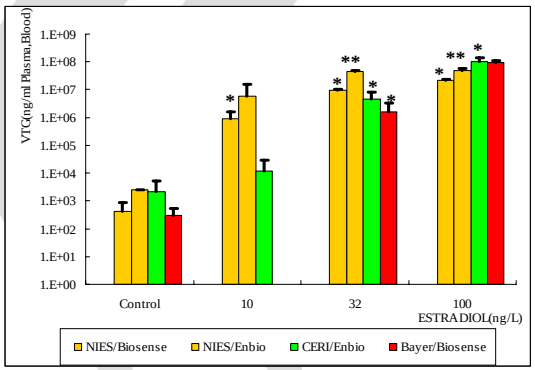
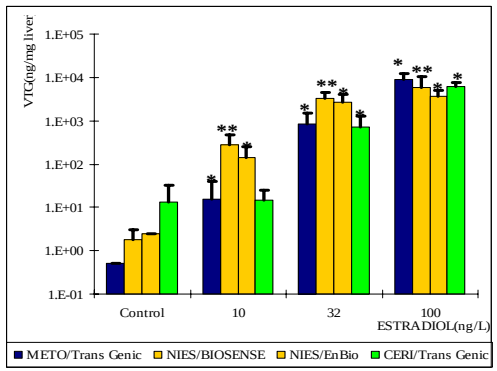
In vivo Fish Screens for ED (3)

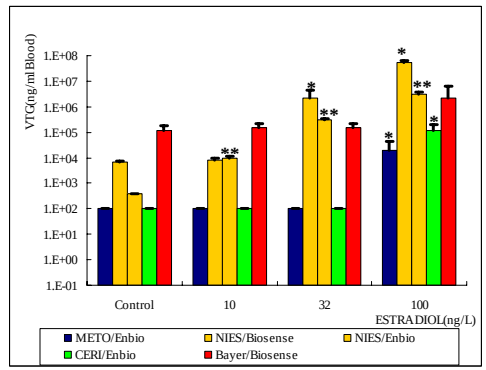
VTG in Males after 17β estradiol exposure (14-d) - Phase 1A



Fathead minnow

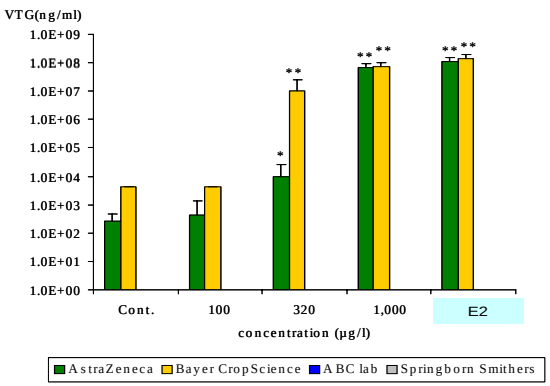


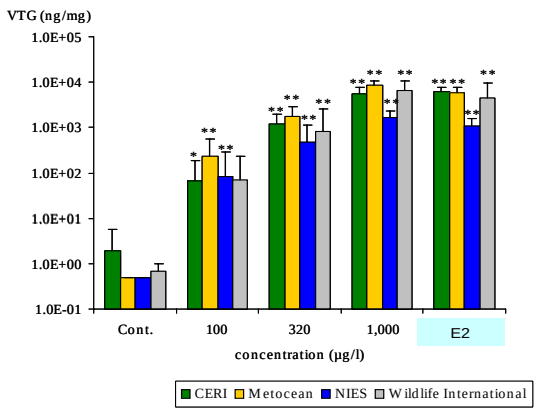
Medaka

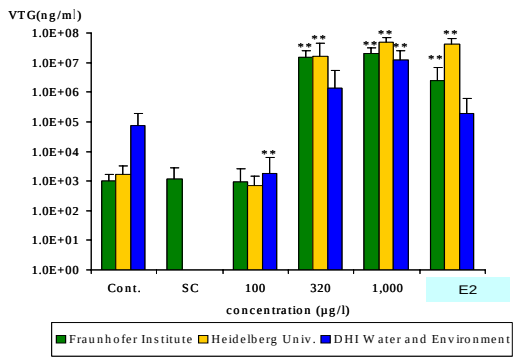


Zebrafish

VTG in Males after 4 tPP exposure (21-d) - Phase 1B

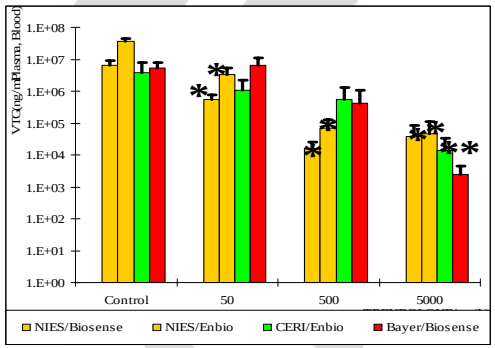




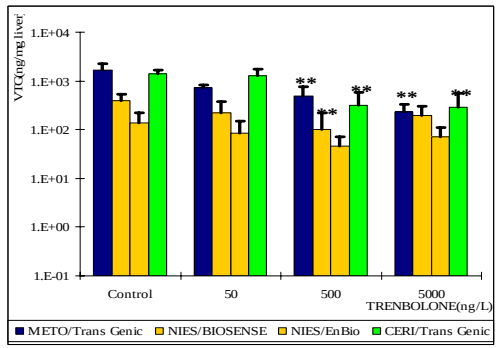


In vivo Fish Screens for ED (4)

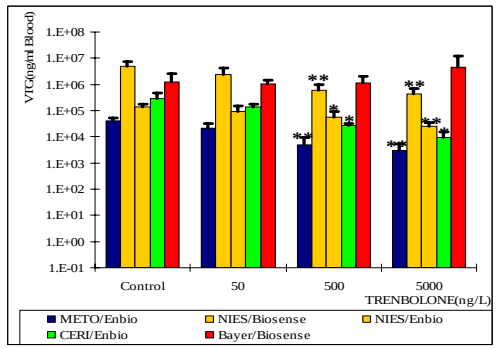
VTG in Females after trenbolone exposure (14-d) - Phase 1A



Fathead minnow

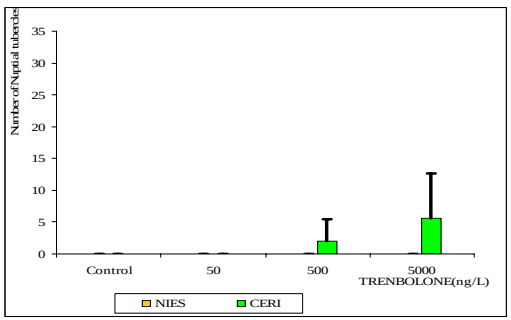


Medaka

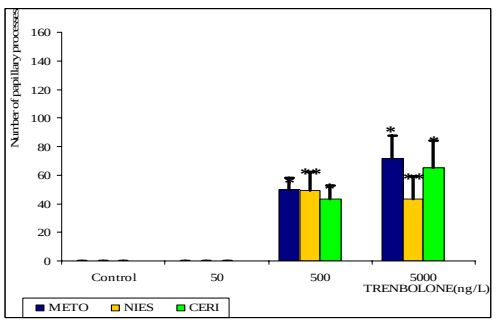


Zebrafish

Secondary sex characteristics in Females after trenbolone exposure (14-d) - Phase 1A



Number of papillary processes in ♀ medaka (♂ sex character)

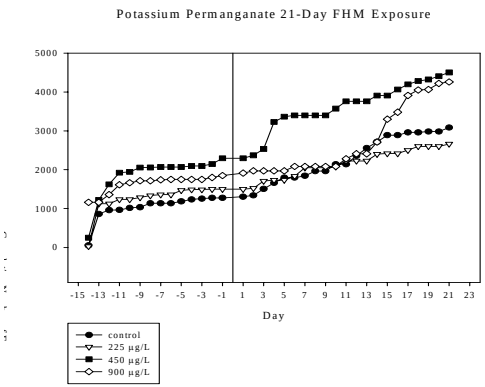


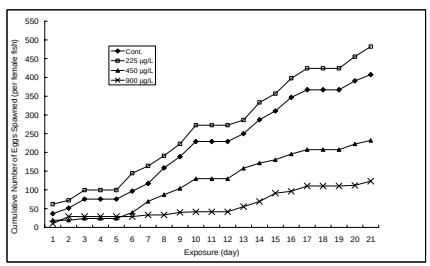
Number of nuptial tubercles in ♀ fathead minnow (♂ sex character)

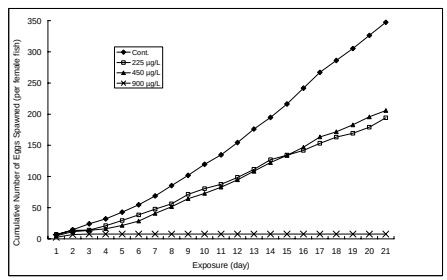
No secondary sex characteristics responsive to endocrine active substances in zebrafish

In vivo Fish Screens for ED (5)

- Negative substances tested (→ false positives rate):
  - Potassium permanganate (225, 450, 900µg/l)
    - No response on VTG
    - ↓ SSC at 900 µg/l in FHM
    - ↓ fecundity in ZF, MDK (//lethality in medaka)





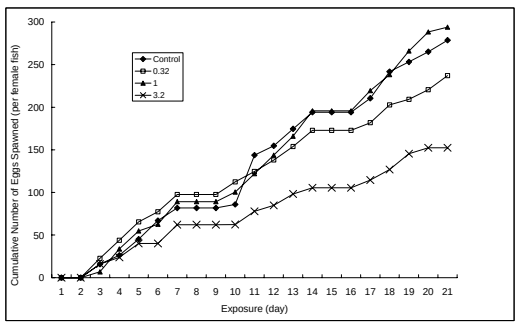


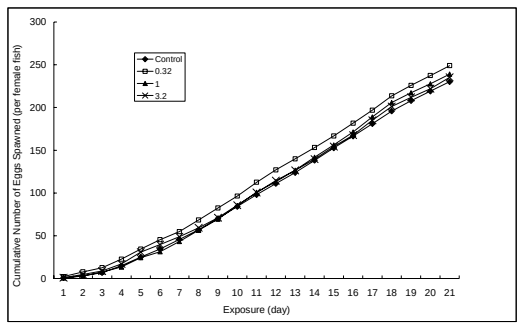
FHM **ZF** **MDK**

Fecundity in potassium permanganate studies

In vivo Fish Screens for ED (6)

- Negative substances tested (→ false positives rate):
  - n-octanol (0.32, 1.0, 3.2 mg/l)
    - No response on VTG (slight ↑ in ZF, but not significant)
    - No response on SSC (FHM results still missing)
    - ↓ fecundity in ZF





ZF **MDK**

Fecundity in n-octanol studies

21-day Fish screening assay: next steps

- VMG-eco to agree on the completion of the validation (Dec. 2005)
  - Strong ref. substances, weak substances, negative substances tested
  - Relevance, reproducibility between labs and over time evaluated
  - Coded substances=probably not appropriate
- Review statistics and power of the assay to minimise animal use and ensure cost-efficiency
- Organise a peer-review of the validation data
- Identify member country to take the lead in the development of the TG

Other fish tests in pre-validation (1)

- Fish sexual development test (Lead: Nordic countries)
  - enhanced OECD TG210
  - duration extended to 60-d
  - additional endpoints : VTG, sex ratio

Substance	FSDT (pre-validation data-DK)		FSCA (Ph.1A-Ph1B)	
	VTG	Sex ratio	VTG	SSC
17β-estradiol	↑ 54ng/l	54ng/l	↑ 32 ng/l	↓ 100ng/l (fhm)
Trenbolone	↓ 117ng/l	9.7ng/l	↓ 500ng/l	↑ 500ng/l
4tPP	↑ 274µg/l	68 µg/l	↑ 320µg/l	↓ 1000µg/l
Flutamide	-	-	-	-
Prochloraz	-	↑ 274µg/l	↓ 300µg/l	-

Increased sensitivity

Other fish tests in pre-validation (2)

- Fish FLC/2-generation tests
  - US/JPN: comparison of FLC/2-gen using common substances on medaka

2-Gen: Adult 21-d repro ⇨	F1(3-4 months) ⇨	F2 (28dph)
fecundity, VTG, histo, sex steroids	hatching, survival, growth, sex ratio, fecundity, histo VTG, sex steroids,	hatchability growth
FLC: F0 eggs (100dph) ⇨	**F1 (60dph)**	
sexual differentiation (ssc, histo), growth, fecundity, VTG	hatching success, survival sexual differentiation (ssc, histo)	

Amphibian Metamorphosis Assay (1)

- Phase 2 of the validation underway
- Single protocol, 21-day exposure
- Endpoints: dev. stage, T histo, body length, hind-limb length
- Multi-laboratory: 6 participants
- Test substances: T4, IOP, perchlorate
- +feeding study, +*ad hoc* chemical studies, +*X. tropicalis* comparison

Amphibian Metamorphosis Assay (2)

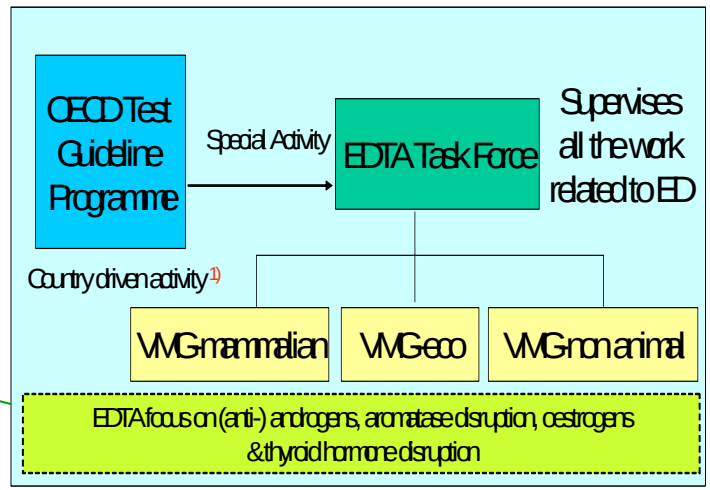
- Phase 2 results available mid-2006
- Detailed Review Paper on thyroid assays in preparation (tox, ecotox, in vitro)

Invertebrate tests on development and reproduction

EDTA-7: focus on invertebrate testing

- Purpose: investigate adverse consequence of potential (Vertebrate) endocrine active substances;
- Life-cycle tests incl. development and reproductive endpoints;
- Level 5 of the EDTA Conceptual Framework: *in vivo assays on adverse effects about endocrine and other mechanisms for risk assessment*

Endocrine Disruptor Testing & Assessment (EDTA): A Special OECD Task Force



1) OECD member countries make proposals to develop new or update existing TG; proposals are prioritized by countries and a lead is designated for the work.

Invertebrate tests on development and reproduction

- Copepod development and reproduction test. Status:

  - ring-test in 2006

  - Test substance: 3,5-DCP

- Mysid 2-generation test. Status:

  - pre-validation ✓fipronil, ✓fenoxycarb, ✓ 3,5-DCP, 4tPP, ketoconazole, prochloraz, flutamide (2006)

- Enhanced Daphnia reproduction test. Status:

  -pre-validation (2005)

  -Strain differences

  - ring test with 3,5-DCP and pyriproxyfen

UPCOMING OECD EVENTS

- VMG-Eco: 12-13 December 2005, Paris
- VMG-non animal: 14-15 December 2005, Paris
- EDTA Task Force: March-April 2006, Stockholm

Thank you for your attention!