

Environmental risk assessment of endocrine disruptors: status and needs of biomarkers



Tom Hutchinson PhD
AstraZeneca R&D, Global Safety Assessment
Södertälje, Sweden

Key Principle of ERA (aquatic)

**Predicted
Environmental
Concentration (PEC)**

**Predicted No Effect
Concentration (PNEC)**

PEC:PNEC ratio

Calculation typically includes data on:

- **Microorganisms
(from Sewage Treatment Works)**
- **Plants**
- **Invertebrates**
- **Fish**

Finally, if the PEC:PNEC ratio ≥ 1 , potential risk and further work (termed 'risk refinement') may be required.

Biomarker definition

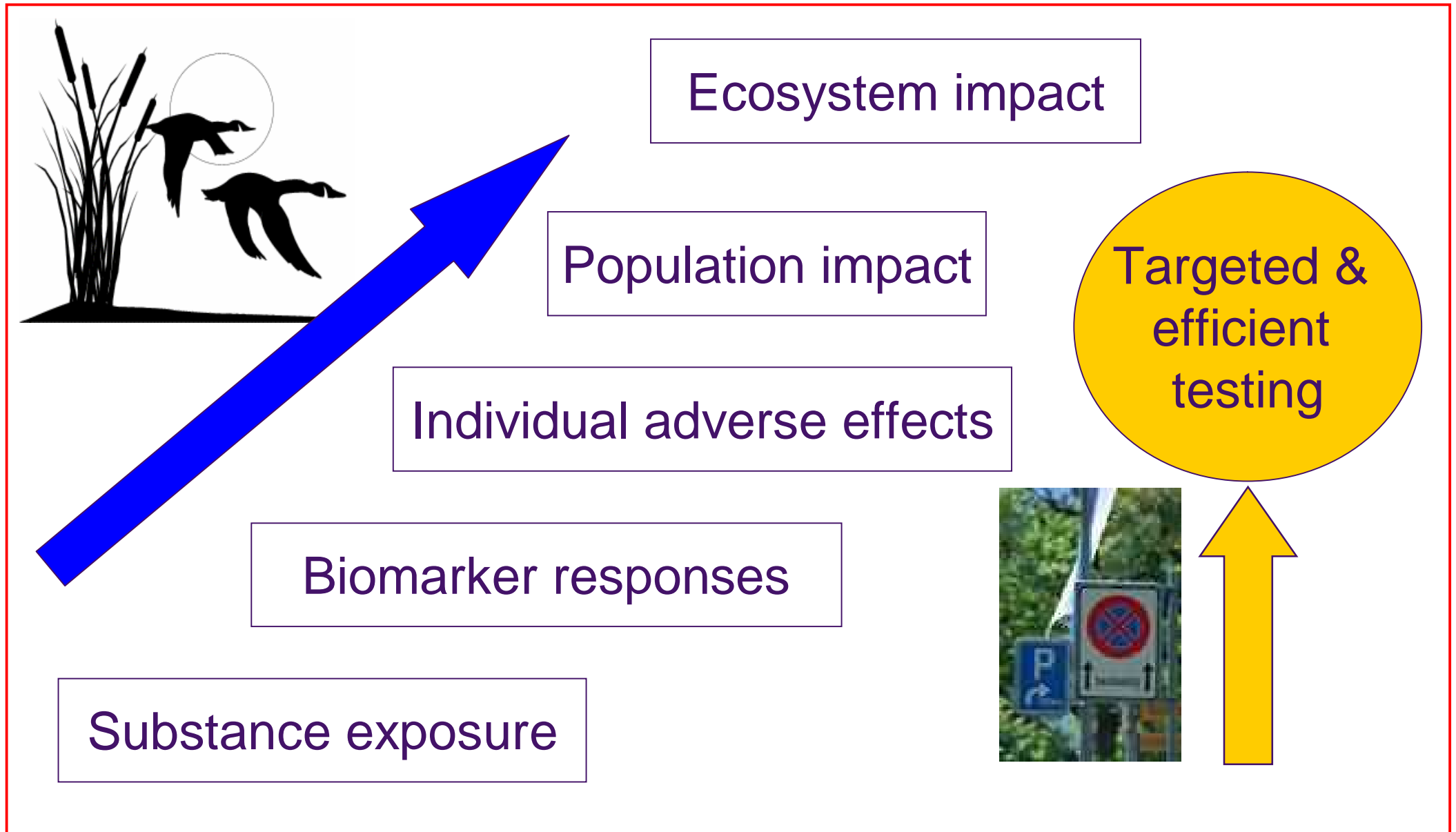
“A molecular, biochemical, cellular or physiological indicator of either exposure to, or effects of, xenobiotic chemicals”.

(after Huggett *et al.* (1992)

Biomarkers – SETAC Special Publication.

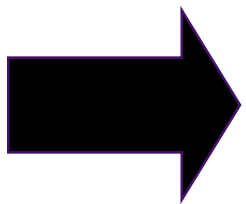
In this context, biomarker responses do not include commonly measured toxicological effects such as reduced development, fecundity, fertilisation, growth or survival.

'Biomarkers as signposts ...' concept



Three key issues to consider ...

- **Which biomarkers are available today for measuring endocrine disruption in field and laboratory studies?**
- **Have the biomarkers of interest been adequately validated in different laboratories?**
- **From a science perspective, how should biomarkers be used in regulatory testing to support environmental risk assessment (ERA)?**



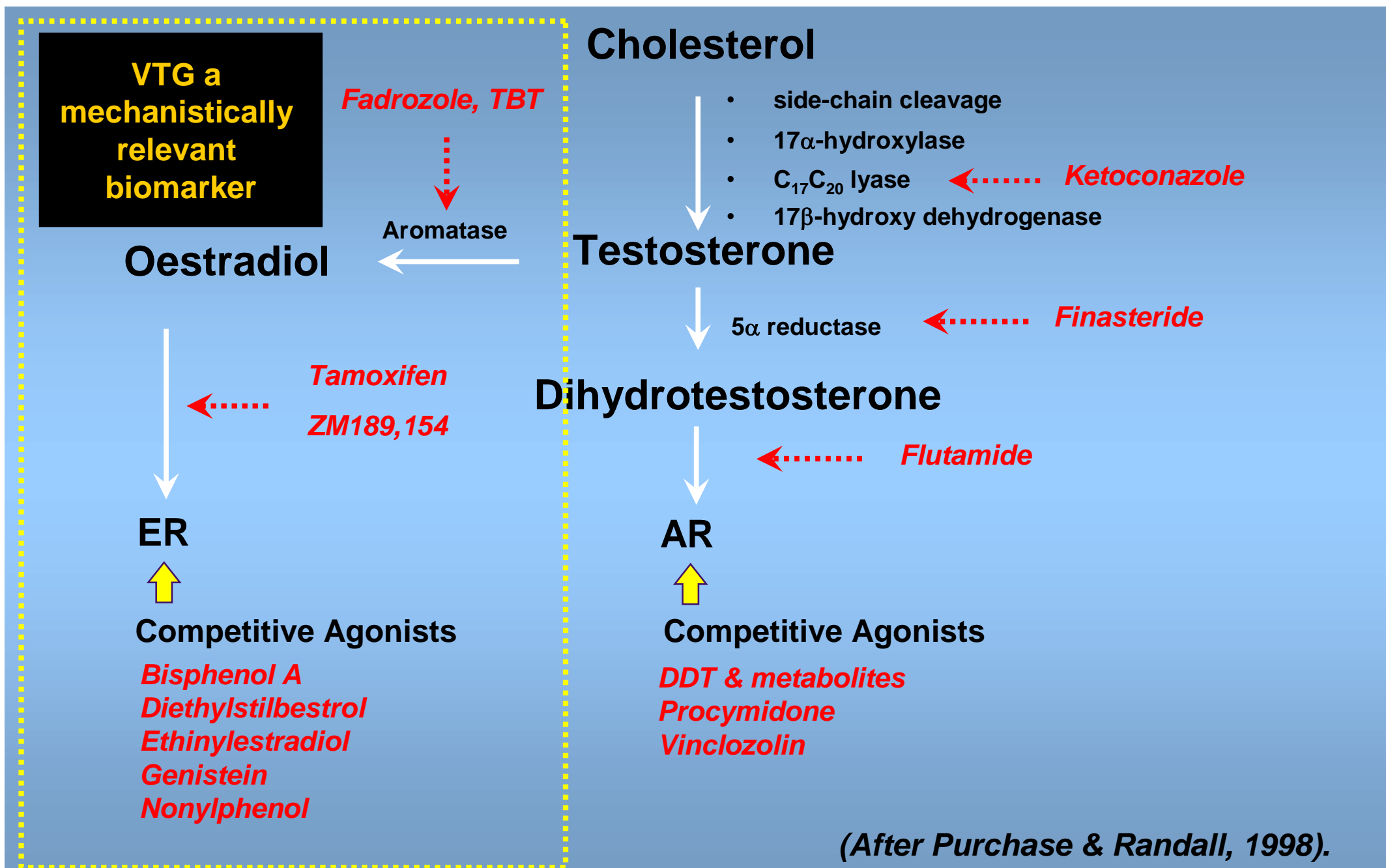
*Major goals: better understanding,
reliable measurements
& accurate predictions.*

1. Which biomarkers are available today for EDCs?

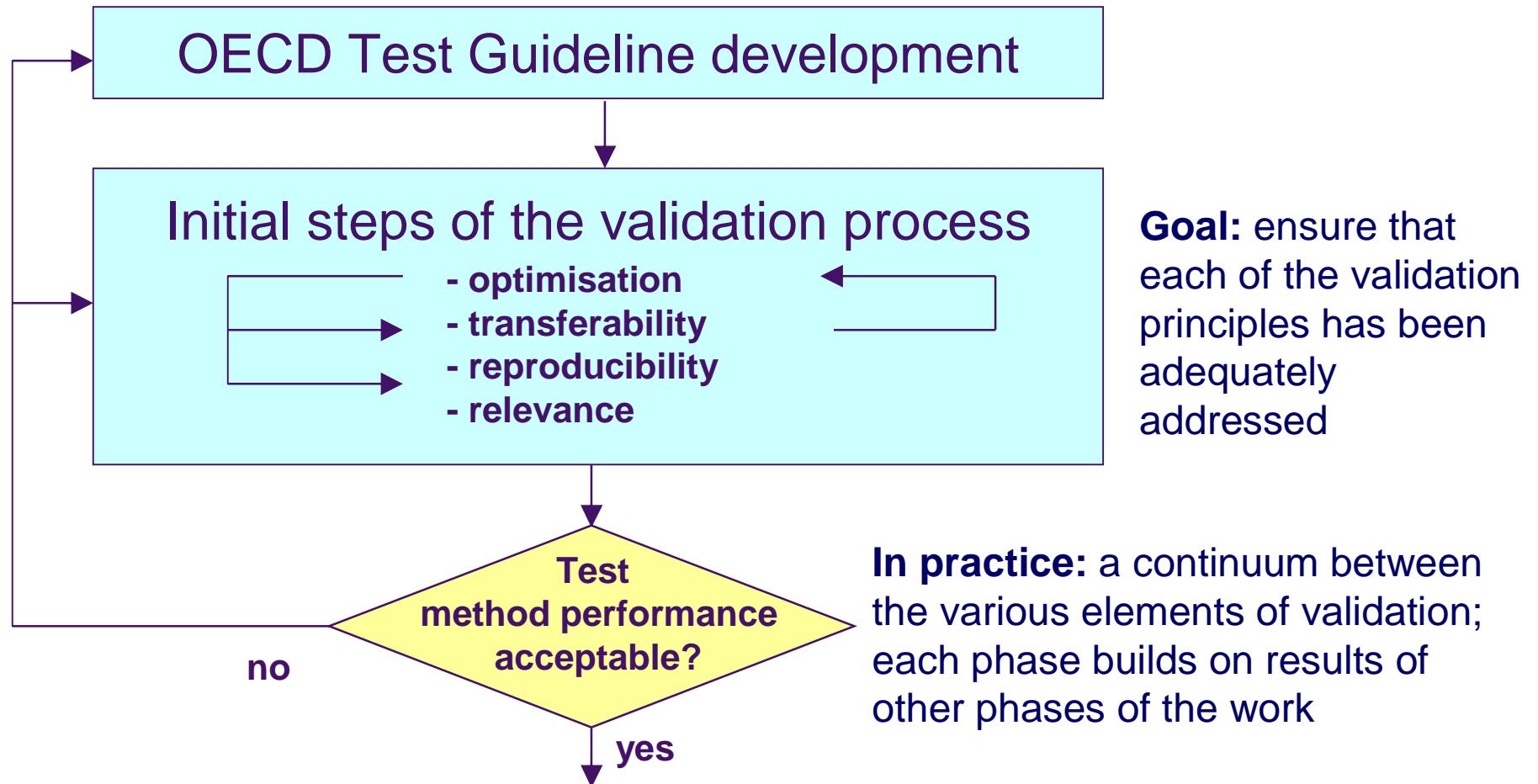
- **Endocrine system biomarkers**
 - gene expression (eg AR, ER, CYP19)
 - plasma hormone levels (eg oestradiol, thyroxine)
 - vitellogenin
 - secondary sexual characteristics
 - gonad histology
- ***In vitro* applications**
 - vertebrate cell lines (eg fish hepatocytes)
 - invertebrate cell lines (eg insect BII cells)
- ***In vivo* applications**
 - field studies
 - laboratory testing

Refs: Fent (2001) Toxicol In Vitro 15: 477–488 (fish cell lines); Dinan et al (2001) Env.Toxicol.Chem. 20: 2038–2046 (insect cell line); Hutchinson et al (2006) Environ.Health Perspect. – in press (fish biomarkers review)

Key mechanisms – examples



2. Have the biomarkers of interest been adequately validated?



Main part of the validation process

Fully assess reliability and relevance. One or more phases including inter-laboratory testing, blind testing and assessment of positive/negative controls

See OECD (2005)
Guidance Document 34.

Validation terminology

- **Repeatability = the variation between repeated tests of the same protocol in the same laboratory. This is also caused intra-laboratory variability.**
- **Reproducibility = the variation between repeated tests of the same protocol in different laboratories. This is also caused inter-laboratory variability.**
- **Comparability = the variation for the same biological endpoint measured using different protocols**

Case study - vitellogenin

Organism	Endpoint	Variable	CV (%)
Fathead minnow	Juvenile whole body VTG	Reproducibility	55
"	Female plasma VTG	Reproducibility	45
"	Male plasma VTG	Reproducibility	38
"	Plasma VTG	Repeatability	3 – 14
"	Plasma VTG	Repeatability	16.4
"	Plasma VTG	Reproducibility	18.6
"	Plasma VTG	Repeatability	32
"	Plasma testosterone	Repeatability	46
"	Plasma estradiol	Repeatability	64
Medaka	Liver VTG	Comparability	52 – 863
	Whole body VTG		100 – 1873
	Liver VTG	Repeatability	<7
Zebrafish	Whole body VTG	Comparability	70.2 – 269
	Whole body VTG	Repeatability	14 - 18
<hr style="border-top: 1px dashed red;"/>			
Medaka	Fecundity	Repeatability	35.7
Rainbow trout	28d growth LOEC	Repeatability	–
"	28d growth LOEC	Reproducibility	19 – 58
Sheepshead minnow	Larval IC25	Repeatability	28 – 42
"	Larval IC25	Reproducibility	44
Zebrafish	Survival NOEC	Repeatability	26 – 33
"	Survival NOEC	Reproducibility	35 – 52
	Fecundity	Repeatability	26 - 63

From: Hutchinson et al (2006) Environ.Health Perspect. – in press (fish biomarkers review).

Validation – overall situation

- **Vitellogenin – probably the leading example:**
 - mechanistic scope is well understood
 - repeatability well-defined
 - reproducibility looks as good as traditional ecotoxicology endpoints (19-55%)
- **BUT more work is needed on other biomarkers:**
 - plasma sex steroid levels
 - plasma thyroid hormones
 - gonad histology
 - molecular responses
- **Historical databases are essential tools for interpretation of biomarker data***

Lesson number 2 from Sumpter and Johnson (2005) Environ. Sci. Technol. 39: 4321-4332

3. How should biomarkers be used in regulatory testing?

- **Validated biomarkers are important in key areas:**
 1. **Linking field and laboratory studies**
 2. **Screening for endocrine specific mechanisms of action**
 3. **Helping efficient design of chronic tests for risk assessment**
 - **species selection**
 - **exposure concentration selection**

YES and YAS for (anti-)oestrogenic and (anti-)androgenic activities

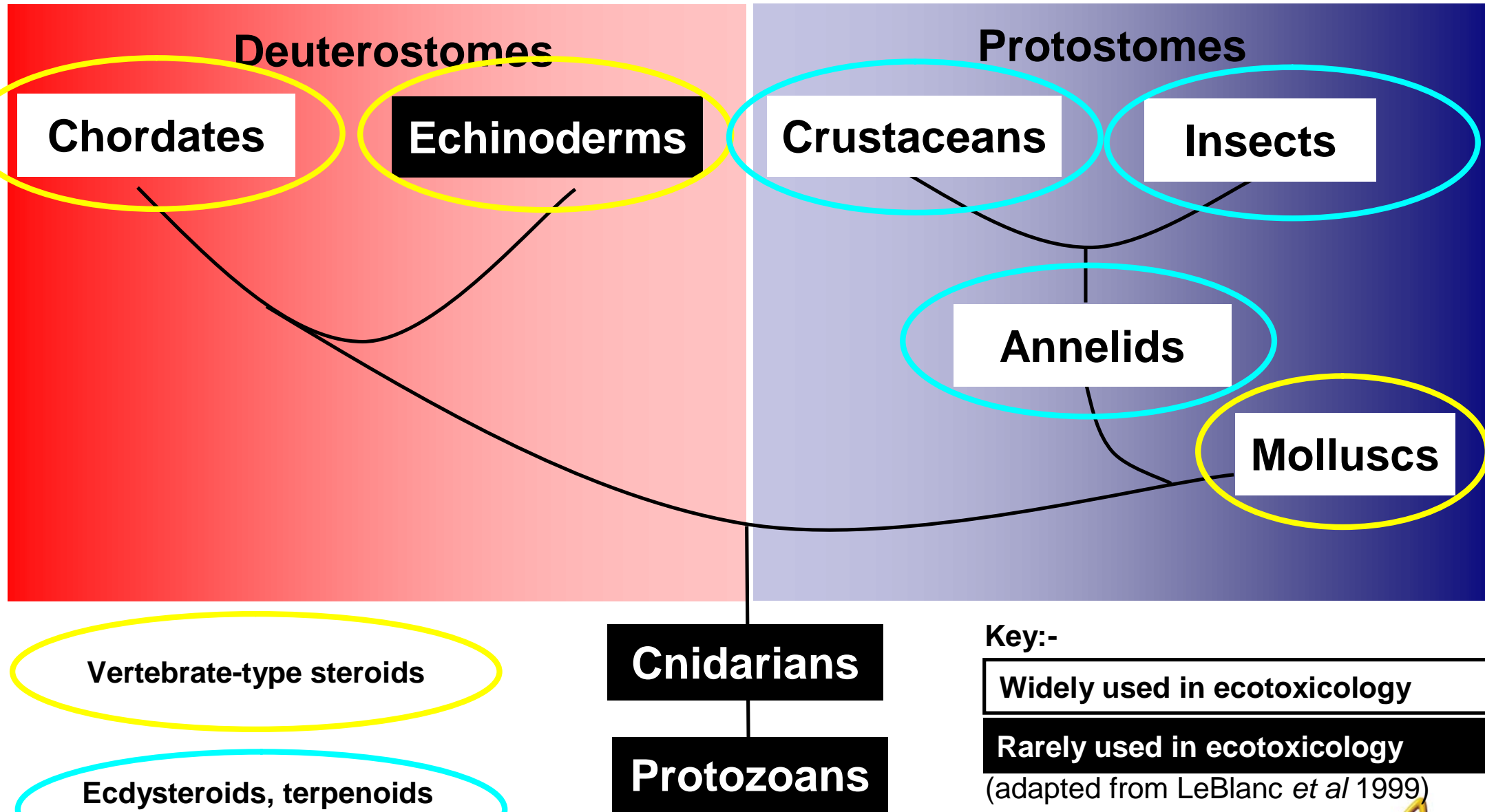


In vitro BII Assay for Ecdysteroid Activity

<u>Substance</u>	<u>Concn</u>	<u>Response (+/-)</u>
Bisphenol A	$3.0 \times 10^{-3}M$	Active antagonist
DES	$1.0 \times 10^{-3}M$	Inactive
Diethylphthalate	$2.0 \times 10^{-3}M$	Active antagonist
E2	$3.0 \times 10^{-5}M$	Inactive
Flutamide	$5.0 \times 10^{-5}M$	Inactive
Genistein	$1.0 \times 10^{-3}M$	Inactive
20-hydroxyecdysone	$7.6 \times 10^{-9}M$ (~4 ppb)	Active agonist
Lindane	$3.0 \times 10^{-3}M$	Active antagonist
Methoxychlor	$1.0 \times 10^{-3}M$	Inactive
Octylphenol	$1.0 \times 10^{-3}M$	Inactive

Dinan et al (2001) *Environ Toxicol Chem* 20: 2038-2046

Phylogeny of major invertebrate groups within Coelomates



Biomarkers for fish screening



Biomarkers guide design of chronic tests

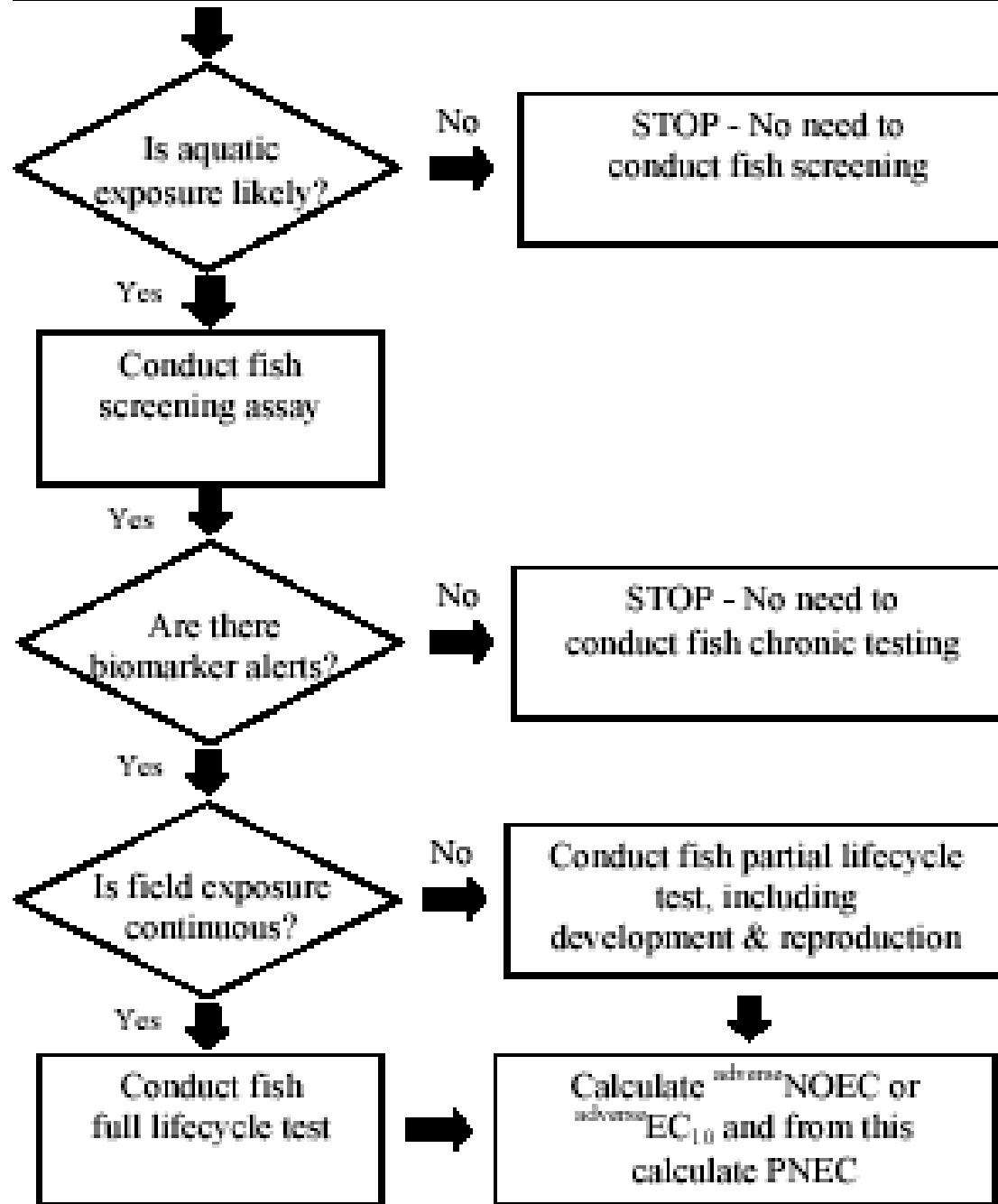
NOEC or EC_x from population endpoints

Calculate PNEC

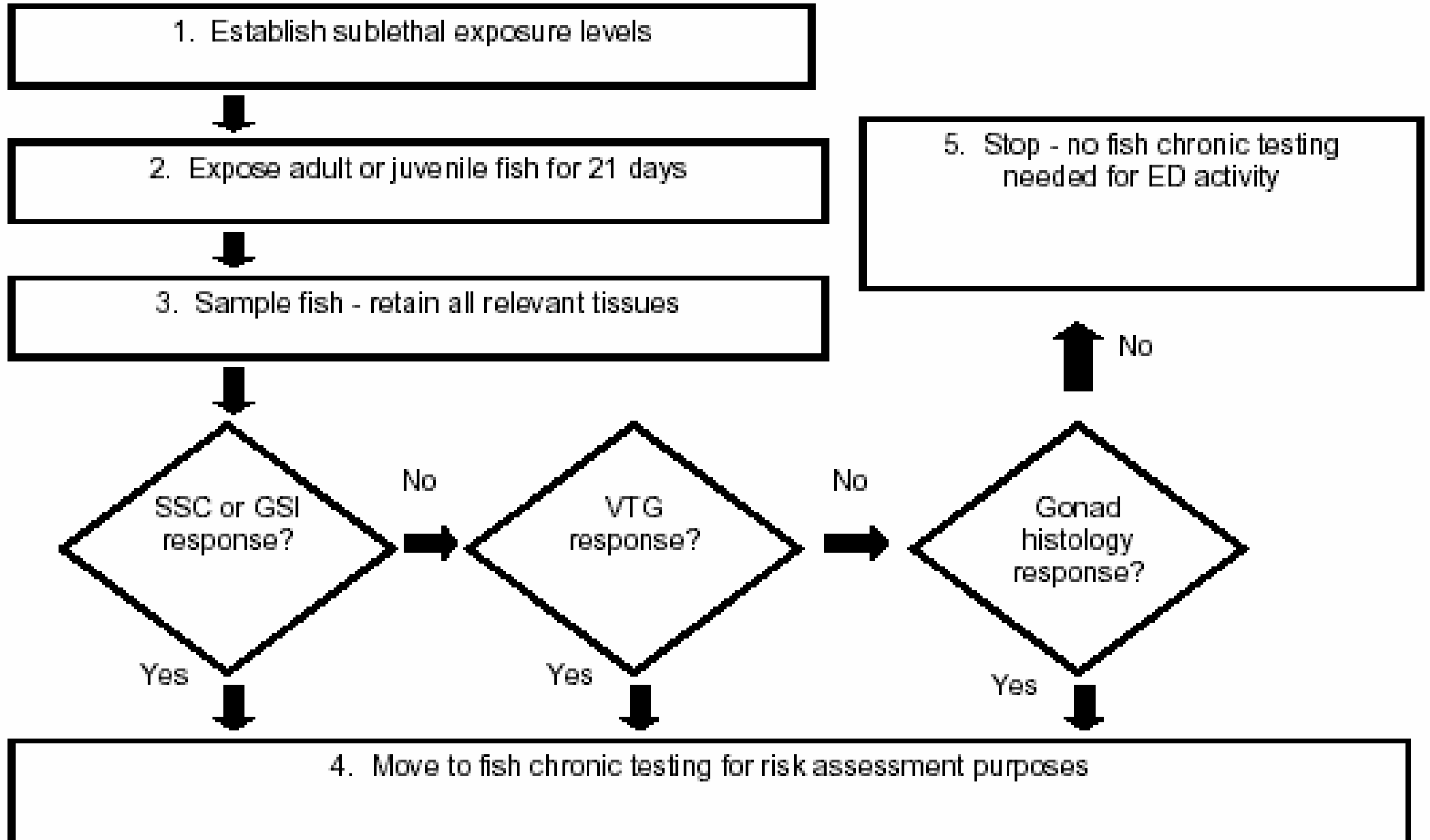
Biomarkers to link lab - field

Review existing information:

- *in vitro* or *in silico* data
- alerts based on chemical structure
- alerts from mammalian, avian or amphibian data



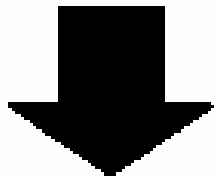
Fish screening – stepwise biomarkers



Biomarker responses are not adverse effects

biomarker EC₁₀, NOEC or LOEC

- VTG
- SSC
- GSI
- Gonad histology
- Plasma steroids
- Enzyme induction
- Gene expression



Biomarker signals provide mechanistic data to guide chronic testing for adverse effects. At present they should not be used to derive PNEC values for EDCs.

adverse EC₁₀, NOEC or LOEC

- survival
- length
- weight
- development
- fecundity
- fertilisation rate
- hatching success



Adverse effect measures address population relevant parameters and should currently be used for Calculating PNEC values for EDCs.

Acknowledgments

- Thanks to Gary Ankley (US Environmental Protection Agency, Minnesota), Helmut Segner (University of Bern, Switzerland) and Charles Tyler (University of Exeter, UK) for contributing to these ideas.
- For further information see article (available online): Hutchinson TH, Ankley GT, Segner H, Tyler CR (2006). Screening and testing for endocrine disruption in fish - biomarkers as signposts not traffic lights in risk assessment. *Environ Health Perspect*

Thank you for your attention!