"From field to lab" and "from lab to field"

chemicals, *in vitro*, *in vivo* vs. *in situ*

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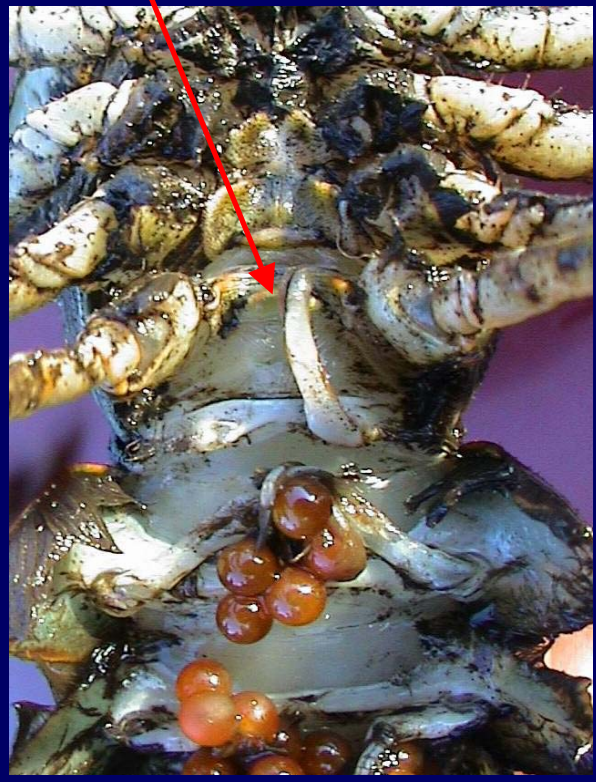
The in situ problem

Pilnok reservoir

- Ostrava-Karvina region

Pontastacus leptodactylus

INTERSEX:
Females w/ male gonopods
Males carrying eggs?



Major questions / Objectives

- What is the cause of intersex occurence ?
- Can ED-chemicals be identified ?
- Can the mechanism be understood ?
- Can we induce ED experimentally ?

Integrated assessment

Sediments collected

- Pilnok
- Reference localities
  Karvina pond, Steinlach creek

Organic extraction

Chemical analyses

In vitro effect testing

In vivo effects

Chemical contamination ?

Organics (ng/g d.w.)

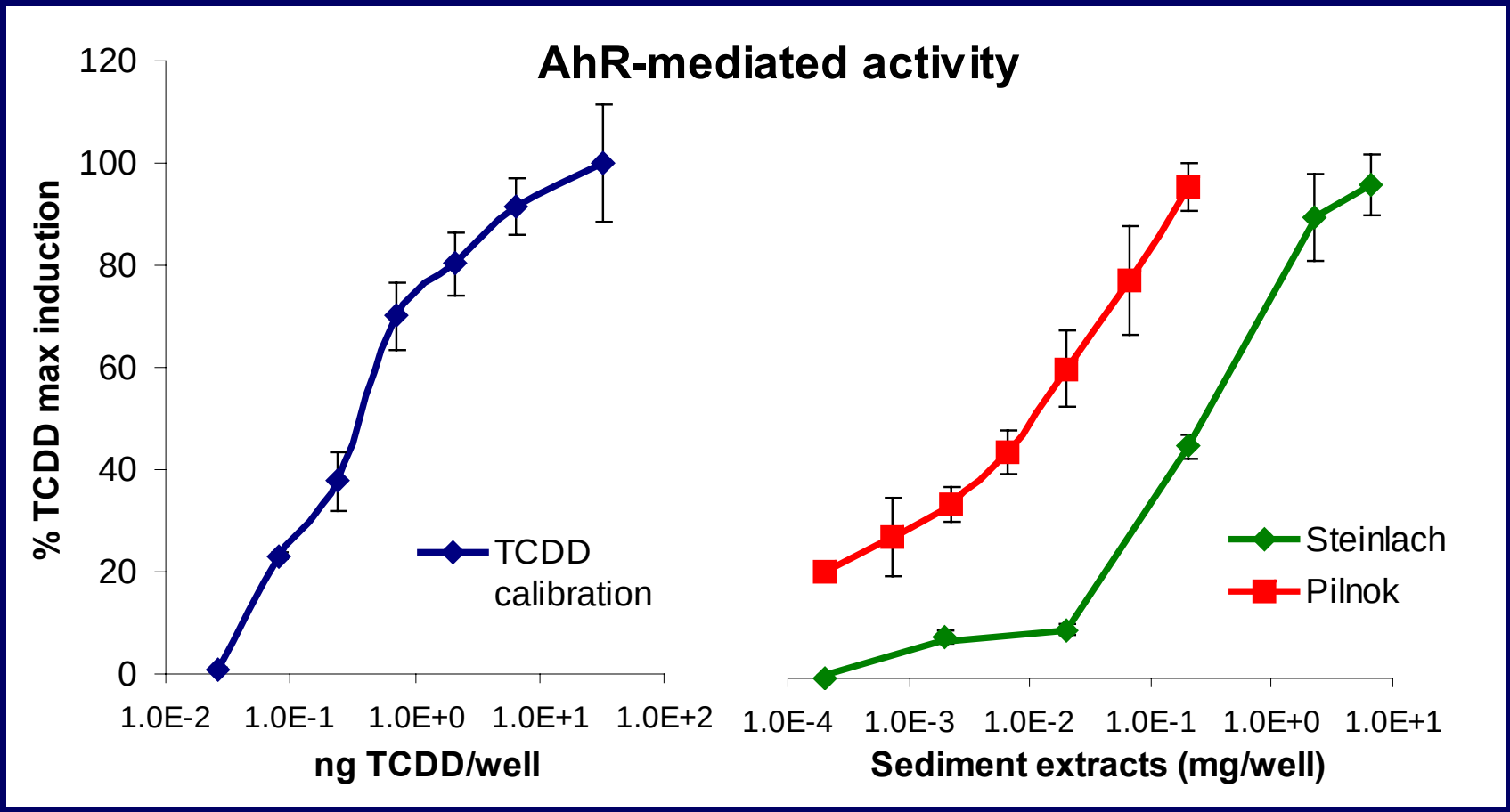
	Pilnok	Karvina	Steinlach	Dřevnice	Morava
16 PAHs	18420	10075	422	9427	5263
Σ PCBs	18.7	6.7	0.86	14.8	13.6
Σ DDTs	1.7	2.8	0.33	5.8	22.4

- High concentrations of PAHs in PILNOK
- Other POPs (PCBs, OCPs) ~ low-average

Metals (μg/g d.w.)

	Pilnok	Karvina	Steinlach
Pb	47	**109**	3.5
Cu	29.1	31	2.9
Zn	45.7	**86**	21.5

Bio-TEQs (H4IIE.luc bioassay)



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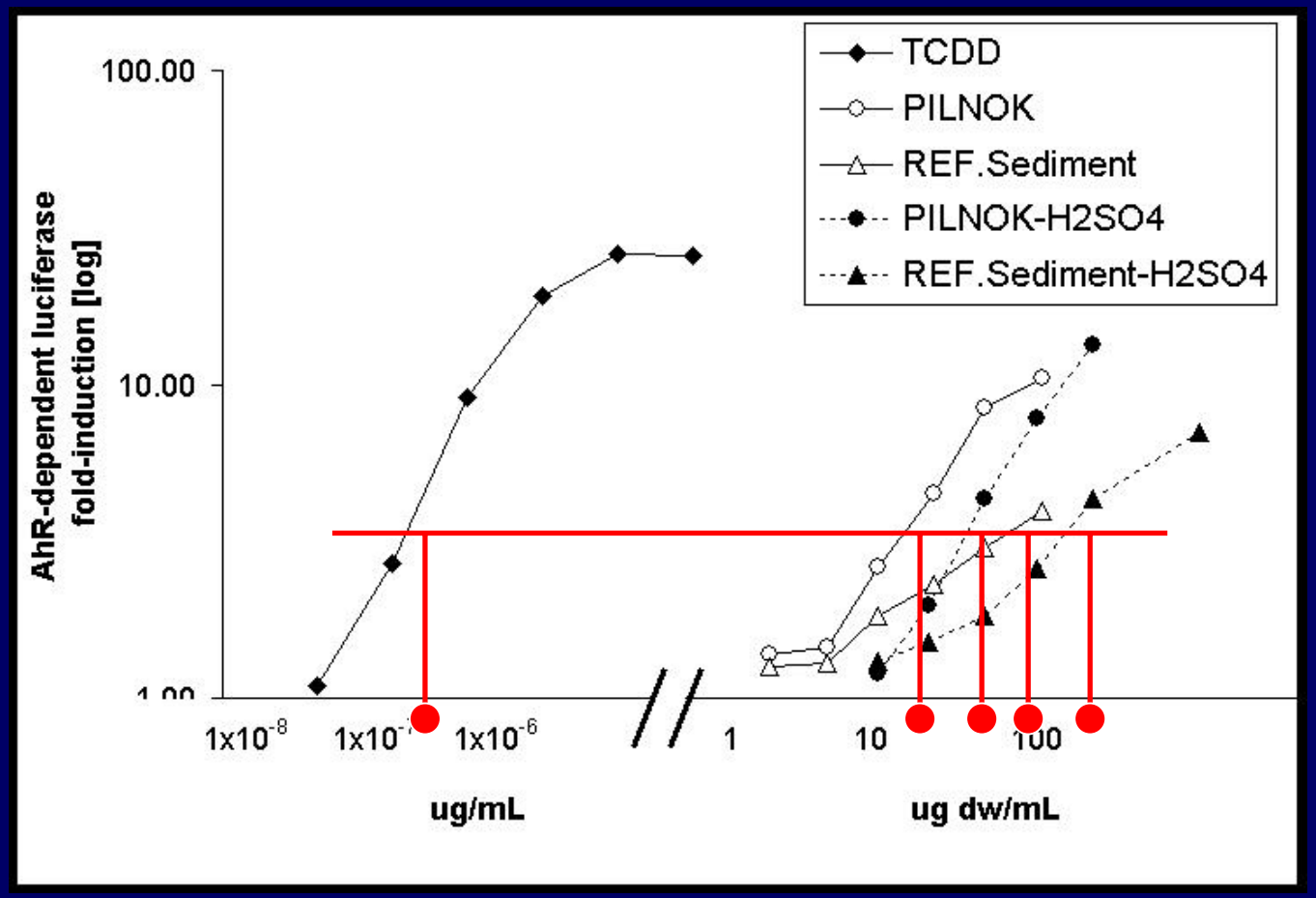
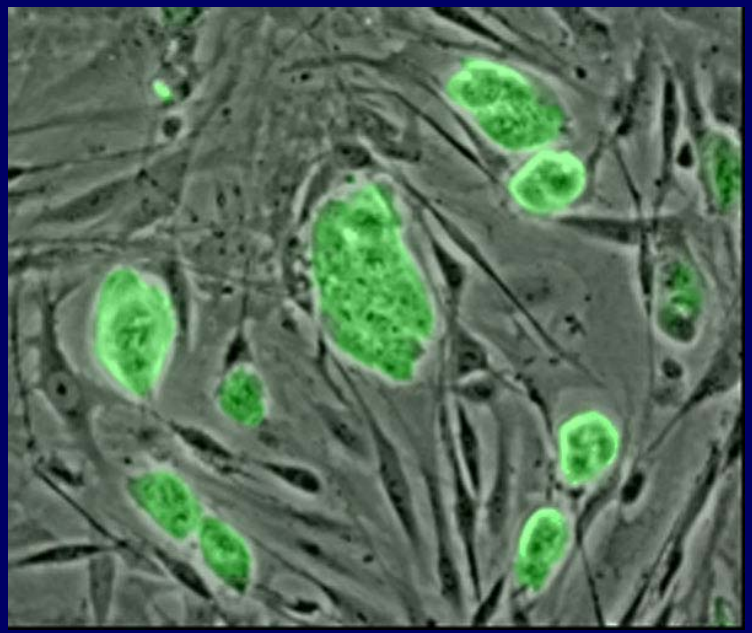


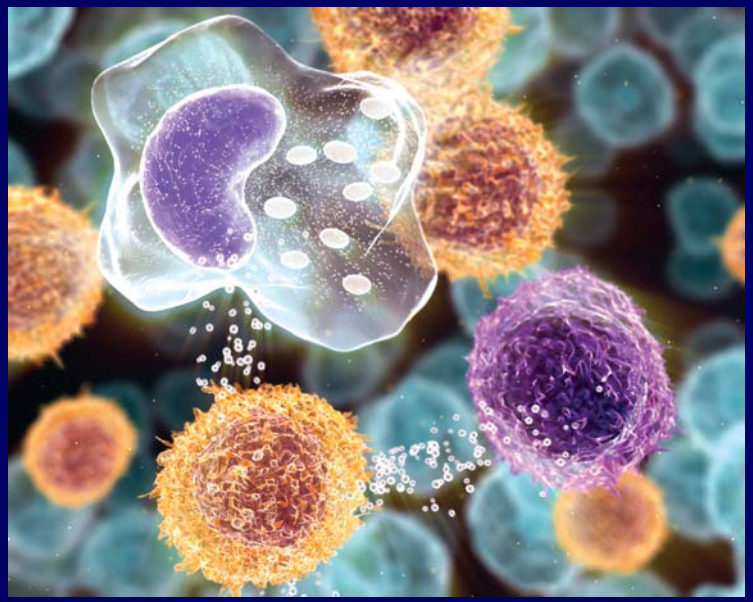
TEQs + Analytical data (ng/g d.w.)

	Pilnok	Karvina	Steinlach	Morava
16 PAHs	**18420**	**10075**	**422**	5263
Σ PCBs	**18.7**	**6.7**	**0.86**	13.6
Σ DDTs	**1.7**	**2.8**	**0.33**	22.4
Chem-TEQs*	**1.1**	**1.2**	**0.002**	0.9
Bio-TEQs$_{crude}$	**70**	**13**	**2.4**	0.7
Bio-TEQs$_{H_2SO_4}$	**6.9**	**0.2**	**<0.005**	<0.005

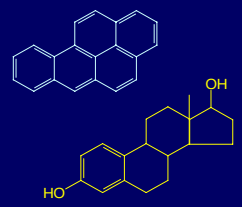
** WHO TEFs for PCBs & IEFs for PAHs (Machala et al. 2001)*

Effects *in vitro* ?

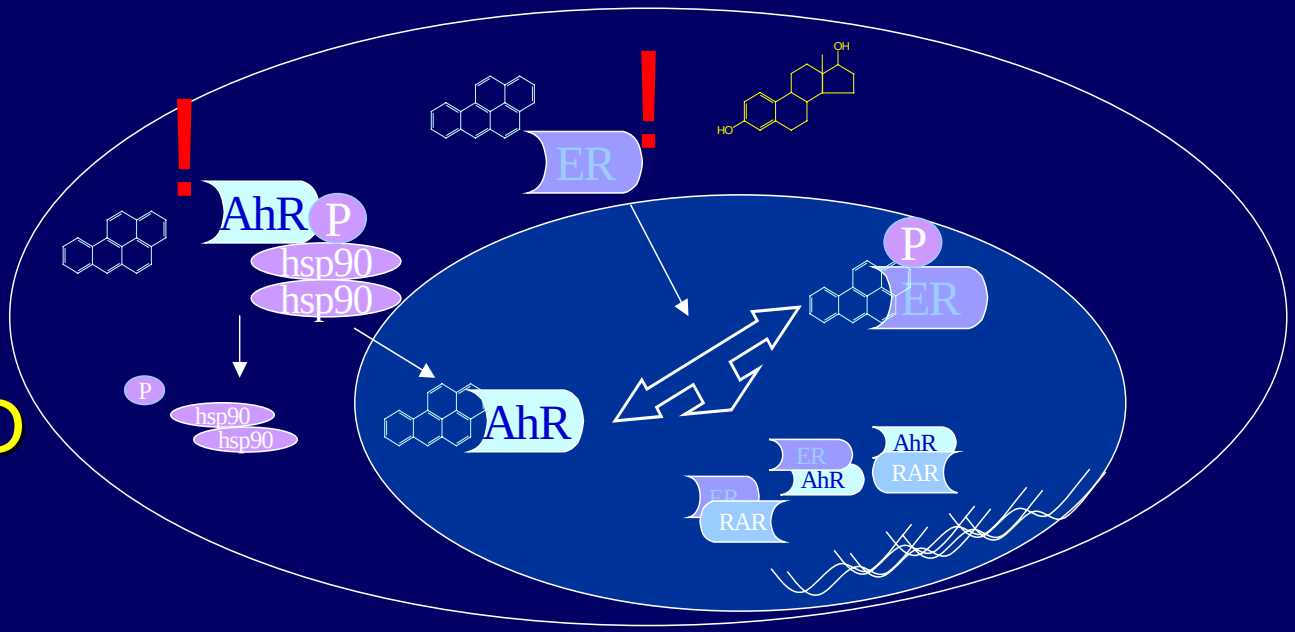




Assessment of *in vitro* effects

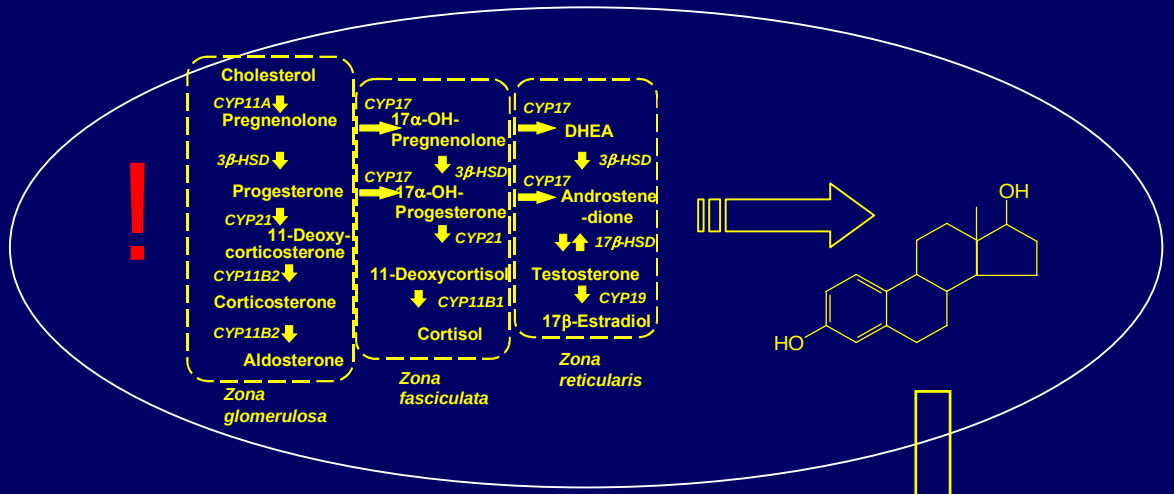


ACTION
OF STEROID
HORMONES

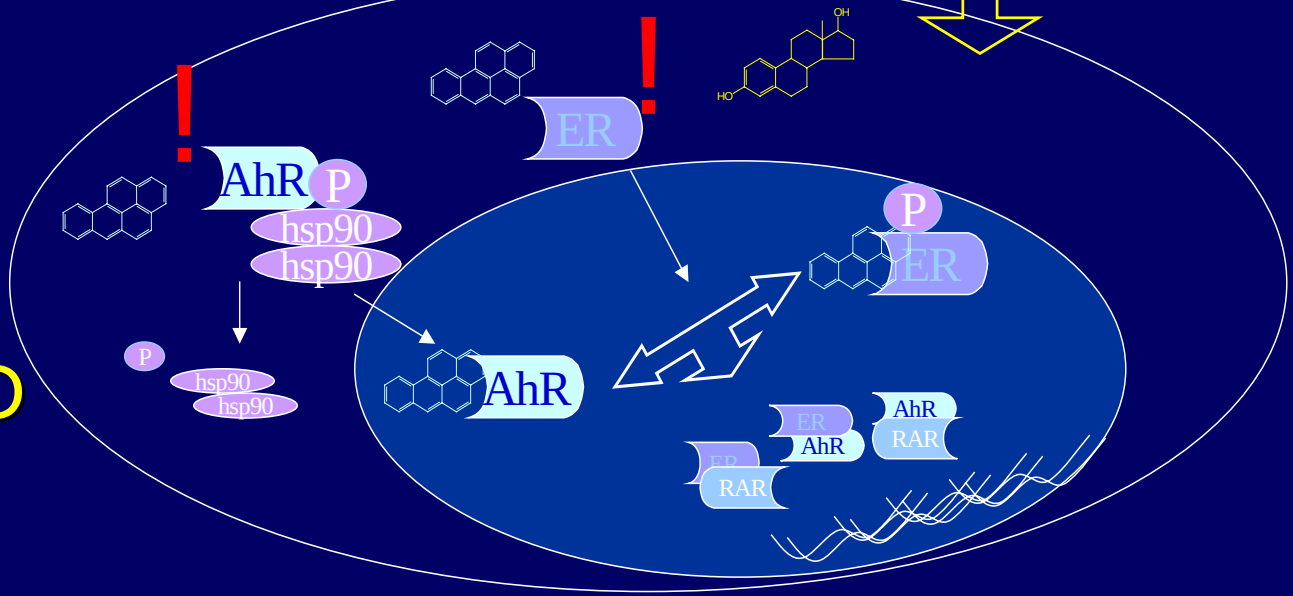


Assessment of *in vitro* effects

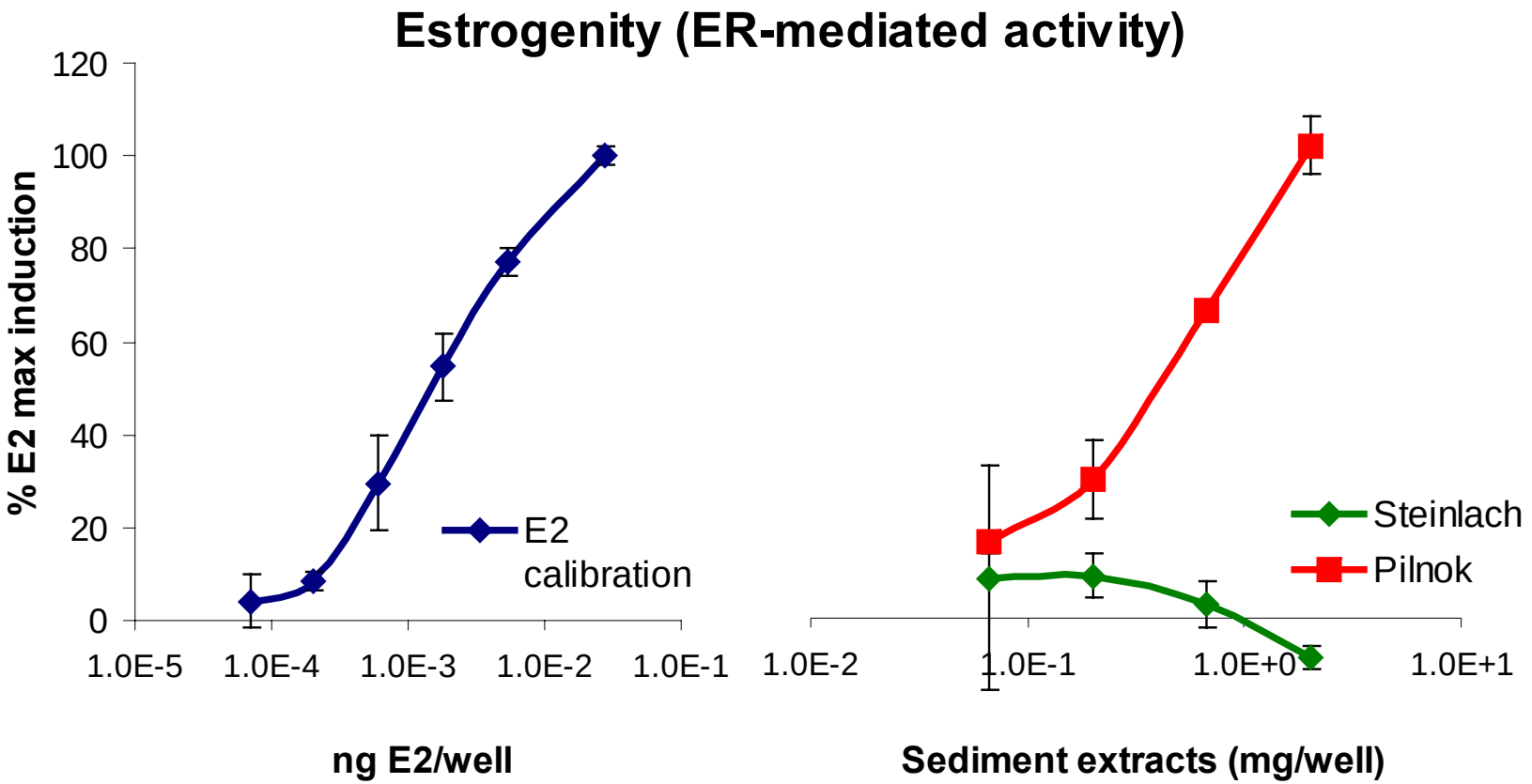
SYNTHESIS OF STEROID HORMONES



ACTION OF STEROID HORMONES

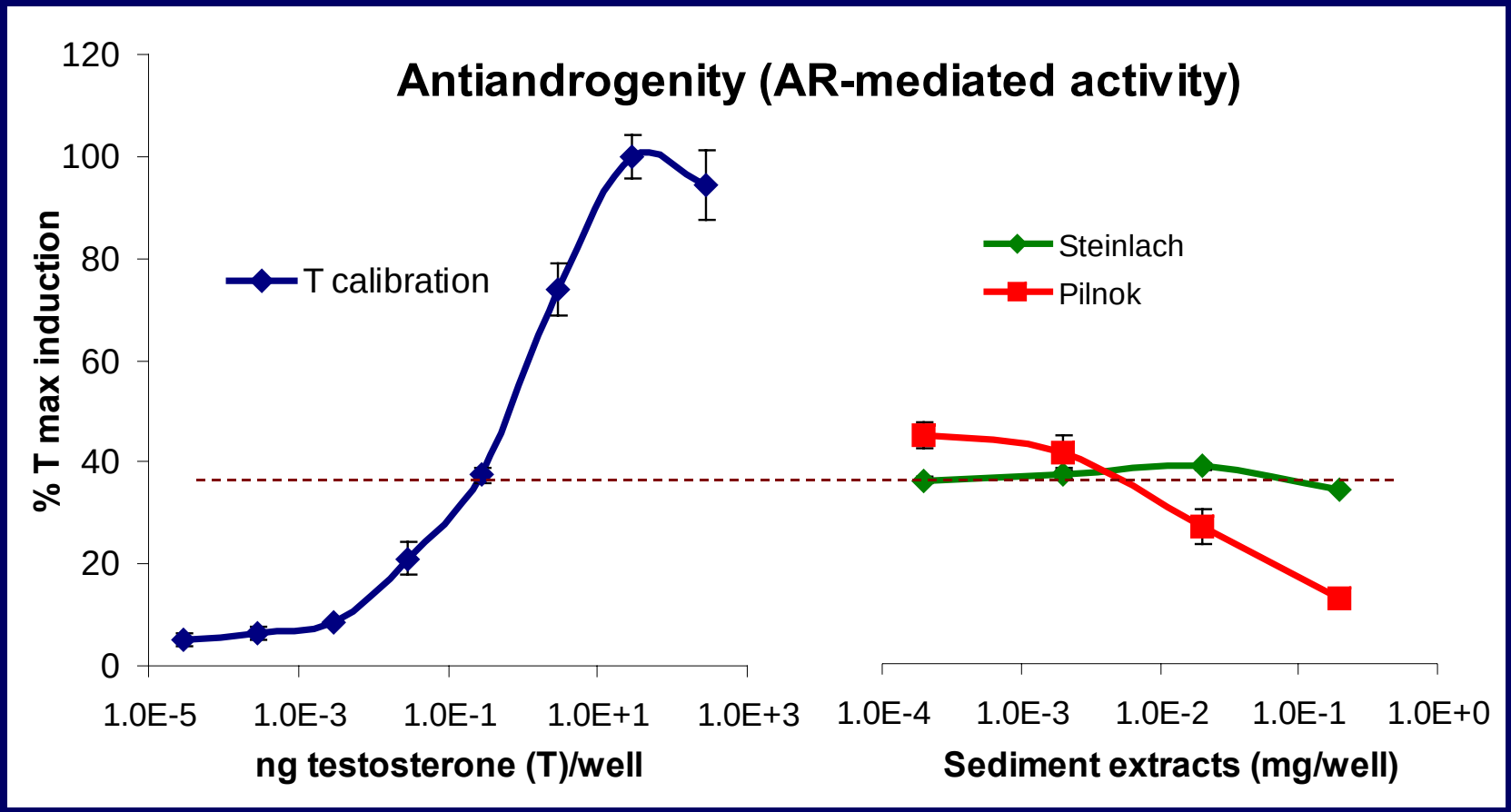


MVLN bioassay



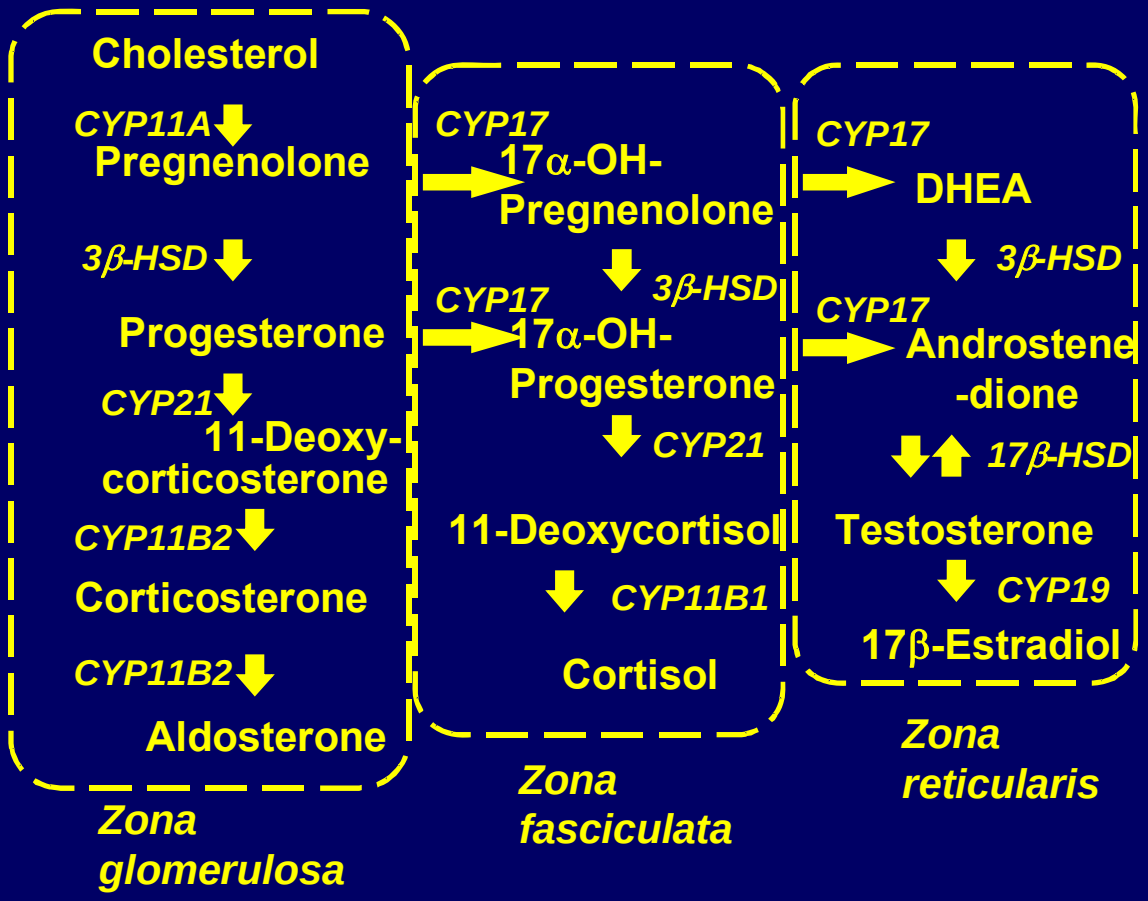
- PILNOK estrogenic
- Reference sediments - no effects

AR-yeast bioassay



- PILNOK anti-androgenic
- Reference sediments - no effects

Effects on steroidogenesis

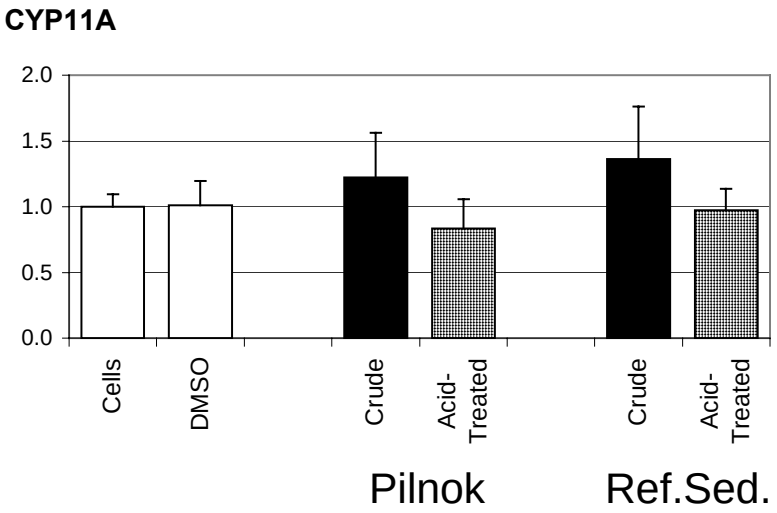


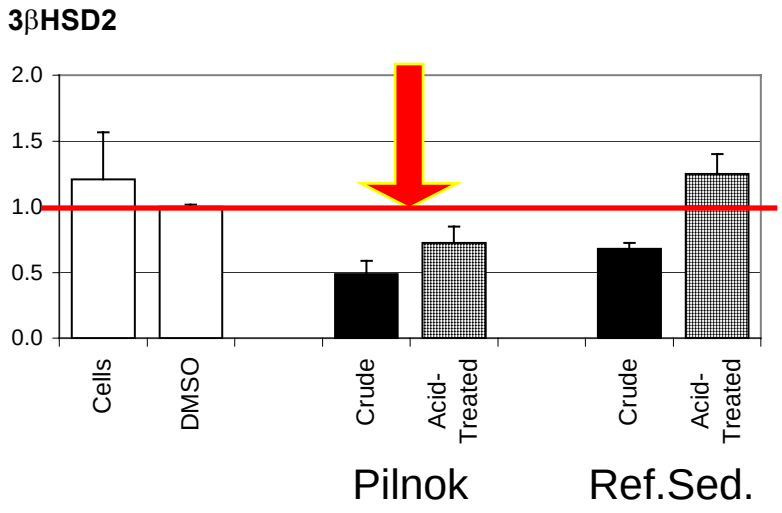
- gene modulation (DNA->mRNA) Real Time PCR
- protein levels
- enzyme activities
- hormones produced

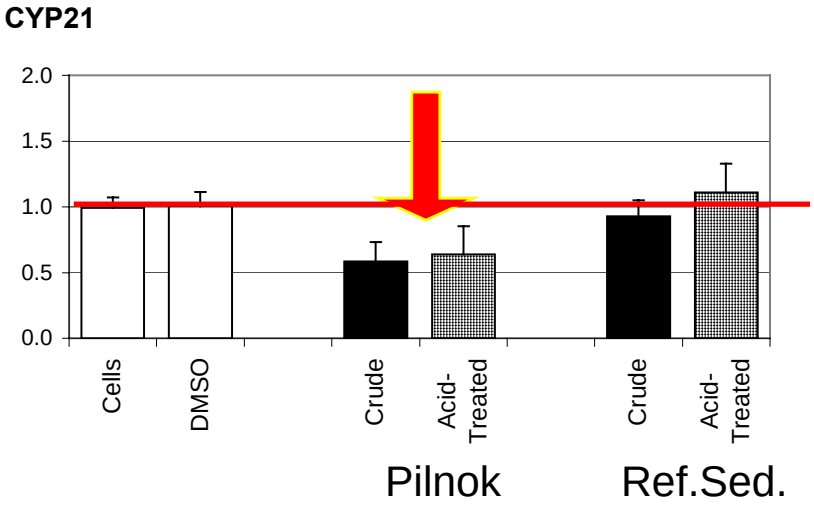
H295R cell line

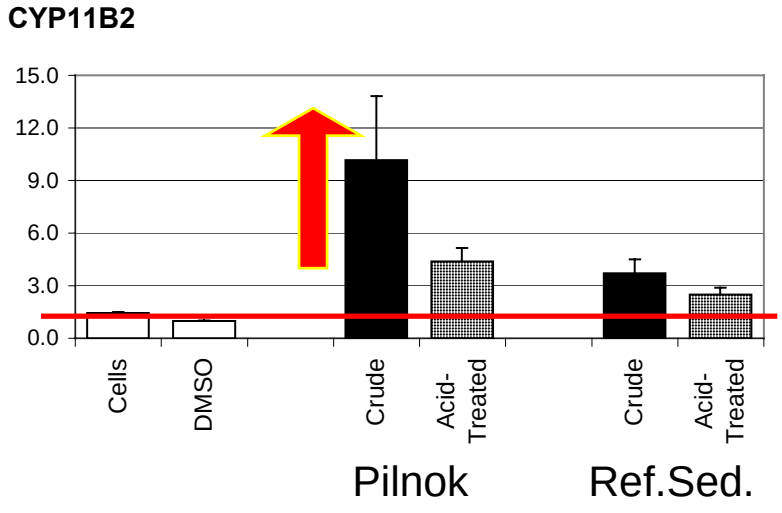
bioassay development supported by US EPA grant

Pilnok sediment extracts modulate steroidogenesis in H295R cells



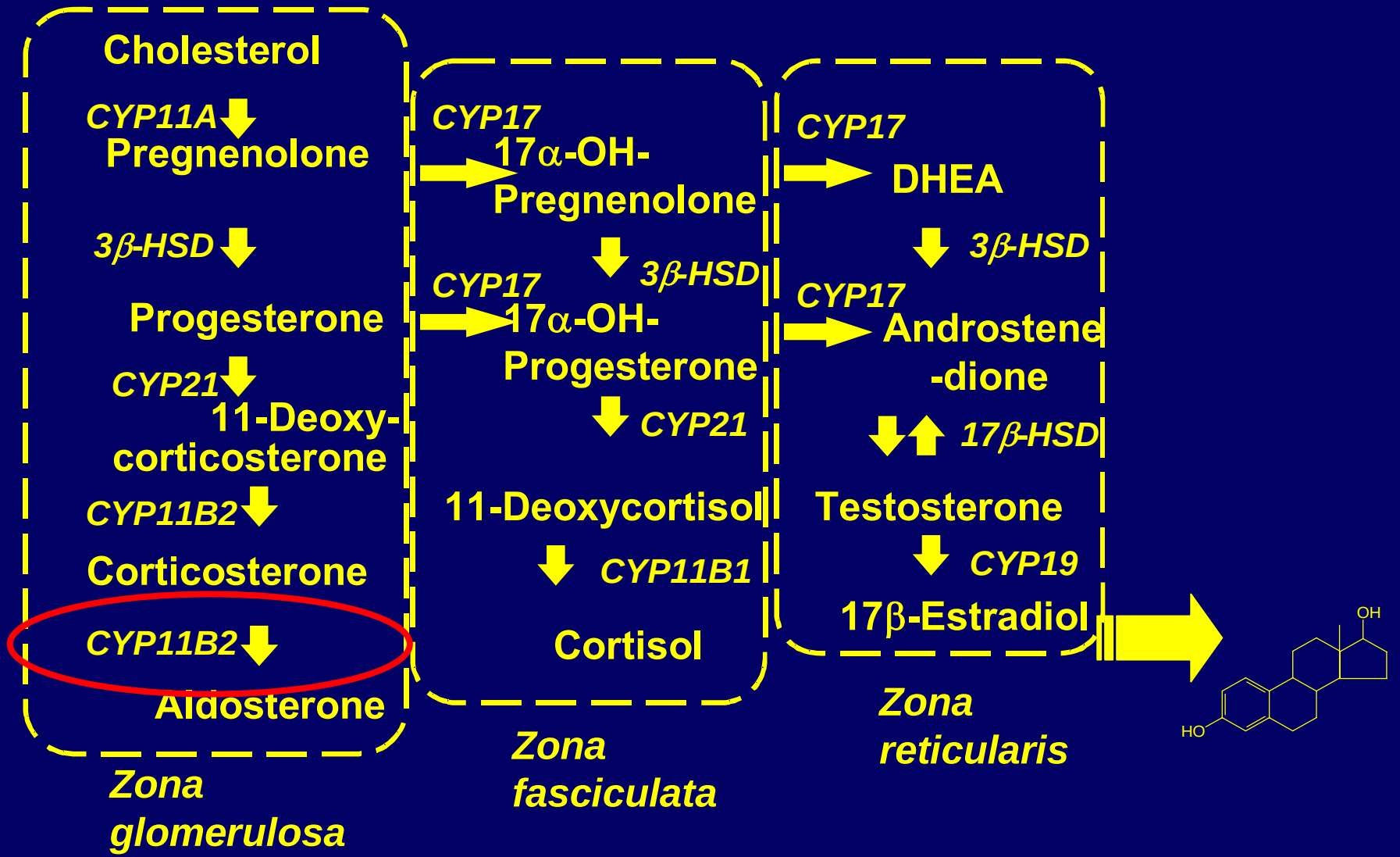




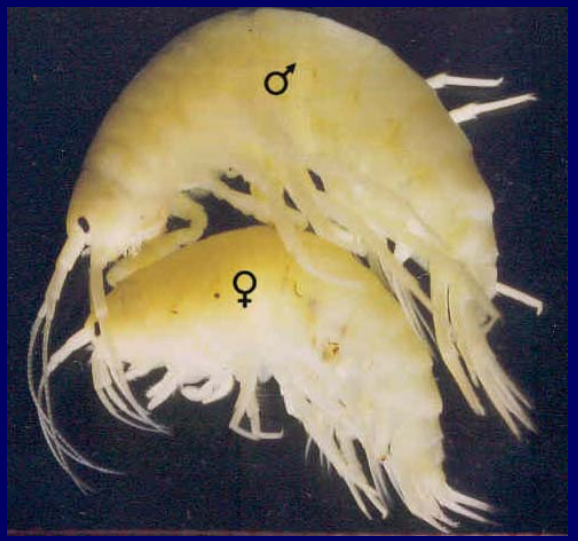


Plahn et al. 2006 Environment International

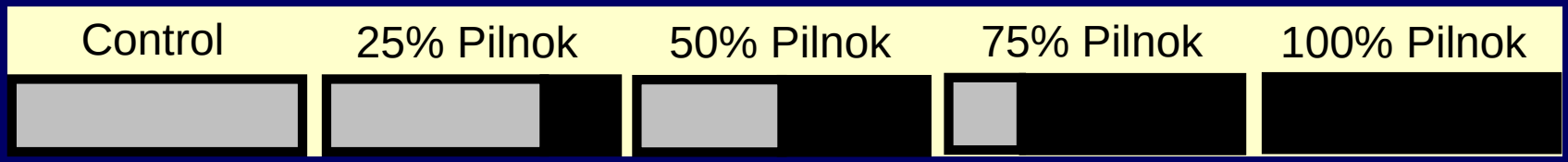
Effects on steroidogenesis

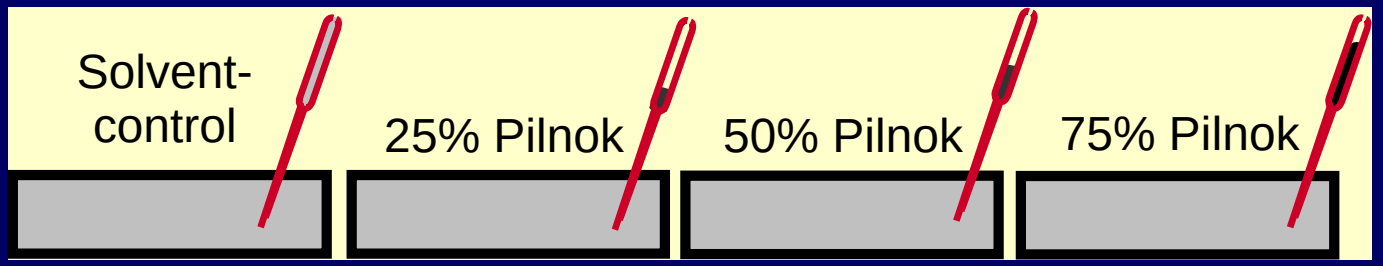


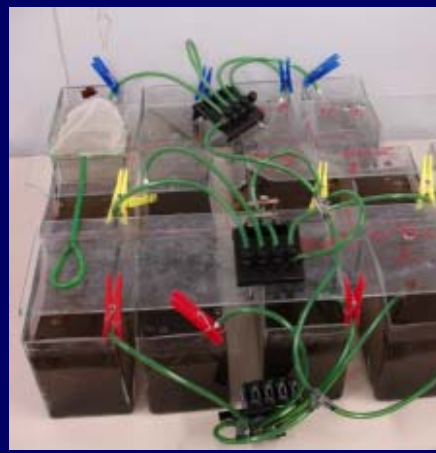
In vivo effects?



Assesment of *in vivo* effects







Potamopyrgus antipodarum

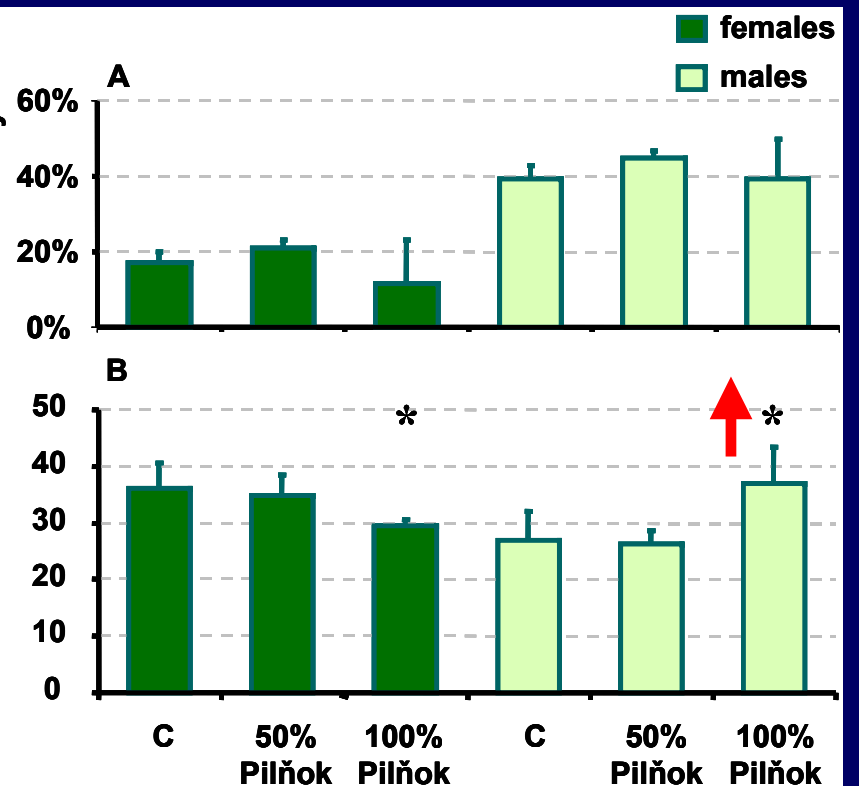
2,5,8 weeks: mortality, embryos, hsp70

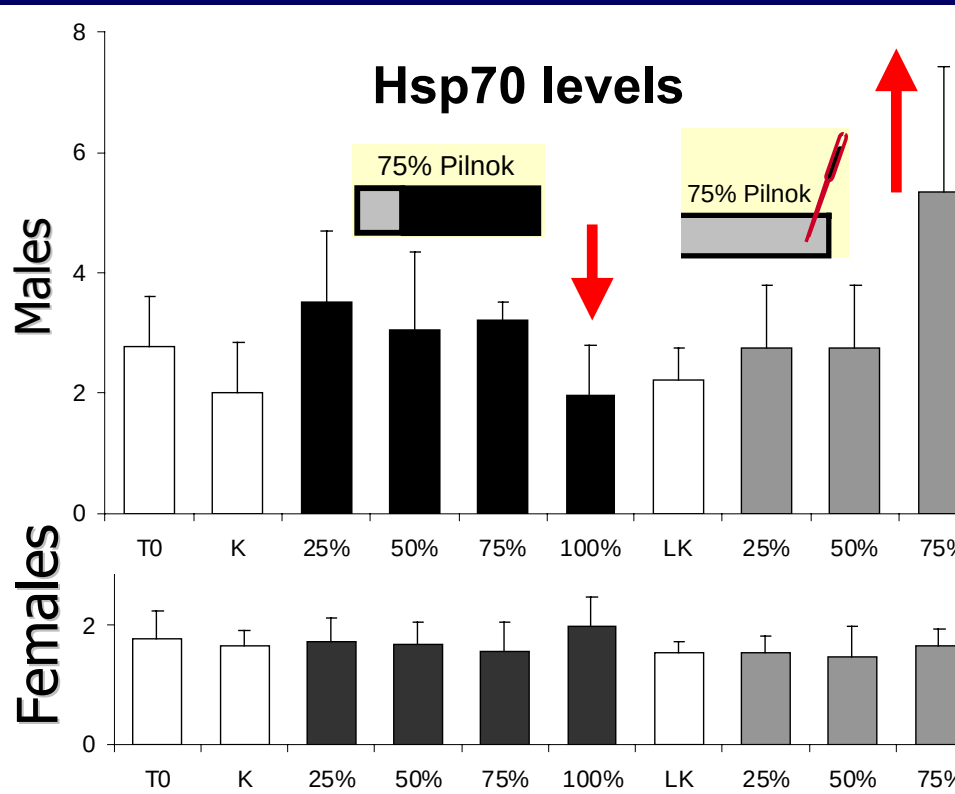
Gammarus fossarum

12 weeks: mortality, juveniles, histopathology

Sex-dependent differences in effects – I

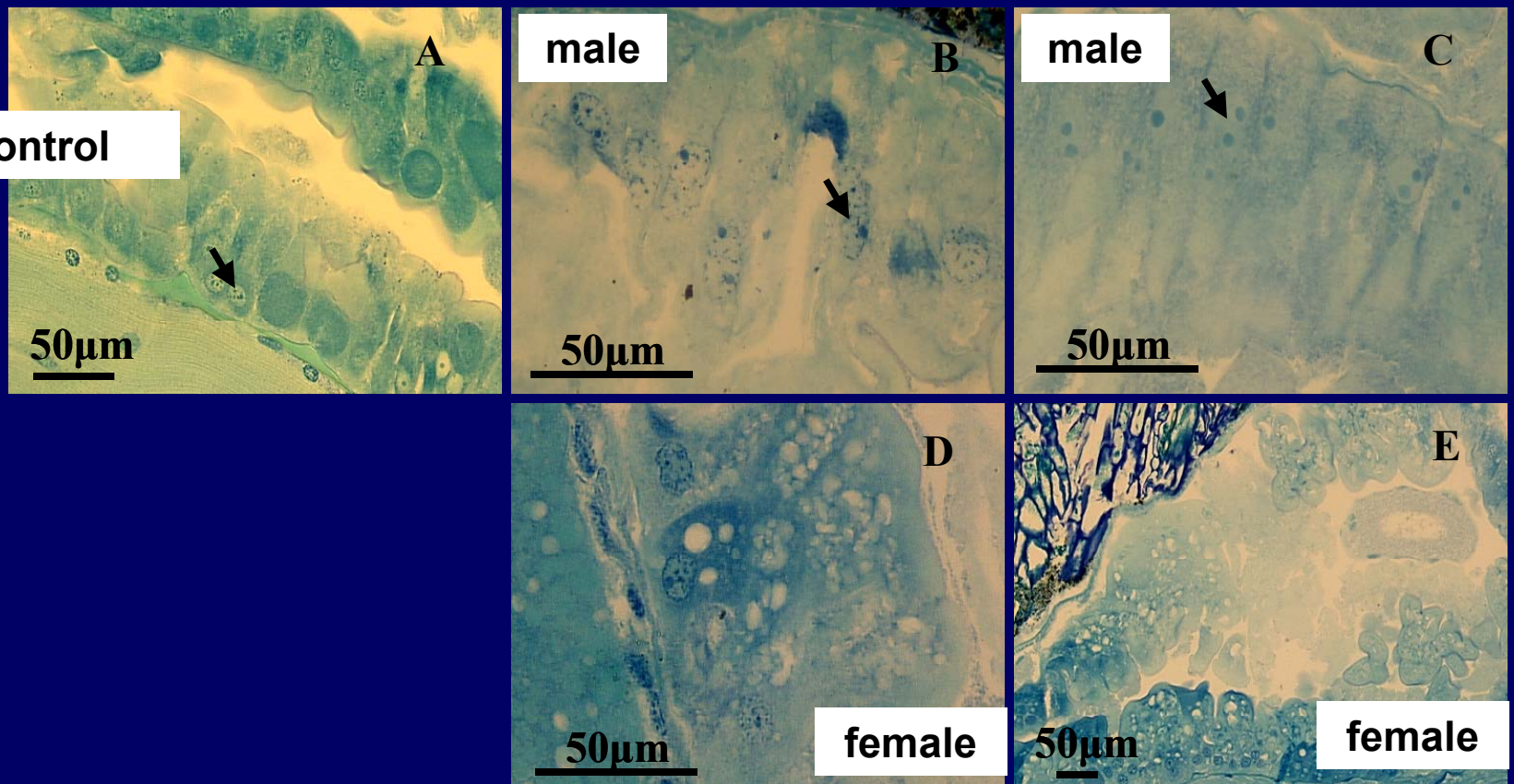
! sensitivity of males



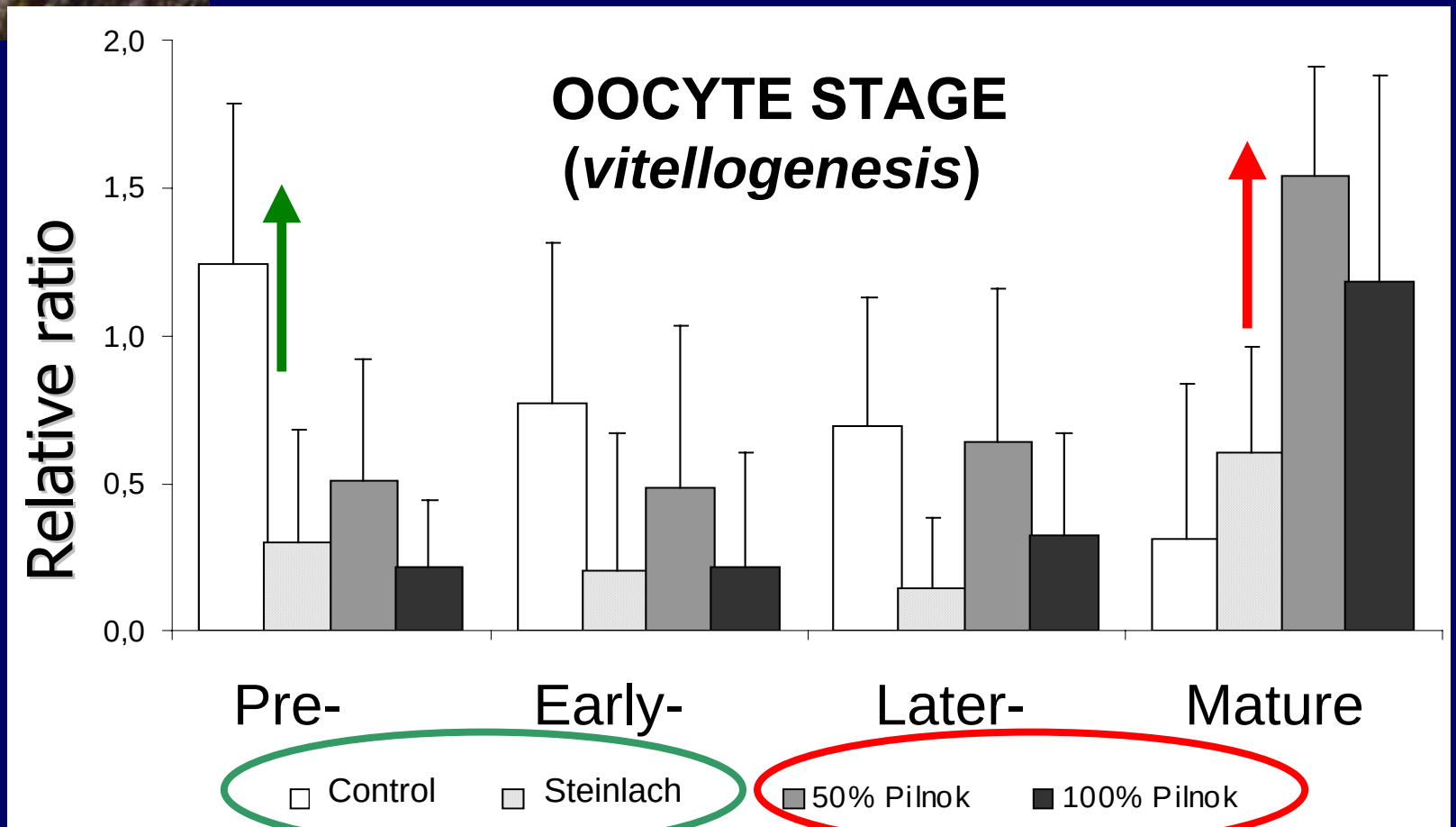


Sex-dependent differences in effects – II

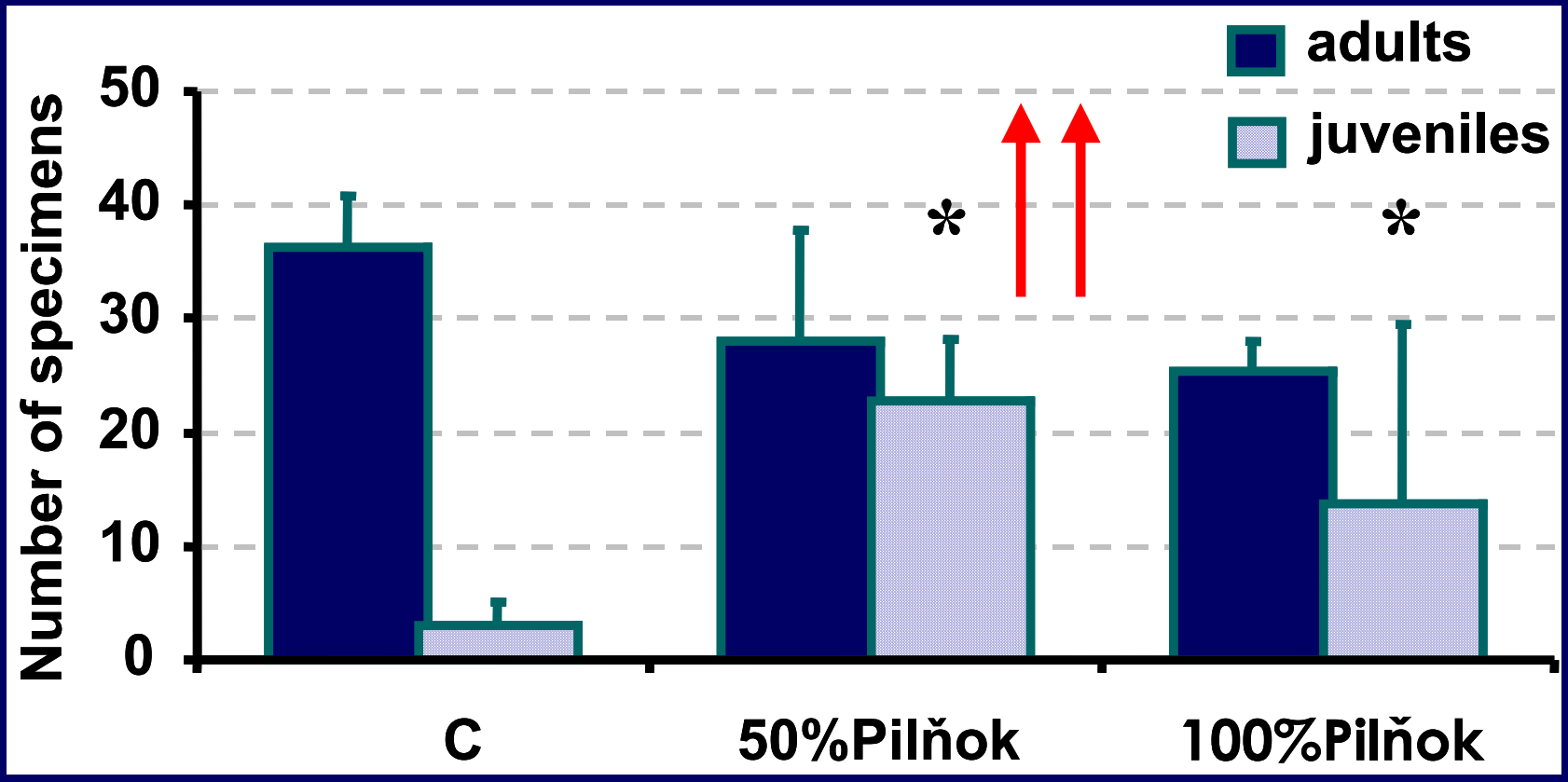
variable effects in hepatopankreas



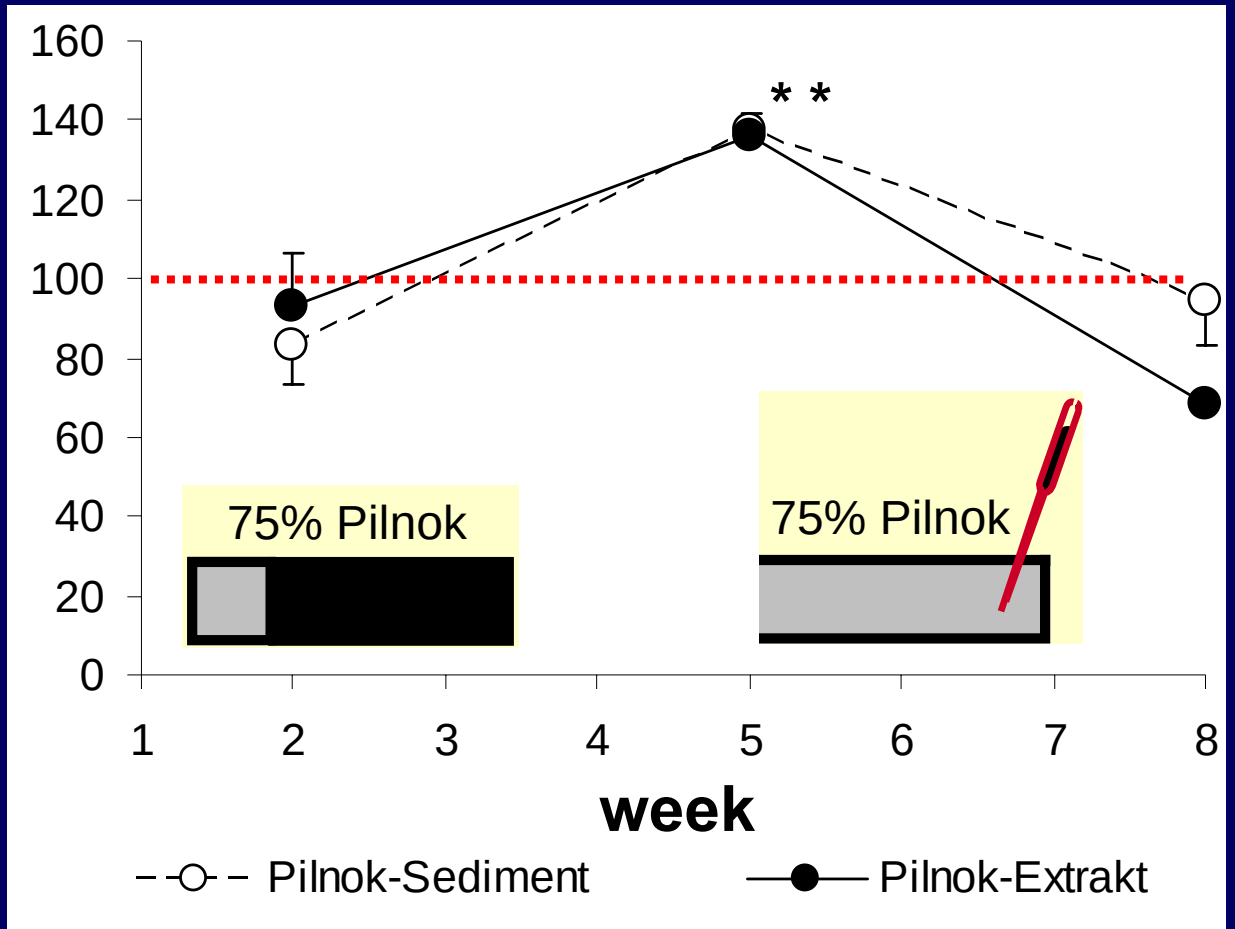
Pilnok sediments shift reproduction cycle in females (towards maturity)



Pilnok sediments induce production and size of F1 juveniles



Pilnok sediments (and organic extracts) stimulate production of embryos



SUMMARY 1

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- estrogenicity and anti-androgenicity

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2) Biological experiments complement chemical analyses and suggest elevated levels of unknown EDs in PILNOK pond *(? PAH derivatives)*

3) In vitro assessment indicate „feminization"
   - estrogenicity and anti-androgenicity

4) New mechanism ? (↑↑CYP11B2 / steroidogenesis)

SUMMARY 2

5) In vivo experiments with two invertebrate species revealed EDCs in Pilnok sediments

„stimulated reproduction“

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5) *In vivo* experiments with two invertebrate species revealed EDCs in Pilnok sediments

„stimulated reproduction"

6) Differences (?)

- vertebrate in vitro:
  „feminization"
- invertebrate in vivo:
  „reproduction"

Answers to our questions ?

- What is the cause of intersex occurence ?
  - contaminants associated with sediments
- Can ED-chemicals be identified ?
  - partially yes: organic (labile) contaminants
- Can the mechanism be understood ?
  - (partially) yes: feminization / speeding up reproduction
- Can we induce ED experimentally ?
  - Yes!

Acknowledgements

- J.P. Giesy, P.D. Jones, M. Hecker

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- D. Jungmann
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K. Hilscherová, I. Holoubek, E.Mazurová

FINANCIAL: GAČR, Ministry of Education CR

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