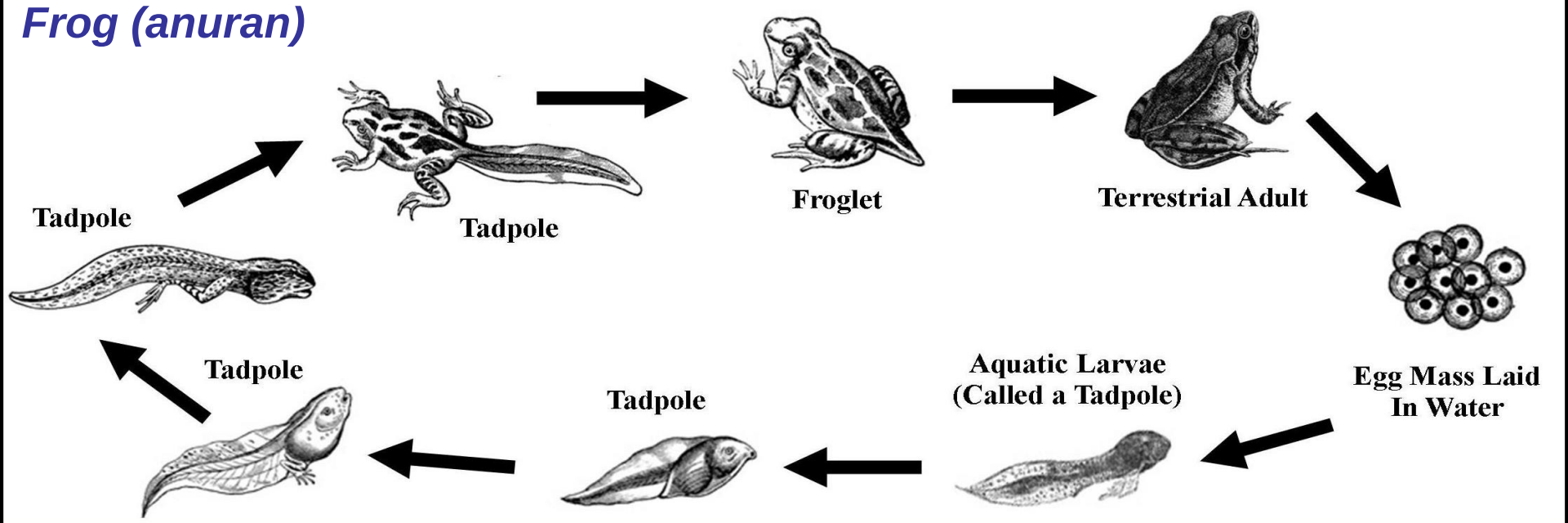
- **OECD TG 231 adopted 7 Sep. 2009.**

Amphibian metamorphosis

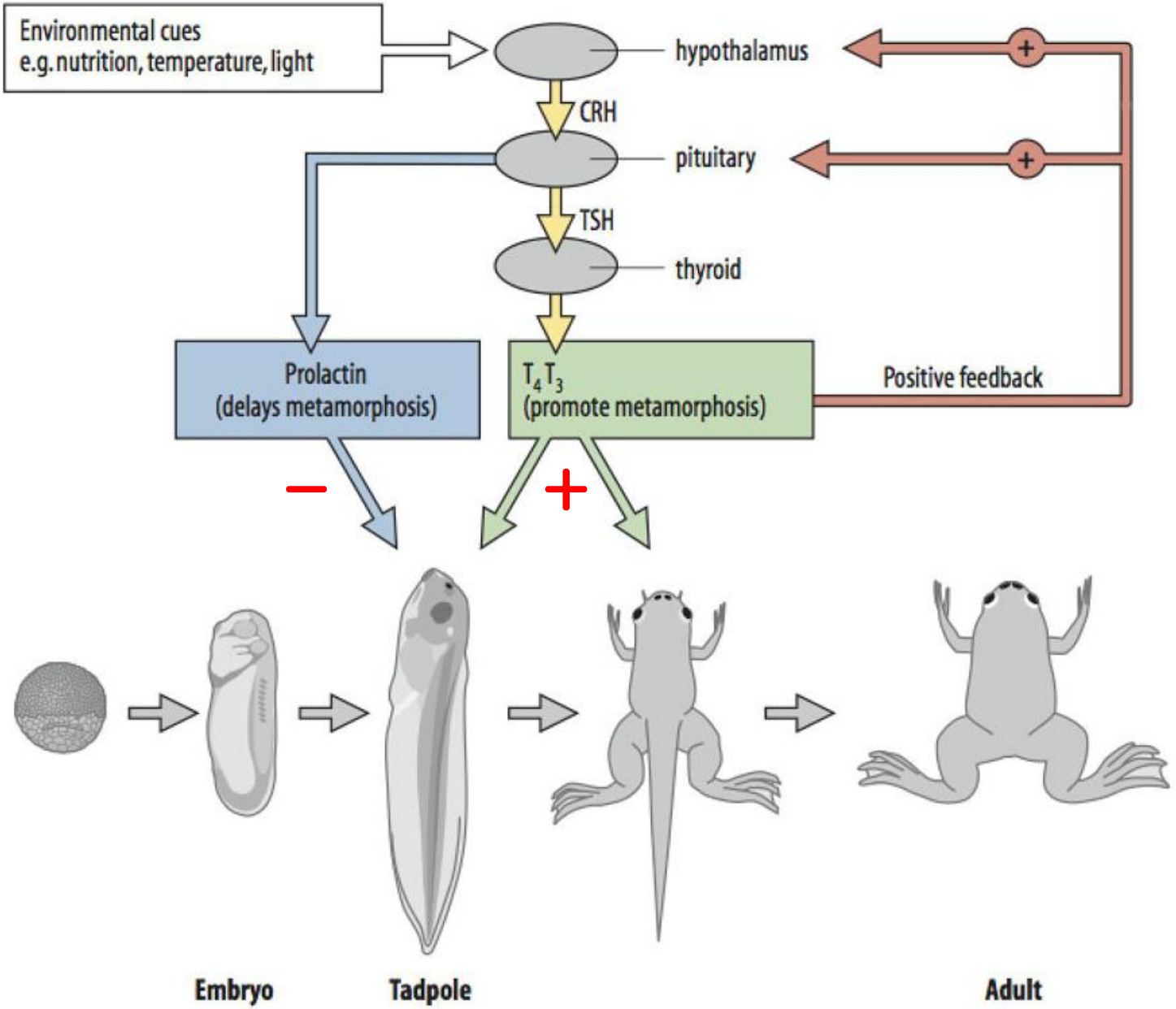
Salamander (Urodela)



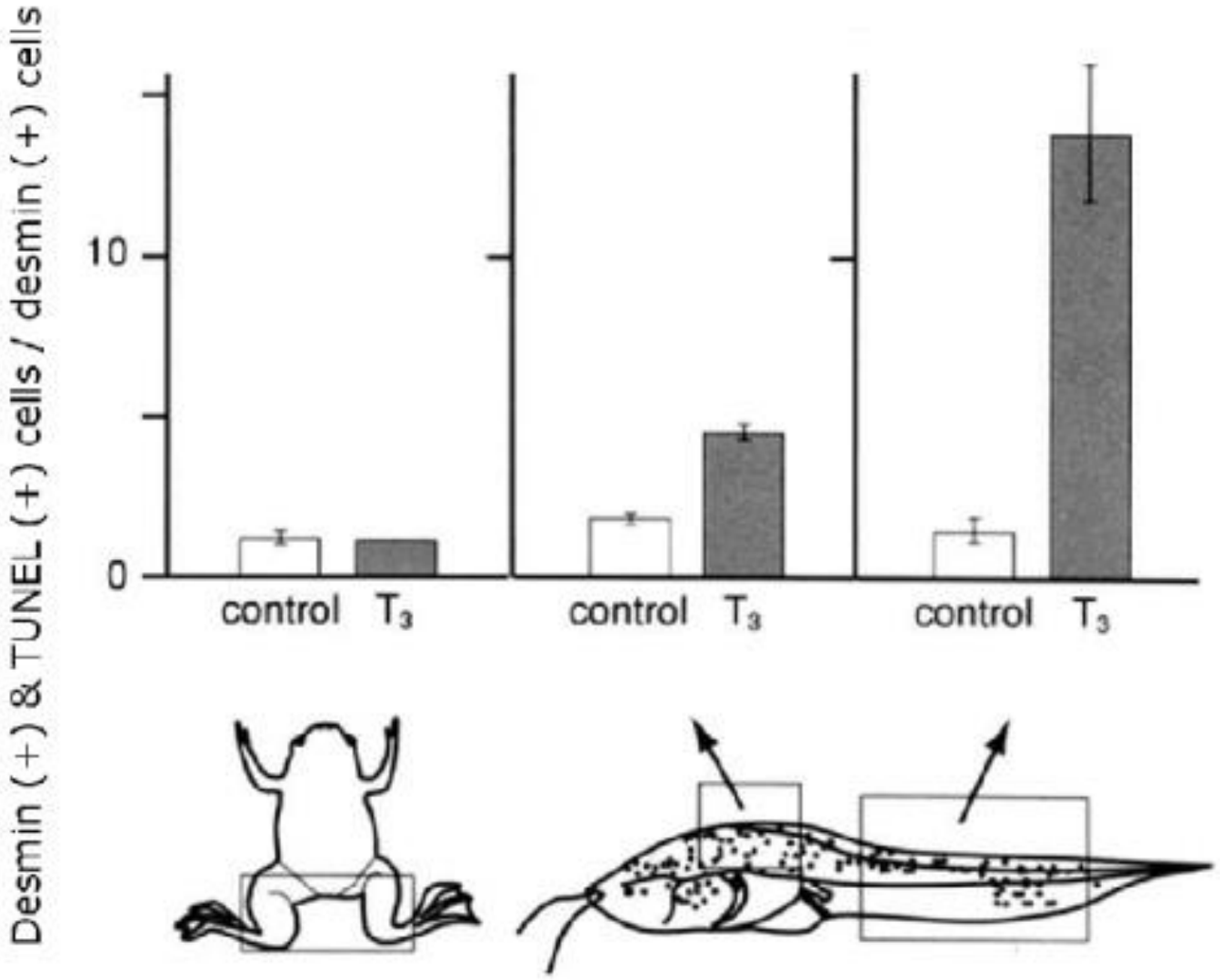
Frog (anuran)



Hypothalamus-Pituitary-Thyroid Axis (HPT axis)

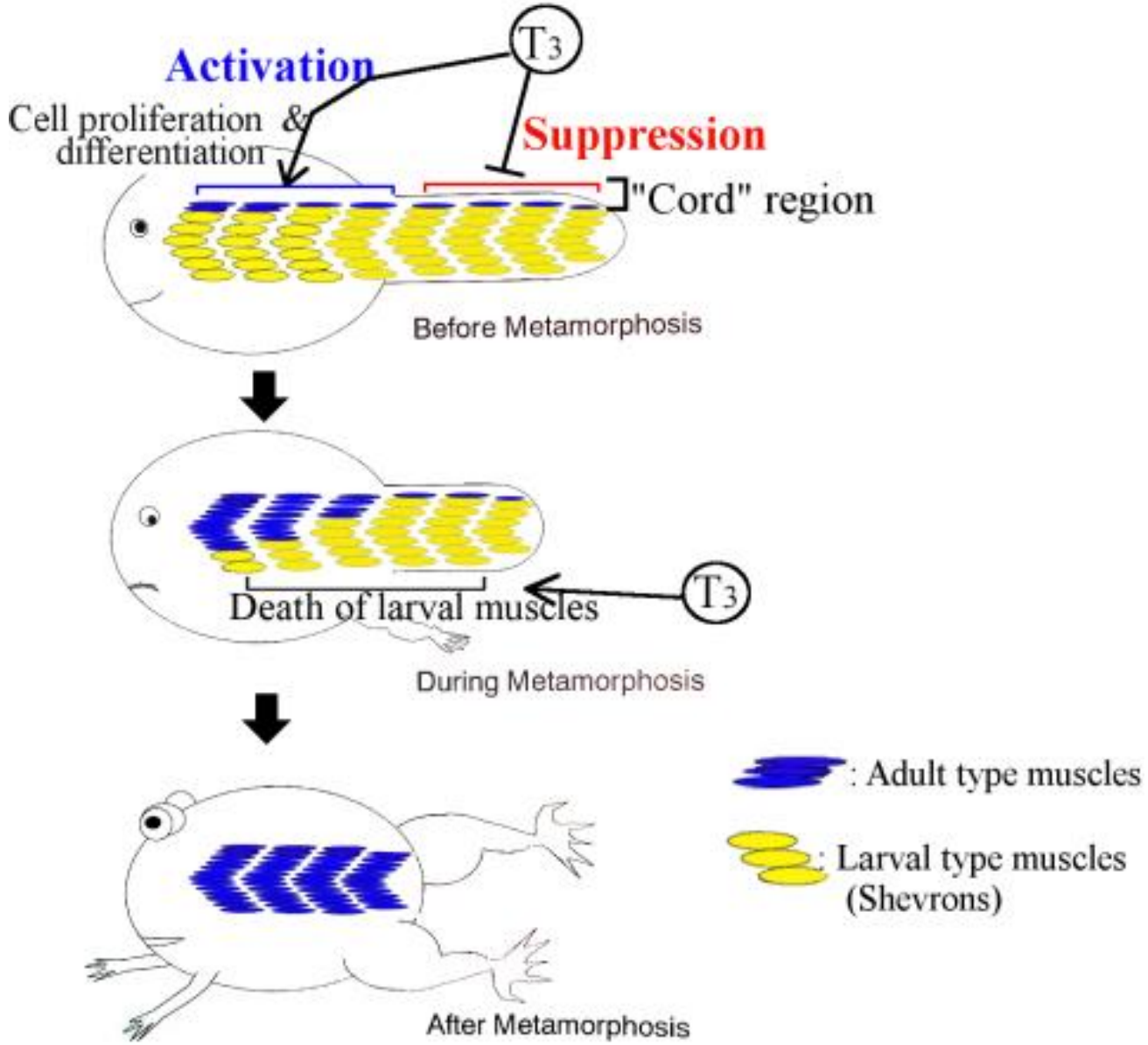


Thyroid hormone(TH)-induced apoptosis



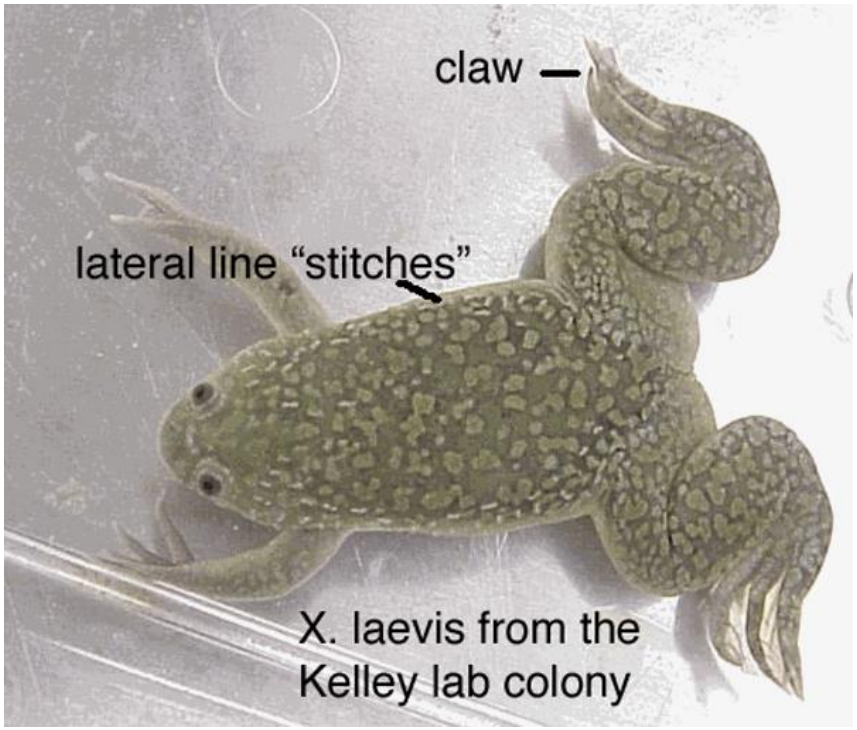
Shimizu-Nishikawa et al., 2002

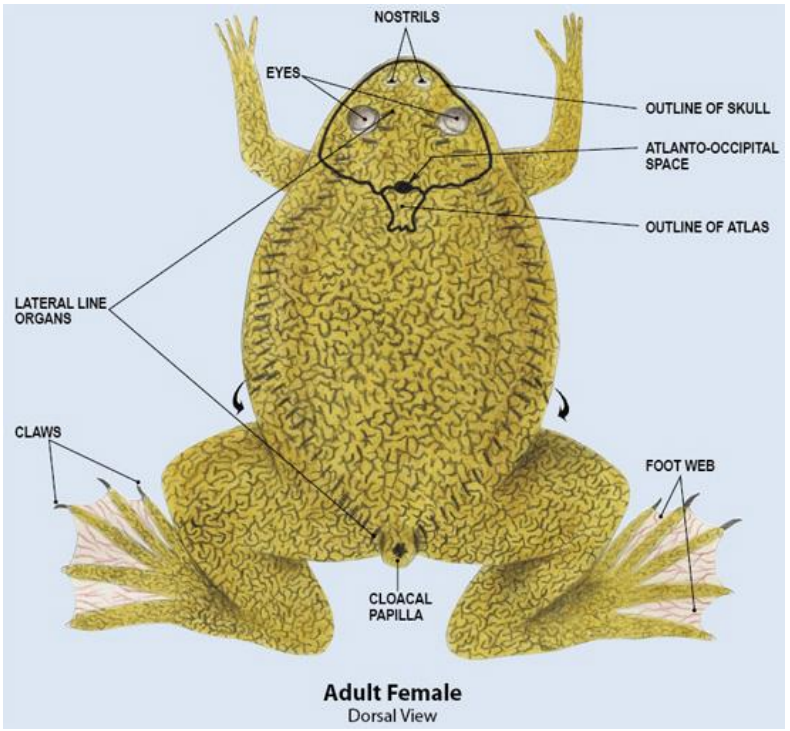
Thyroid hormone(TH)-induced apoptosis



Nishikawa and Hayashi, 1995

African clawed frog (*Xenopus laevis*)





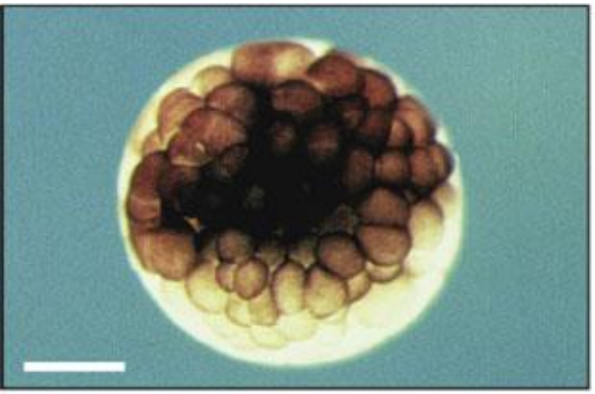
Developmental biologists studied amphibians because…

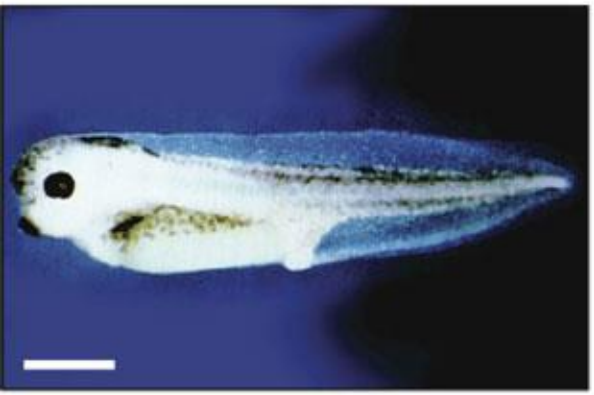
1) the eggs and early embryos were large

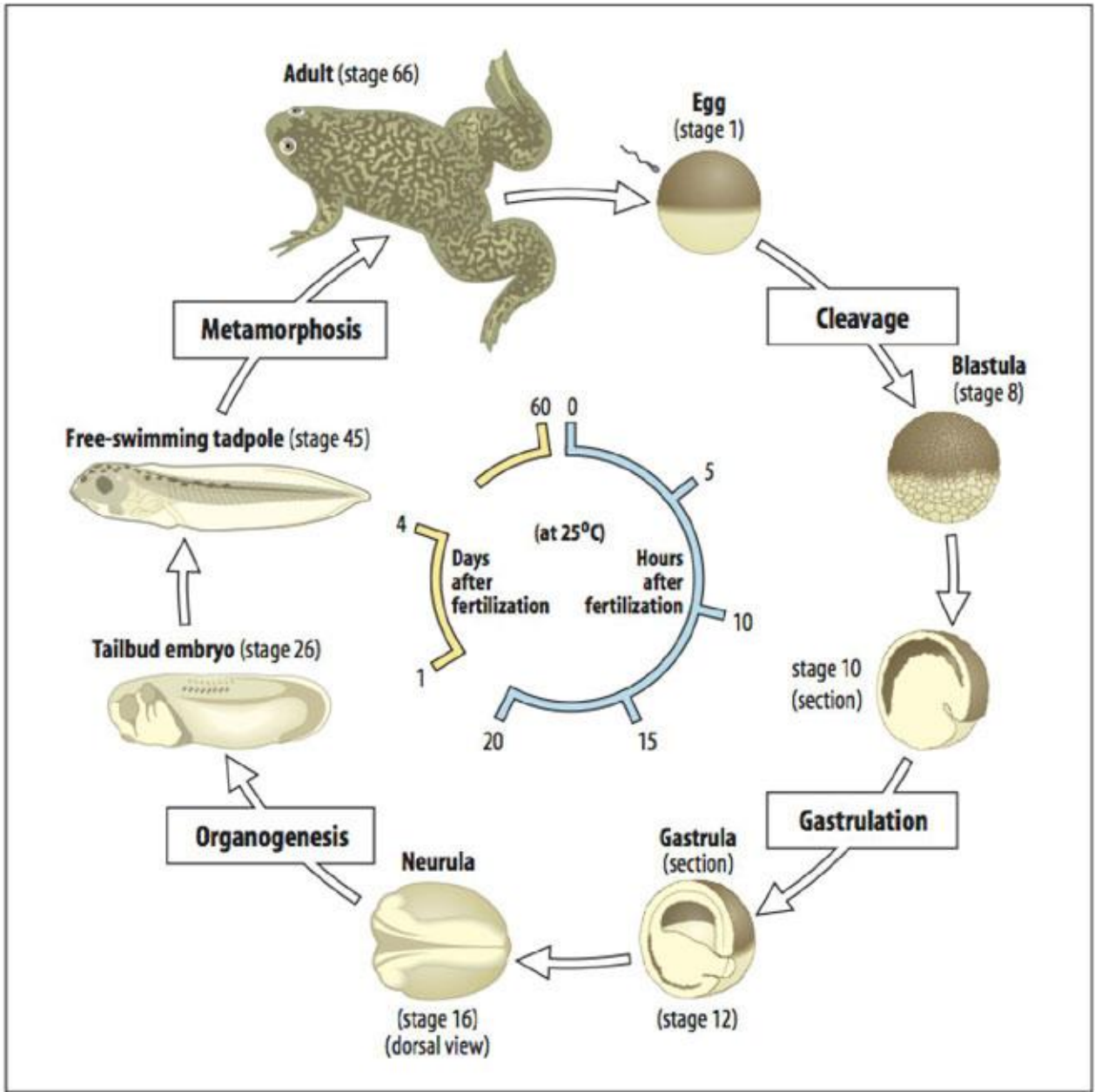
2) experimental manipulations were thus relatively easy

3) development outside of the uterus or egg meant that results could be tracked throughout development

Life cycle of *X. laevis*

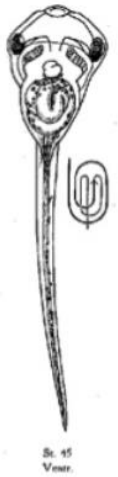






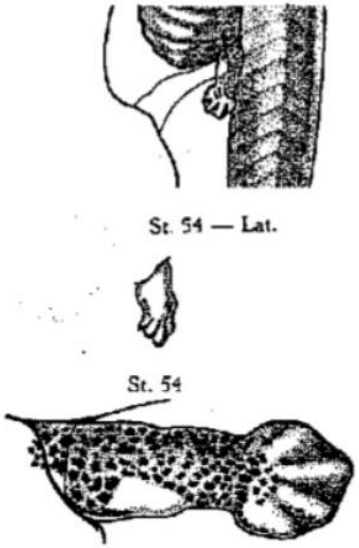
Metamorphosis of *X. laevis*

→ before stage 46 = no need for thyroid hormones = tadpole
→ stage 46 to 53 (pre-metamorphosis) = hind limb visible
→ stage 57/58 (post-metamorphosis) = front limbs visible
→ stage 66 (climax) = tail and gills absorbed = froglet

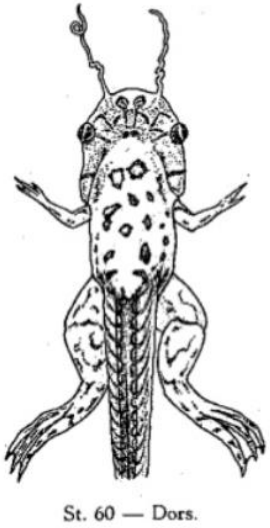


Feeding begins

Exposure begins



Day 7



Optimal Day 21 stage

Amphibian metamorphosis assay (AMA)

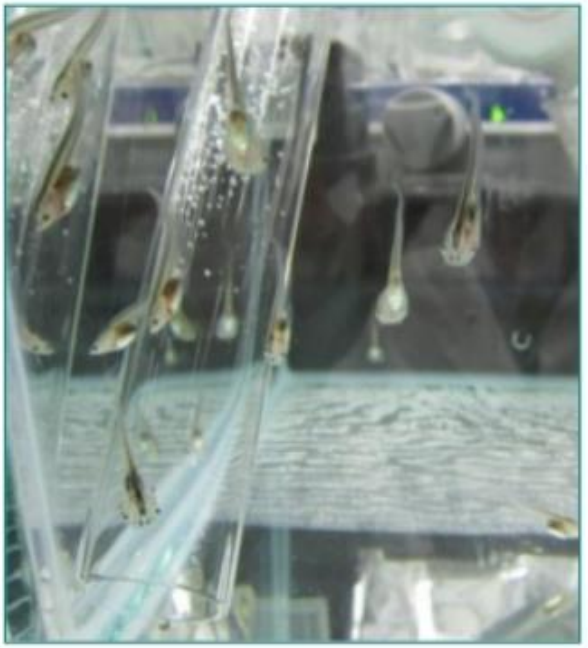
- Duration of 21 days (controls from stage 51 to 60)
- Minimum 3 test concentration plus control (s) with 4 replicates and 20 tadpoles/vessel
- Concentrations separated by factor between 0.1 (max) to 0.33 (min) over at least one order of magnitude
- Highest test level = maximum tolerated concentration (MTC; 10% acute mortality), limit of solubility or 100 mg/L; whichever is lowest

- if no relevant data, range finding test is recommended
  - wide spaced concentrations
  - 1 replicate/concentration with 10 tadpoles
  - 7 to 14 days duration

Amphibian metamorphosis assay (AMA)

Day 0

- Tadpoles pooled and individually staged = 51
- Measure whole body length of a sample of 20 tadpoles ± 3 mm
  (mean: 24-28 mm for stage 51)
- Verify test concentrations achieved
- Water quality in all vessels = temperature, dissolved oxygen, pH
- Water quality in control(s), low and high concentrations = hardness, alkalinity and TOC
- Randomly distributed to control & test vessels : 20 in each



Amphibian metamorphosis assay (AMA)

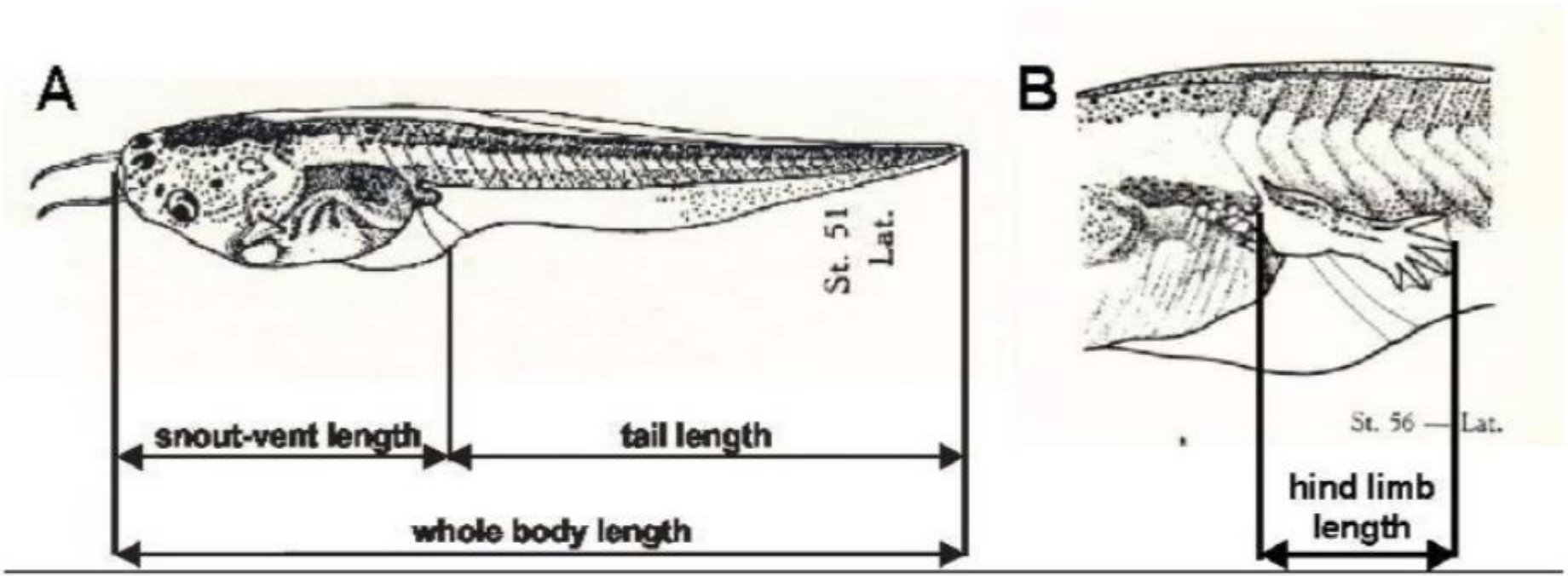
Day 7

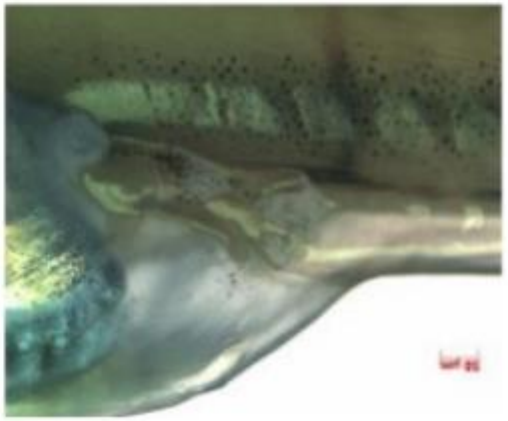
- 5 tadpoles/vessel – euthanised, measured and discarded
  - wet weight (mm)
  - developmental stage
  - snout-vent length (SVL)
  - hind limb length (HLL; left)

Day 21

- Remaining tadpoles/vessel – euthanised, measured and placed in fixative and retained
  - as for Day 7 plus
  - select 5 tadpoles/vessel for thyroid gland histology

Amphibian metamorphosis assay (AMA)

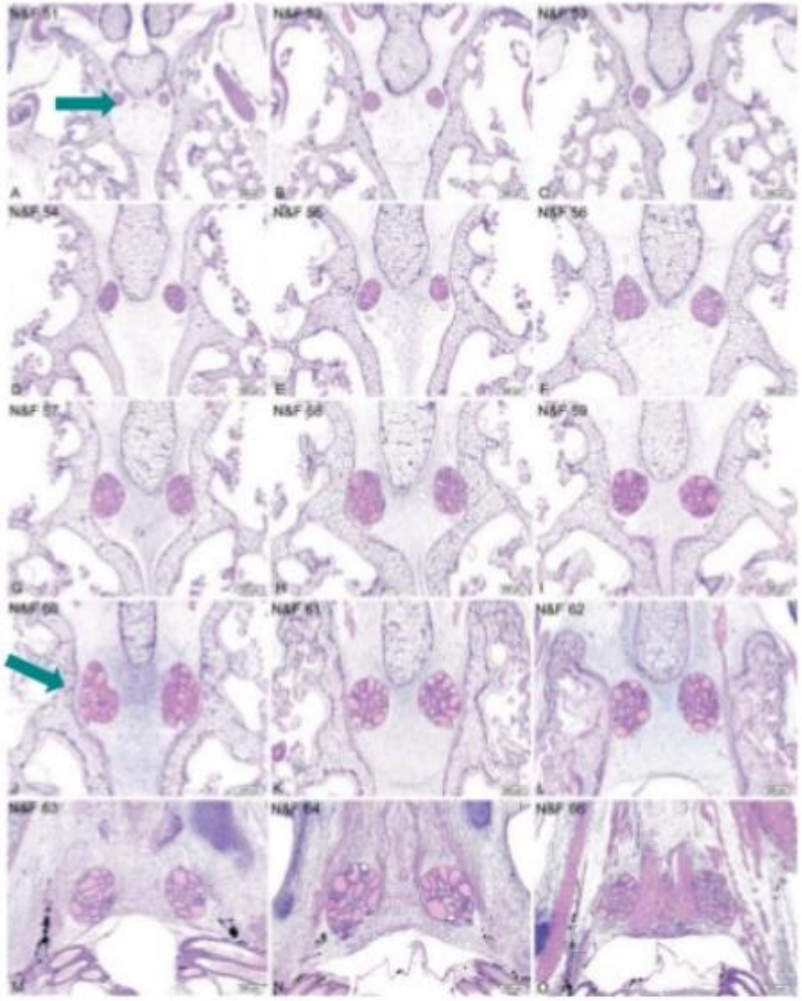




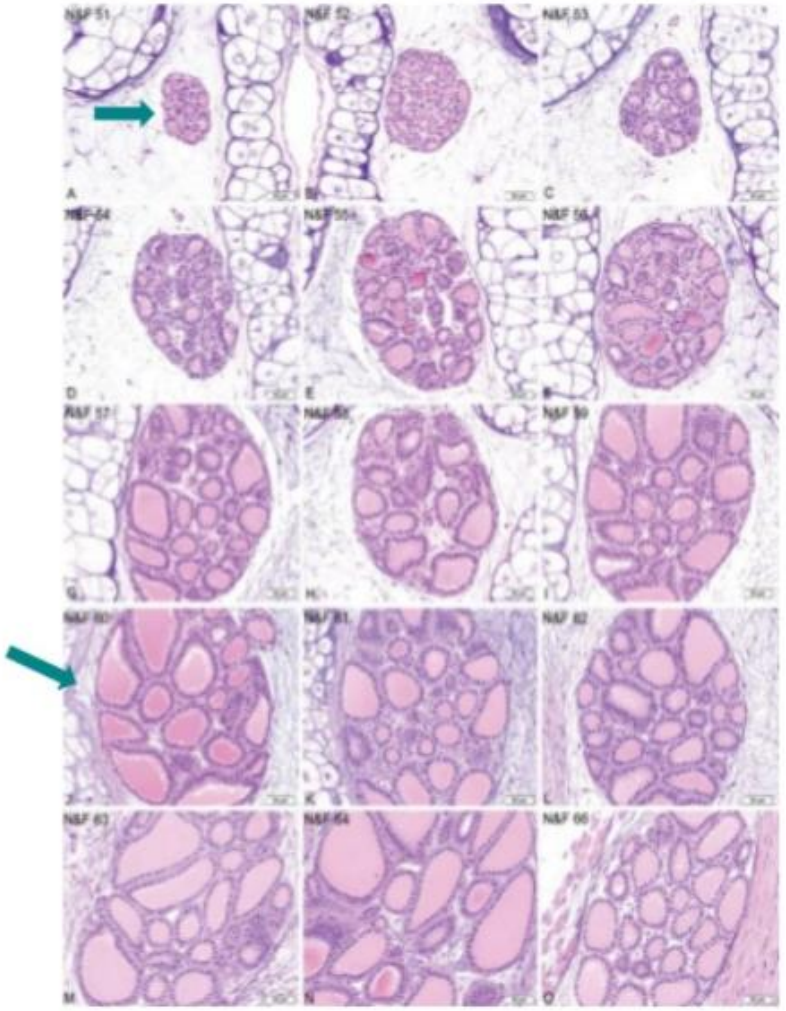
Scale marker = 0.1cm

Amphibian metamorphosis assay (AMA)

development of thyroid gland



thyroid gland



Toxicologic Pathology, 37: 415-424, 2009; K. Christiana Grim *et al*

Amphibian metamorphosis assay (AMA)

Criteria

- thyroid gland : atrophy / hypertrophy (decrease/increase in gland size)
- follicular cell : hypertrophy (change in cell shape – monitor number of tall columnar cells)
- follicular cell : hyperplasia (cell crowding, stratification or papillary infolding)

- Other (qualitative) : colloid quality, follicular lumen area and follicular cell height/shape

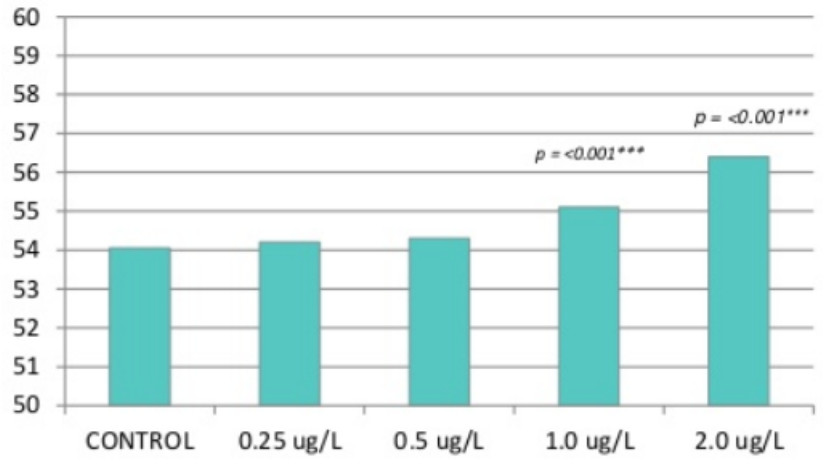
4 severity grade

- 0 = none to minimal (<20% effect)
- 1 = mild or slight (30 to 50% effect)
- 2 = moderate (60 to 80% effect)
- 3 = severe (>80% effect)

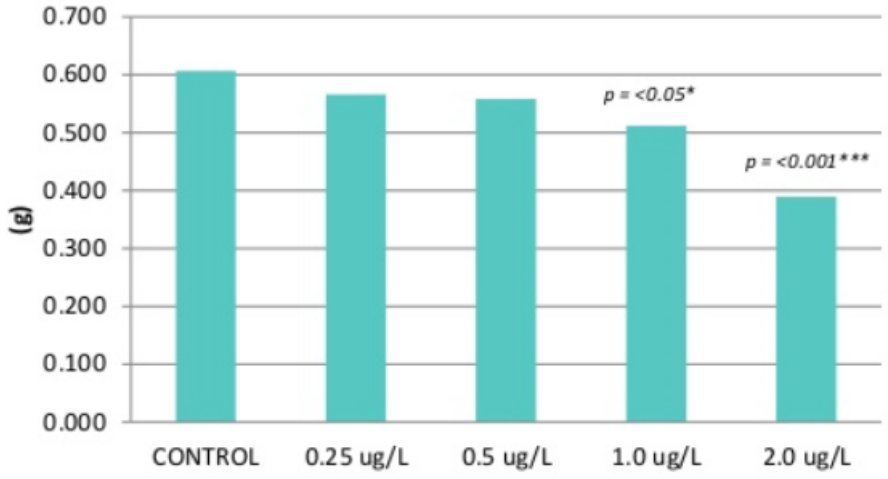
Results of AMA (validation)

Day 7 - Thyroxine

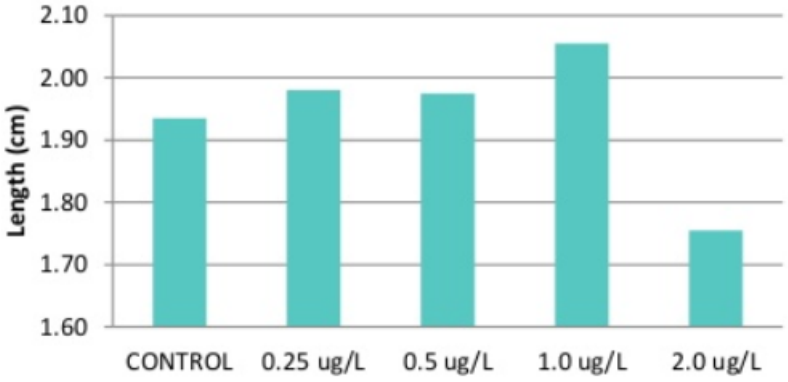
Stage



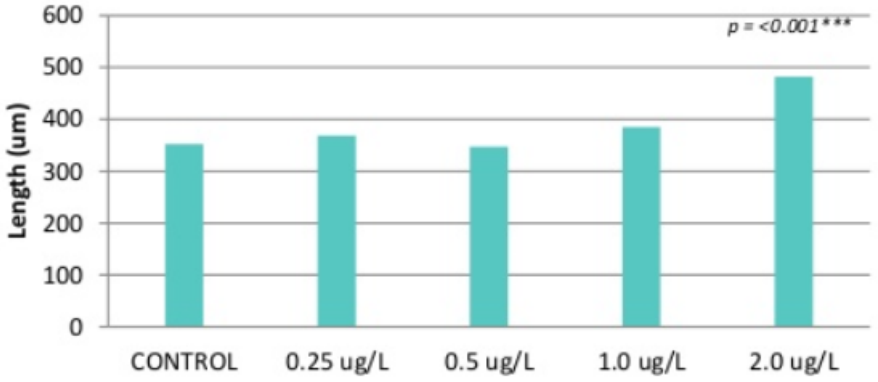
Body Weight



Snout-Vent Length



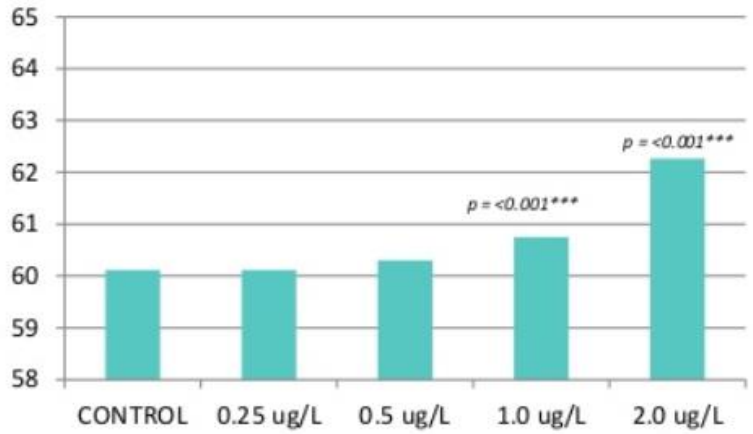
Hind Limb Length



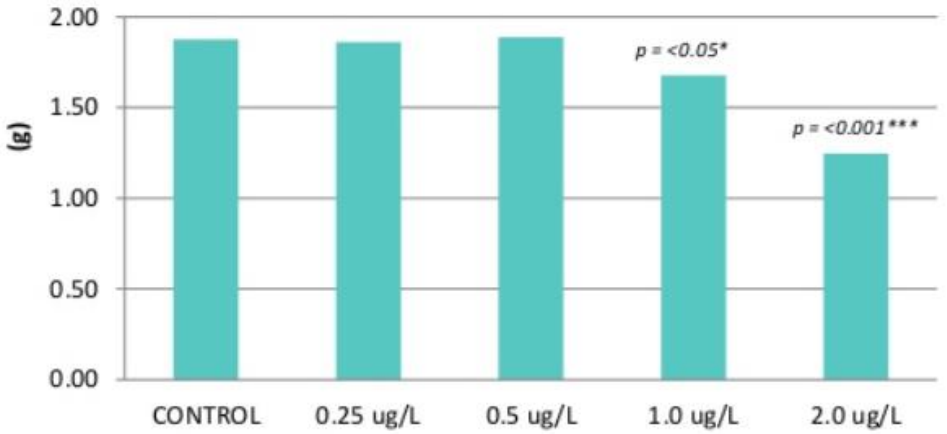
Results of AMA (validation)

Day 21- Thyroxine

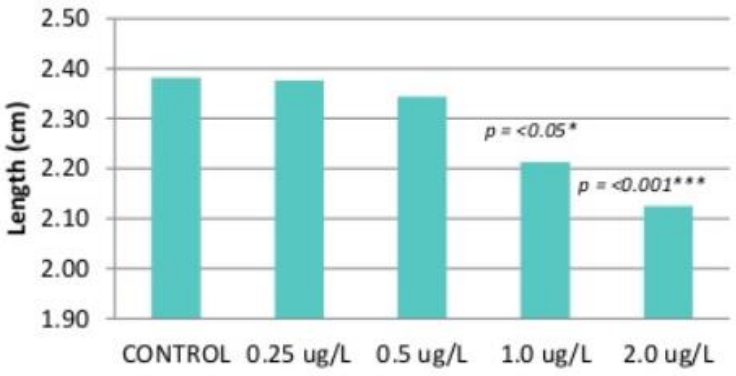
Stage



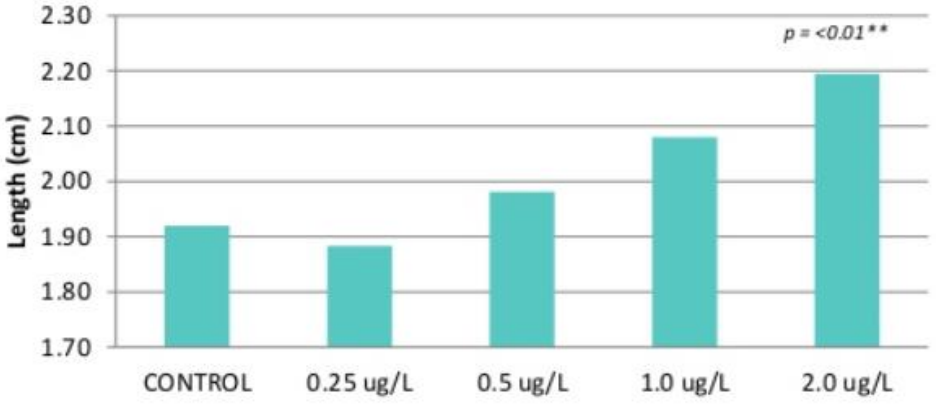
Body Weight



Snout-Vent Length



Hind Limb Length



Results of AMA (validation)

Results - Thyroxine

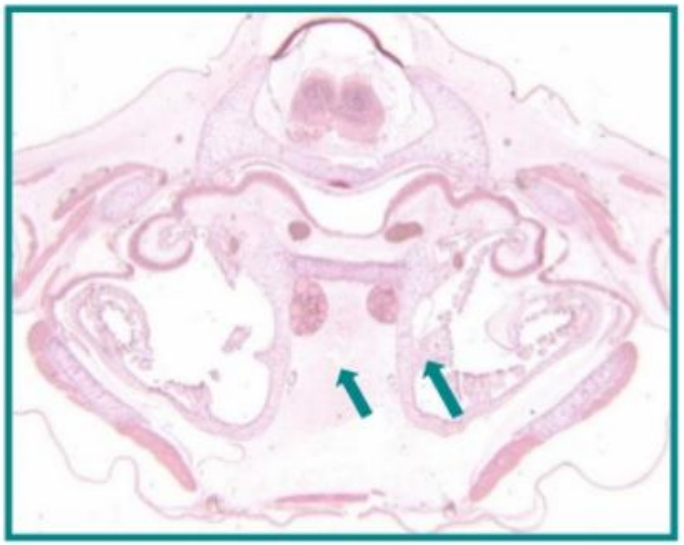
Scale marker = 0.1cm

Day 21: Control

Day 21: 2.0 µg/L Thyroxine

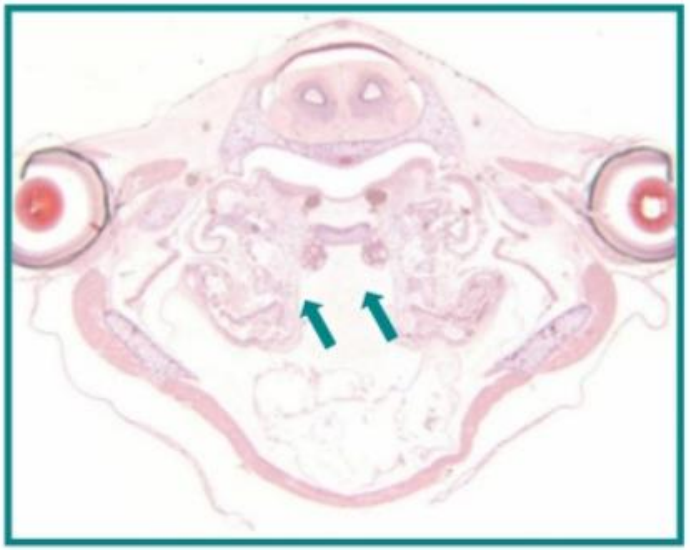
Results of AMA (validation)

Day 21: Control



x 10

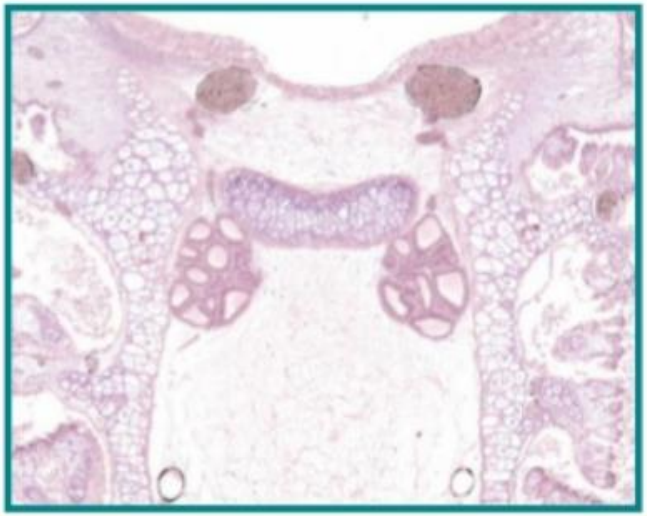
Day 21: 2.0 µg/L Thyroxine



Normal thyroid

Thyroid atrophy

x 100

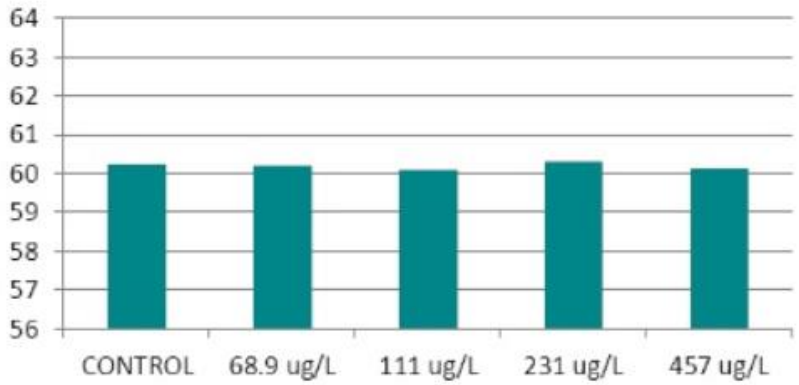


Results of AMA

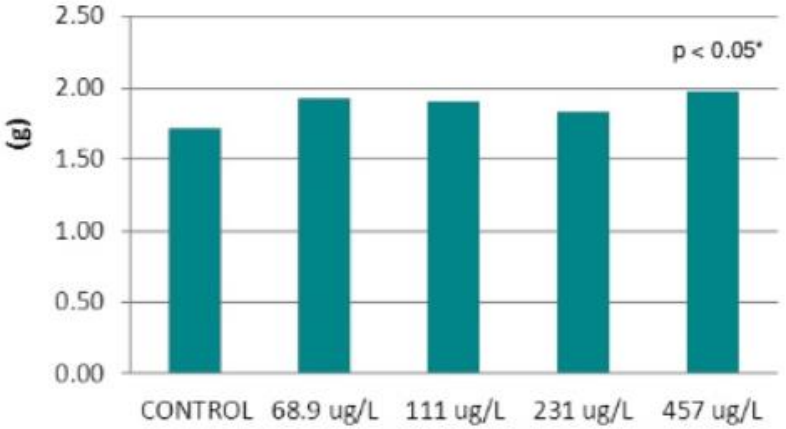
Day 21 - Sodium perchlorate

Iodine uptake blocker

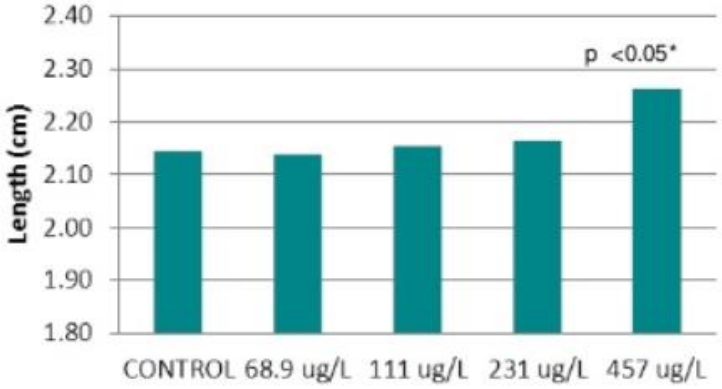
Day 21 Stage



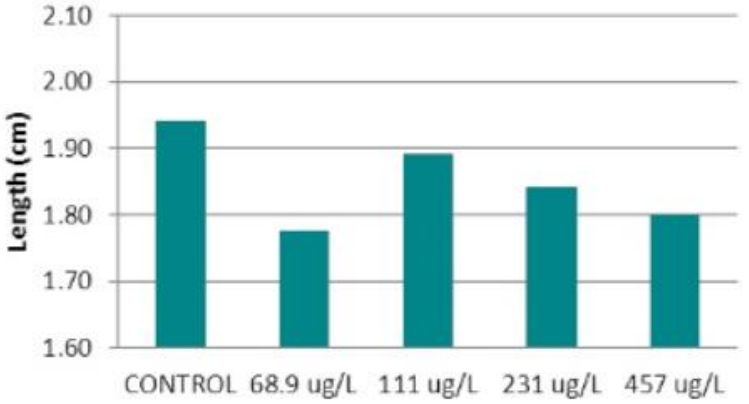
Body Weight



Snout-Vent Length



Hind Limb Length

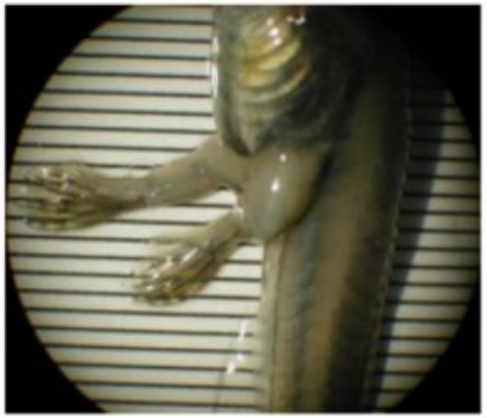


Day 21 – Sodium perchlorate

Iodine uptake blocker

Control

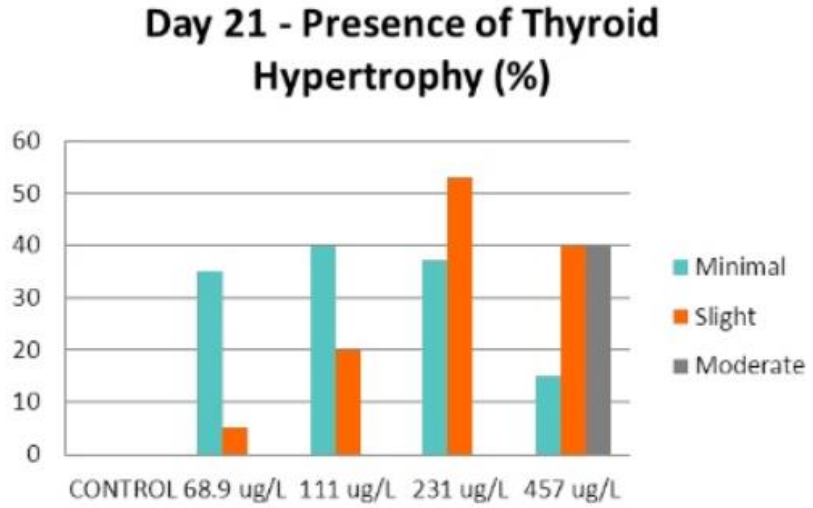
Treatment

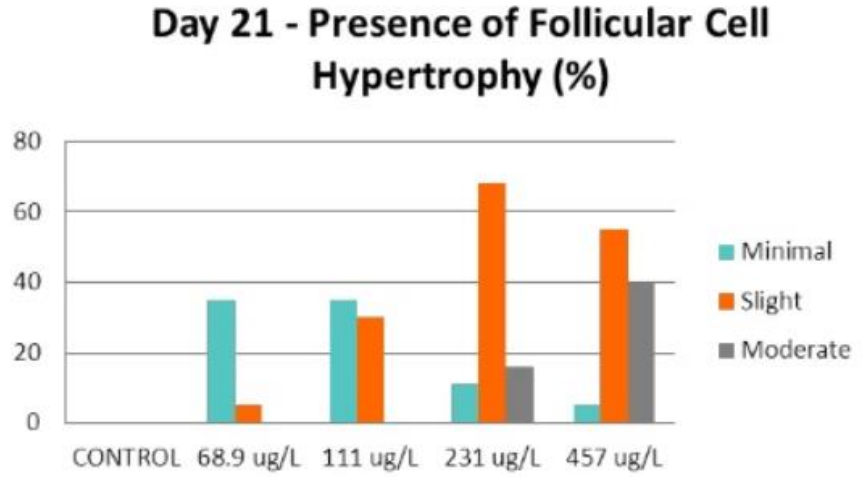


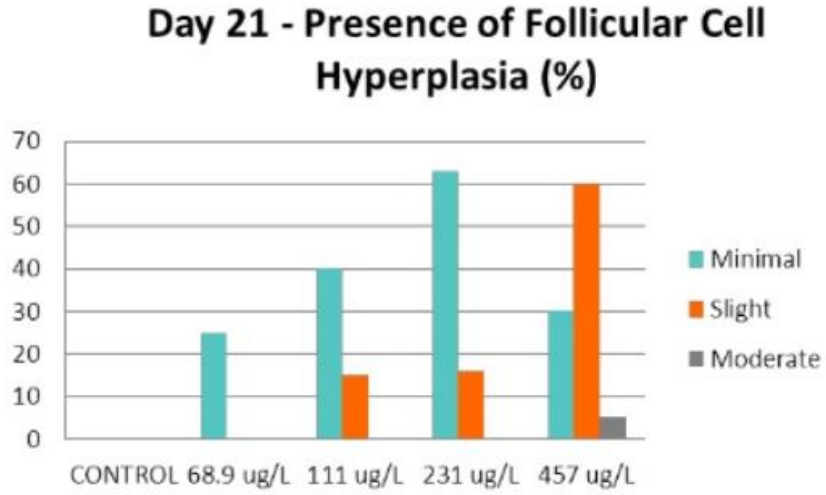
Scale marker = 0.1cm

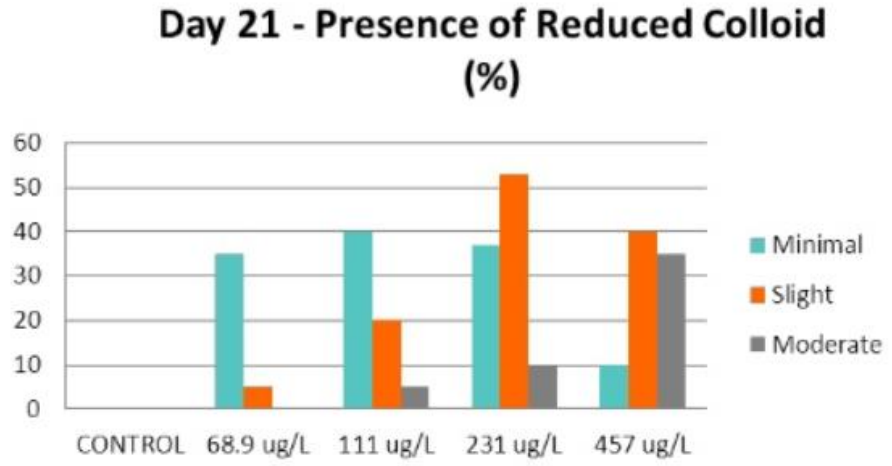
Results – Sodium perchlorate

Iodine uptake blocker

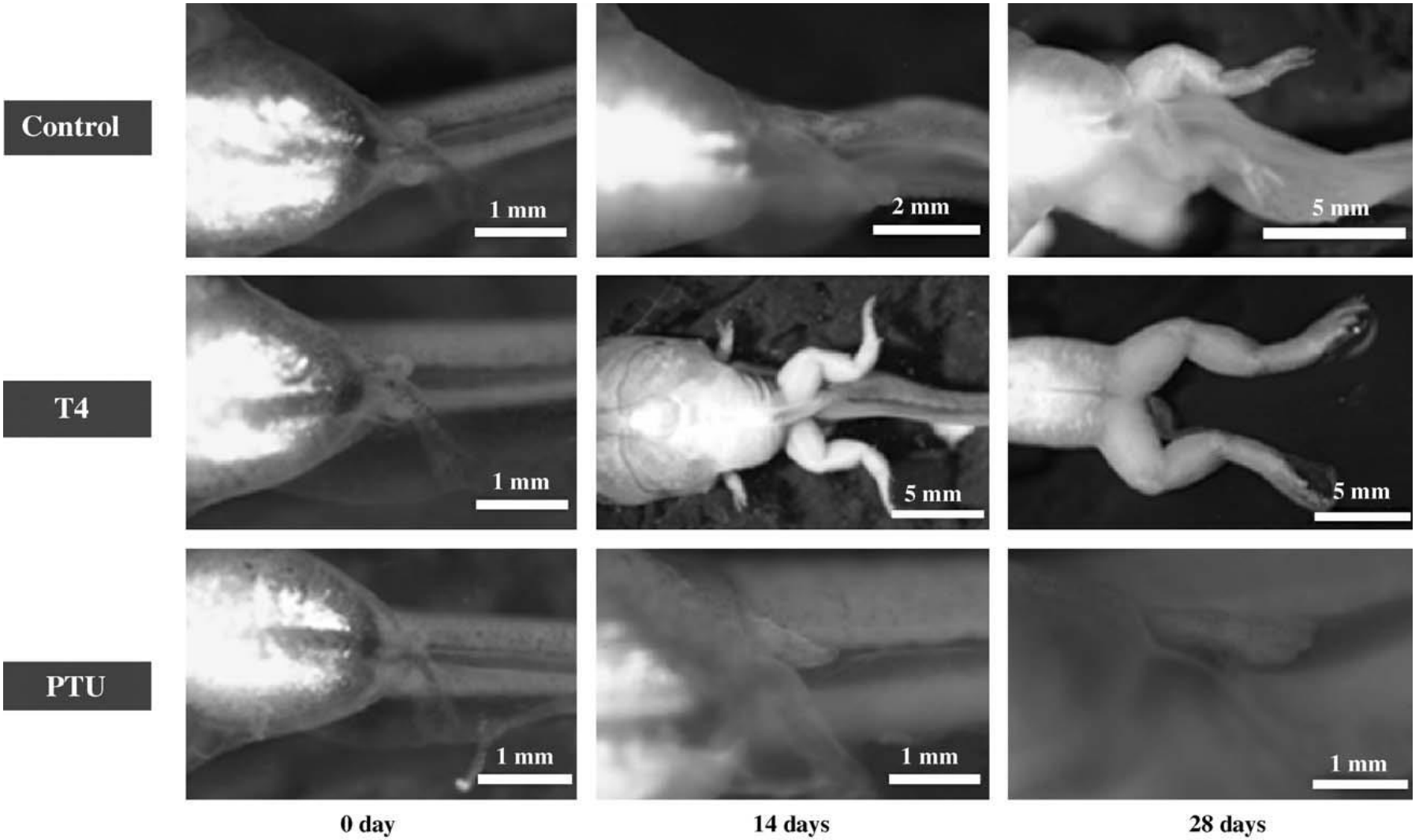




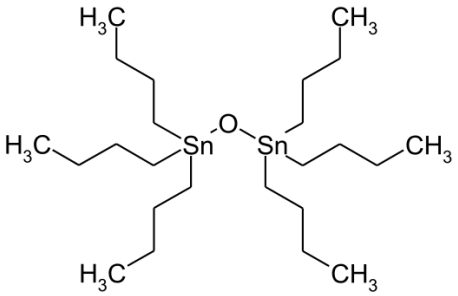




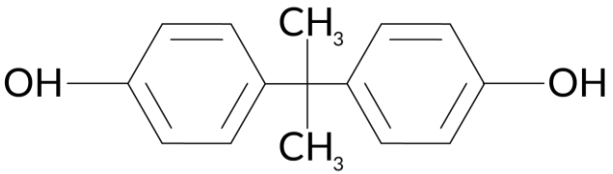
Results of AMA (Propylthiouracil; PTU)



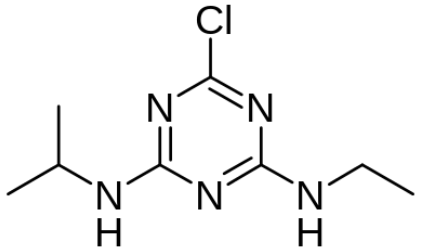
Thyroid disrupting chemicals in AMA screening



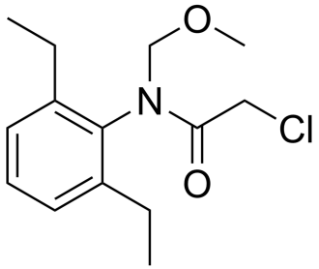
Tributyltin



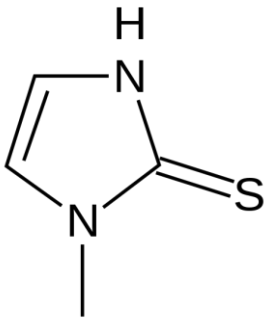
Bisphenol-A



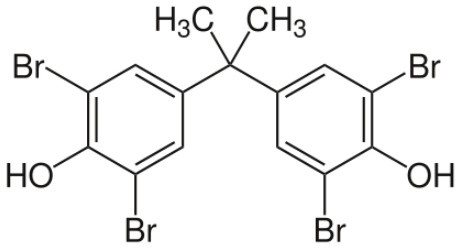
Atrazine



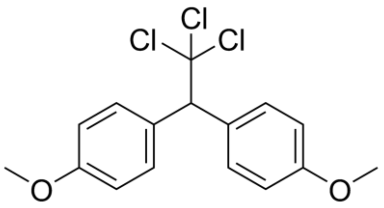
Alachlor



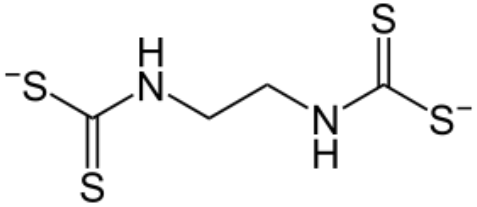
Methimazole



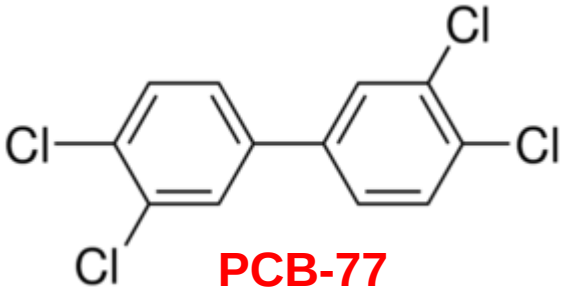
TBBPA



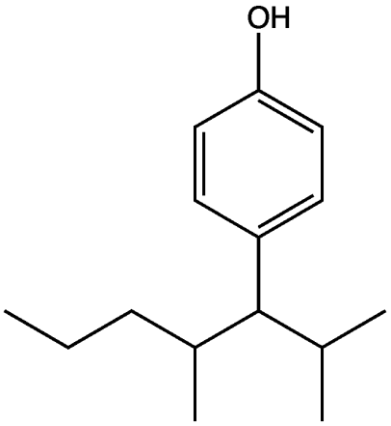
Methoxychlor



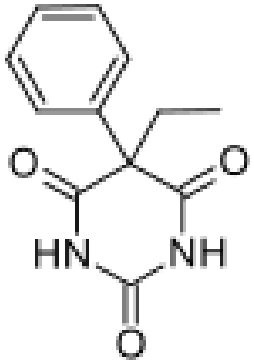
Zineb



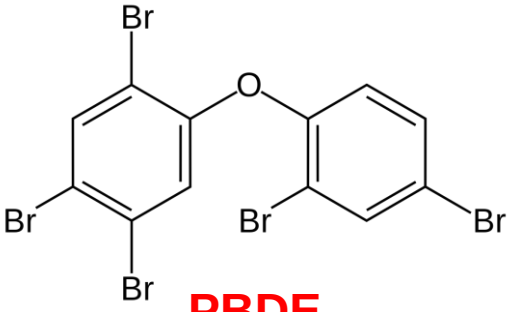
PCB-77



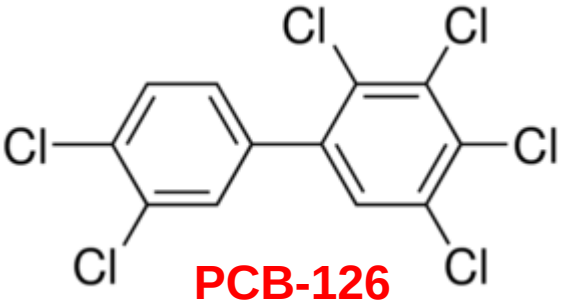
Nonylphenol



Phenobarbital



PBDE
(Pentabromodiphenyl ether)



PCB-126

Alternative species

Wrinkled frog (*Rana rugosa*) - Japan

Ecotoxicology and Environmental Safety 72 (2009) 1400–1405

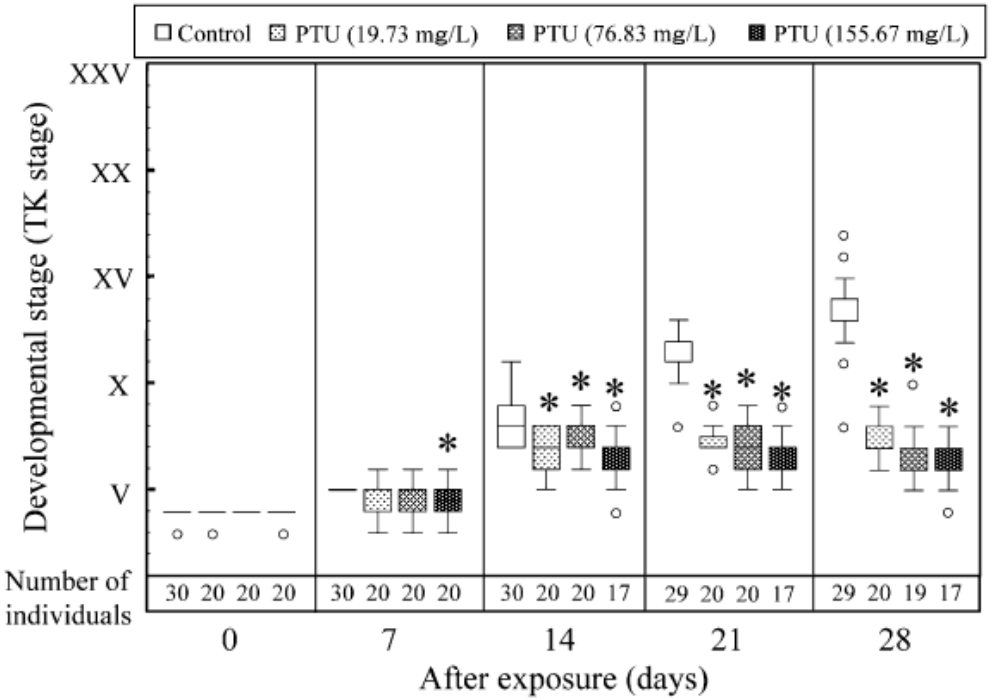
Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Application of metamorphosis assay to a native Japanese amphibian species, *Rana rugosa*, for assessing effects of thyroid system affecting chemicals

Tomohiro Oka [a], Maki Miyahara [a], Jun Yamamoto [a], Naoko Mit
Keiko Kashiwagi [c], Minoru Takase [c], Akihiko Kashiwagi [d], Taise



Alternative species

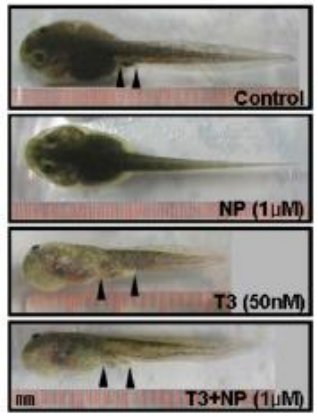
Fire-bellied toad (*Bombina orientalis*) - Korea

Chemosphere 81 (2010) 1292–1300

Contents lists available at ScienceDirect

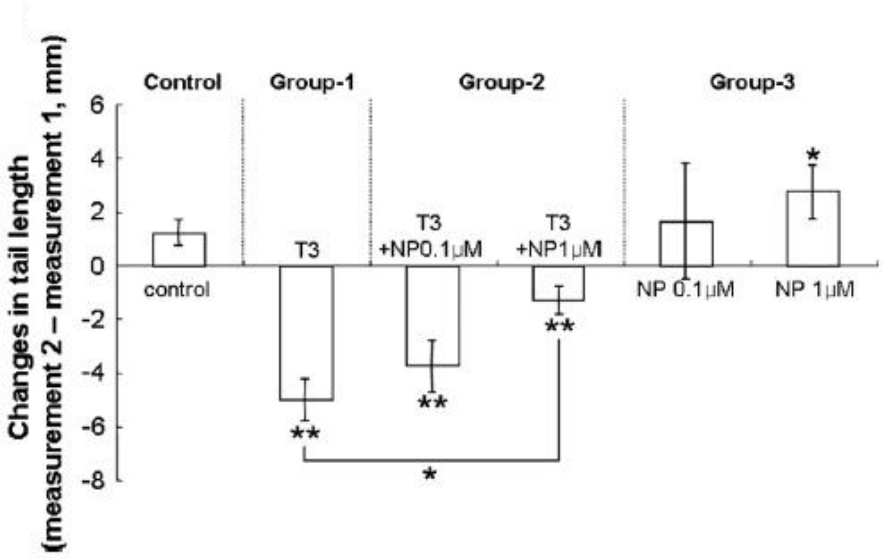
Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere



Effects of nonylphenol on early embryonic development, pigmentation and 3,5,3′-triiodothyronine-induced metamorphosis in *Bombina orientalis* (Amphibia: Anura)

Chan Jin Park, Han Seung Kang, Myung Chan Gye*







Thank you ！