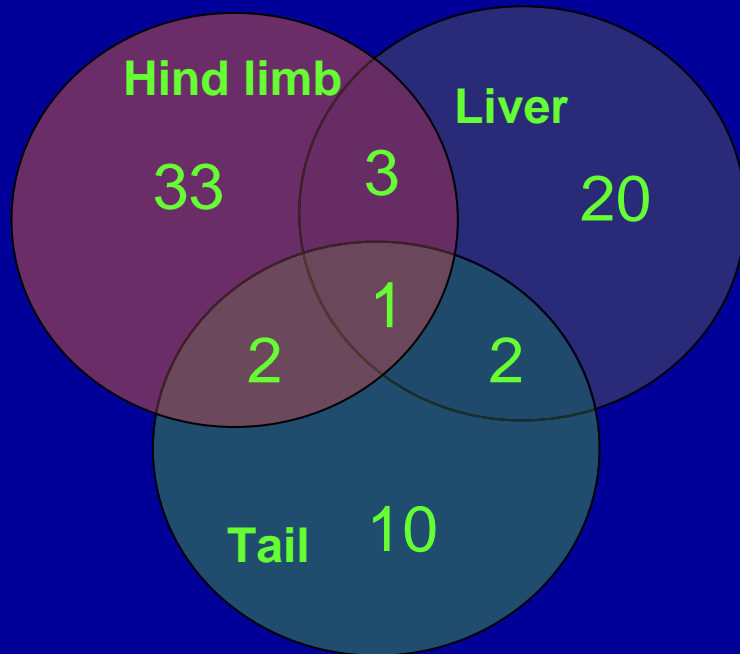


Conditions for the Amphibian Metamorphosis Assay

Test animal		<i>Xenopus laevis</i> larvae
Test substance		XXX
Age at test initiation (post fertilization day)		About 14 days (from fertilization to stage 51)
Exposure period		21 days (from stage 51)
Concentration		5 (4 concentrations and control)
Replication		4 replications per test concentration and control
Number of tanks		20 tanks
Larval density		20 tadpoles per 4L tanks
Number of larvae		400 tadpoles (20 tadpoles / tank × 20 tanks)
Number of determination	Day 7	100 tadpoles (5 tadpoles / tank × 20 tanks)
	Day 21	300 tadpoles (15 tadpoles / tank × 20 tanks)
Endpoints		5 points (Whole body length, snout-vent length, hind limb length, developmental stage, thyroid histology)
Number of thyroid histology		100 tadpoles (5 tadpoles / tank × 20 tanks)
Anal ysis chem istry	Sample schedule from tanks	4 times (Once a week)
	Number of sample from tanks	64 (4 concentration /times × 4replication × 4 times)
	Sample schedule from stock solution	5 (Day 0, 4, 8, 13, 17)
	Number of sample from stock solution	10 (1concentration /time × 2 replication /concentration 5 times)
	Number of total sample	74

Identified T₄ Responsive Genes on Metamorphosis Assay

About a gene identified by a gene expression profile, acquired the individual genetic information such as sequence and the gene function from database and classified it in known gene or unknown gene.



Identified genes on each tissues

簡易同定名	Group	発生における発現量の増減	機能別	T4応答に関する文献	相同性の高い遺伝子					
					a	b	c	d	e	f
actin, alpha, cardiac muscle	I	+	1	-	○					
fibrin (Homo sapiens)	I	+	1	-						
MyHC (myosin heavy chain) (<i>Cynops pyrrhogaster</i>)	I	+	1	Sachs 1997						
myosin light chain, fast skeletal muscle (<i>Xenopus laevis</i>)	I	+	1	Moutou 2002	○					
myozenin 1	I	+	1	-						
nebulin (<i>Mus musculus</i>)	I	+	1	-						
titin (<i>O. zuniculus</i>)	I	+	1	-						
tropomyosin I, skeletal, fast 2	I	+	1	-						
Xenopus laevis, myosin light chain (MLC1)	I	+	1	-						
activated protein kinase C receptor (RACK1) (<i>Xenopus laevis</i>)	I	+	4	Moutou 2002	○					
caspase 6, cysteine protease	I	+	5	-						
mitochondrial (12S rRNA, tRNA-Val, 16S ribosomal RNA gene)	I	+	8	-						
unknown 15 (EST/NIH)	I	+	13	-						
unknown 16 (EST/NIH)	I	+	13	-						
unknown 18 (EST/NIH)	I	+	13	-						
unknown 20 (EST/NIH)	I	+	13	-						
unknown 21 (EST/NIH laevis)	I	+	13	-						
unknown 24 (EST/NIH)	I	+	13	-						
unknown 25 (EST/NIH)	I	+	13	-						
unknown 26 (EST/NIH)	I	+	13	-						
unknown 27 (EST/NIH)	I	+	13	-						
unknown 28	I	+	13	-						
Unknown (要再解析)	I	+	13	-						
thymopoietin	I	-	7	-						
RNA-binding protein (TPA) (<i>Mus musculus</i>)	I	-	9	-						
calnexin	I	-	10	-						
unknown 19 (EST/NIH)	I	-	13	-						
unknown 22 (EST/NIH)	I	-	13	-						
polycystin-2 (pkd2) (<i>Drosophila melanogaster</i>)	II	+	2	-						
betaine-homocysteine methyltransferase (<i>Homo sapiens</i>)	II	+	6	-						
unknown 23	II	+	13	-						
thioredoxin-like 1	II	-	3	-						
proliferating cell nuclear antigen (PCNA)	II	-	4	-						
ran GTP-binding protein, RAS family (<i>Xenopus laevis</i>)	II	-	4	-						
homeo box A7	II	-	9	-						
Unknown (要再解析)	II	-	13	-						
similar to CRYSTATN A1 (<i>Xenopus laevis</i>)	II	+	5	-						
DEAD-box protein p72 (P72) (Human)	II	+	11	-						
Unknown (要再解析)	II	+	13	-						
nonmuscle myosin II heavy chain A (<i>Xenopus laevis</i>)	I	+	1	Sachs 1997						
ATPase, H+ transporting, V0 subunit B	I	+	2	Das 2006						
thioredoxin-like 1	I	+	3	-						
serine (cysteine) proteinase inhibitor, clade C, member 1 (<i>Xenopus laevis</i>)	I	+	6	Δ						
mitochondrial	I	+	8	-						
mitochondrial (<i>X.laevis</i>)	I	+	8	-						
mitochondrion	I	+	8	-						
MTERF domain containing 1	I	+	8	-						
CCAAT-enhancer binding protein delta (<i>Xenopus laevis</i>) (C/EBP)	I	+	9	-						
zinc finger transcription factor slug (slug) gene (SNAI2)	I	+	9	-						
eukaryotic translation elongation factor 2	I	+	10	-						
unknown 12 (EST/NIH)	I	+	13	-						
Unknown (要再解析)	I	+	13	-						
Unknown (要再解析)	I	+	13	-						
HMG-CoA reductase mRNA (<i>Xenopus laevis</i>)	I	-	6	Ness 2000						
unknown 10	I	-	13	-						
unknown 8 (EST/NIH)	I	-	13	-						
serine (or cysteine) proteinase inhibitor, clade A (alpha-1), member 1	II	+	6	Δ						
unknown 11 (EST/NIH)	II	+	13	-						
unknown 14	II	+	13	-						
ribosomal protein S15	II	+	10	-						
unknown 13 (EST/NIH)	II	+	13	-						
similar to chemokine receptor 1 (<i>Xenopus laevis</i>)	II	-	12	-						
unknown 9 (EST/NIH)	II	-	13	-						
unknown 17 (EST/NIH)	II	-	13	-						
betaine-homocysteine methyltransferase (<i>Homo sapiens</i>)	II	+	6	-						
titin (<i>Homo sapiens</i>)	I	+	1	-						
mitochondrion (16 rRNA, tRNA-Leu, Ile, Gln, NADH dehydrogenase 1)	I	+	8	Das 2006						
eukaryotic translation elongation factor 2	I	+	10	-						
unknown 2 (EST/NIH)	I	+	13	-						
14-3-3	I	-	5	Valdhoen 2002						
thyroid hormone receptor interactor 12 (<i>Mus musculus</i>)	I	-	9	-						
unknown 1 (EST/NIH)	I	-	13	-						
betaine-homocysteine methyltransferase (<i>Homo sapiens</i>)	II	+	6	-						
similar to CRYSTATN A1 (<i>Xenopus laevis</i>)	II	+	5	-						
ribosomal protein L17 (<i>Xenopus laevis</i>)	II	-	10	-						
unknown 4 (MGC laevis)	II	+	13	-						
unknown 5 (EST/NIH)	II	+	13	-						
unknown 6 (EST/NIH)	II	+	13	-						
translocated promoter region (<i>Xenopus laevis</i>)	II	+	4	-						
membrane bound C2 domain containing protein (<i>Xenopus laevis</i>)	II	-	4	-						

- a ニシツメガエルで同定されている遺伝子
- b アフリカツメガエル他、カエルで同定されている遺伝子
- c カエルでは同定されていない遺伝子
- d ニシツメガエルのESTデータベースが存在する遺伝子
- e アフリカツメガエルのESTデータベースならば存在する遺伝子
- f 相同性の高いデータベースが存在しない遺伝子

Summary of gene expression by T4

Enhanced TG 211: *Daphnia magna* Reproduction Test



Different Endocrine Systems Between Vertebrates and Invertebrates

Vertebrates
(e.g., Mammals)

Androgen
Estrogen

DES
EE2, etc.

Nonylphenol
Bisphenol A, etc.

Invertebrates
(e.g., Arthropods)

Ecdysteroid
Juvenile hormone

Insect Growth Regulators
(IGRs)

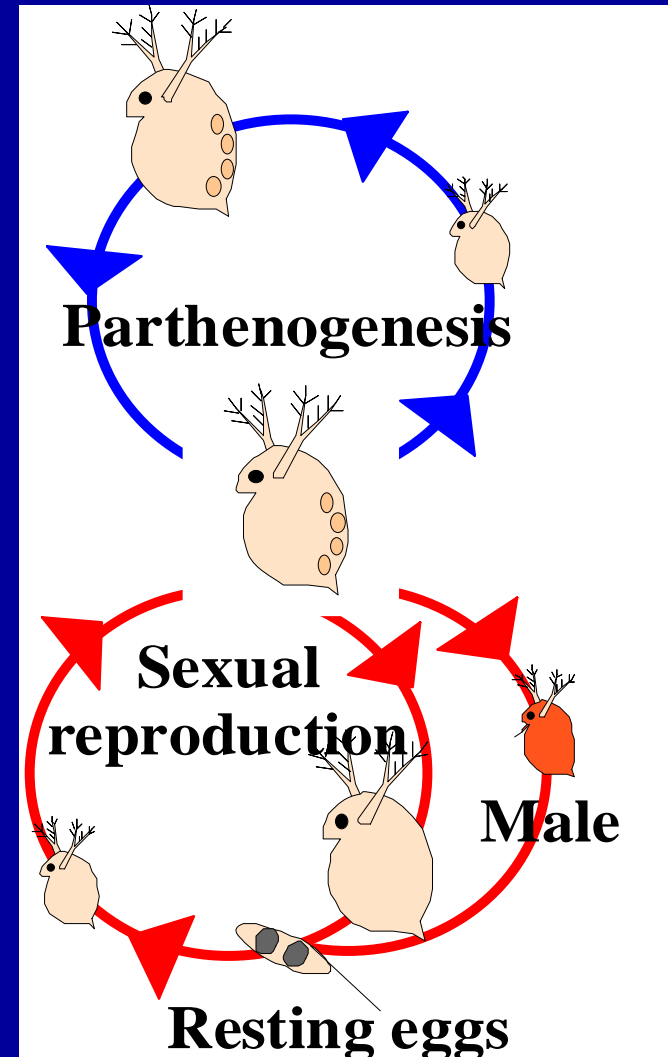
Dieldrin?

Crustacean Reproduction Toxicity Tests in OECD

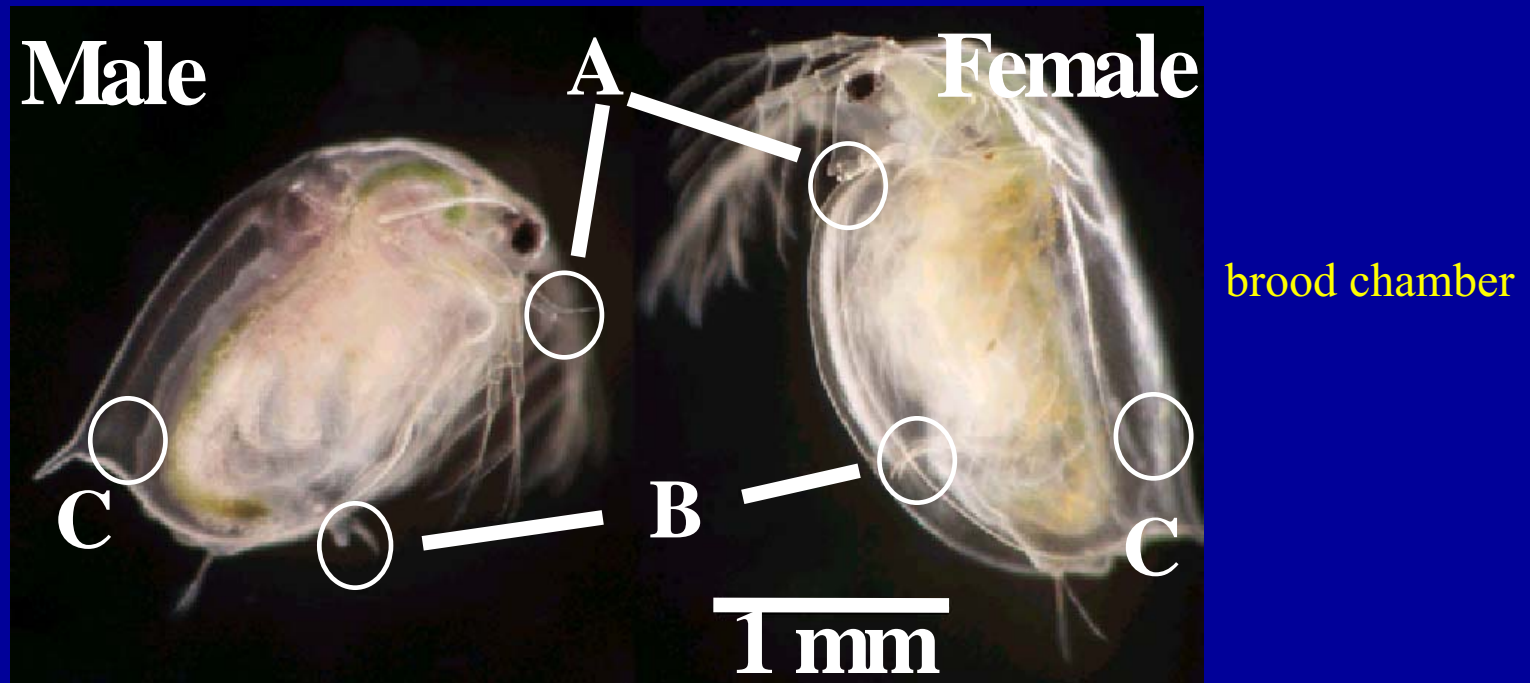
- Draft DRP on **mysid** life cycle toxicity test
(U.S.A)
- Draft proposal on a new test guideline
copepod development and reproduction test
(Sweden)
- *Daphnia magna* reproduction test
(TG 211; OECD 1998)
 - **Proposal for enhanced TG 211** for endocrine
disrupting chemicals

Reproductive System and Sex Determination in Cladocerans

- Cyclic parthenogenesis
 - Environmental sex determination
 - Emergence of males and initiation of sexual reproduction by
 - Short photoperiod
 - Low food concentration
 - High population density, etc.
- adverse conditions → sexual cycle



Sex Differentiation



A: 1st antenna

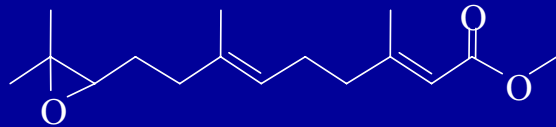
B: post abdominal claw

C: abdominal process

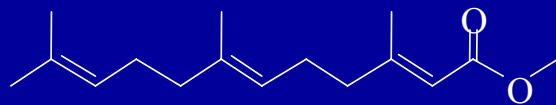
Juvenile Hormones and Their Analogs

Juvenile hormones

juvenile hormone III

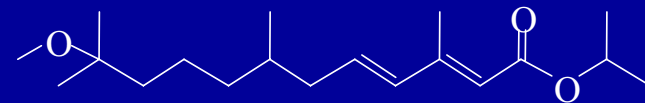


methylfarnesoate

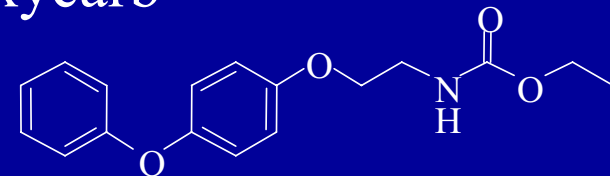


Juvenile hormone analogs (Insect Growth Regulators; IGRs)

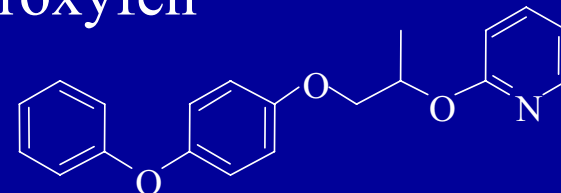
methoprene



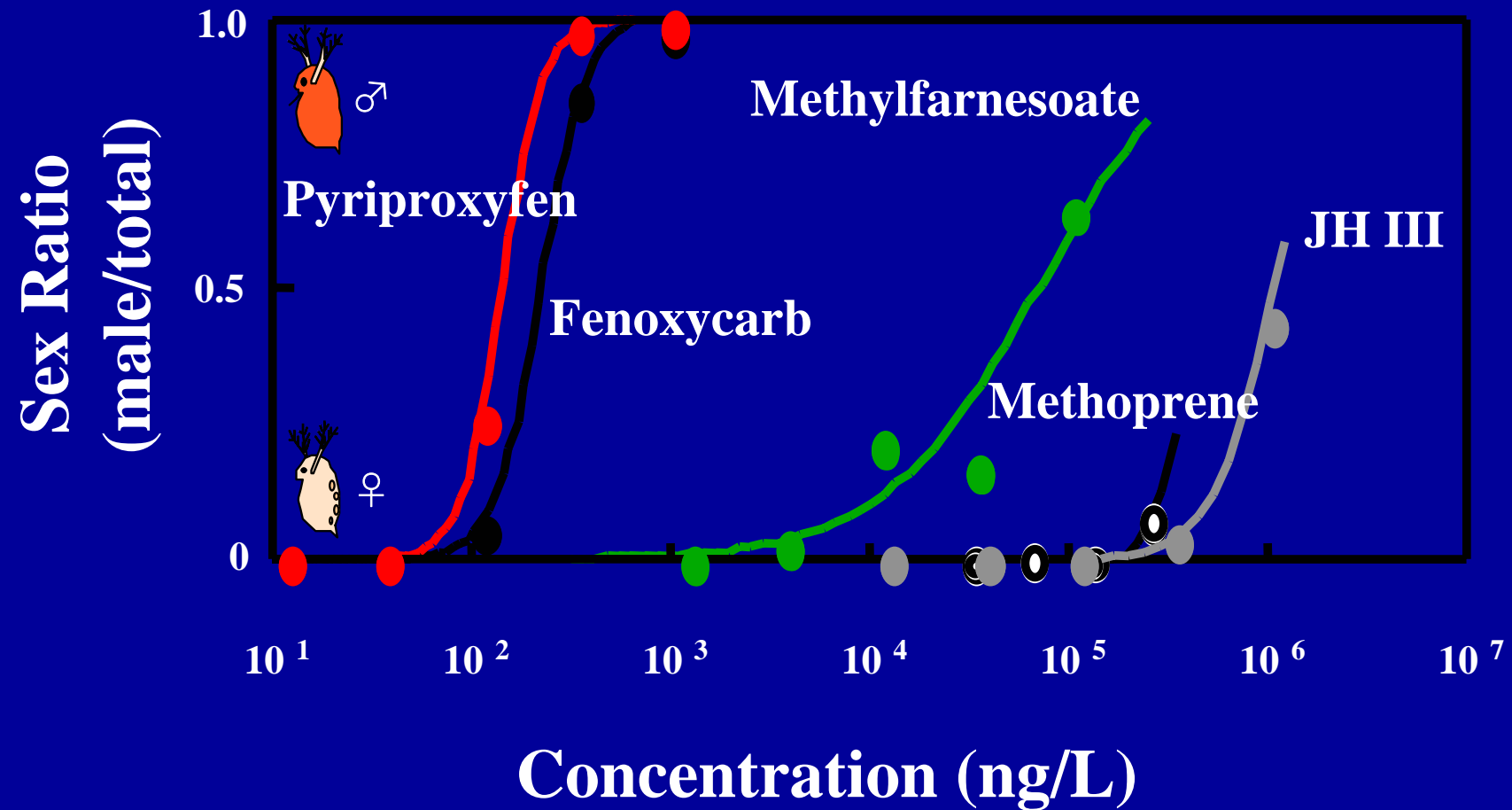
fenoxycarb



pyriproxyfen

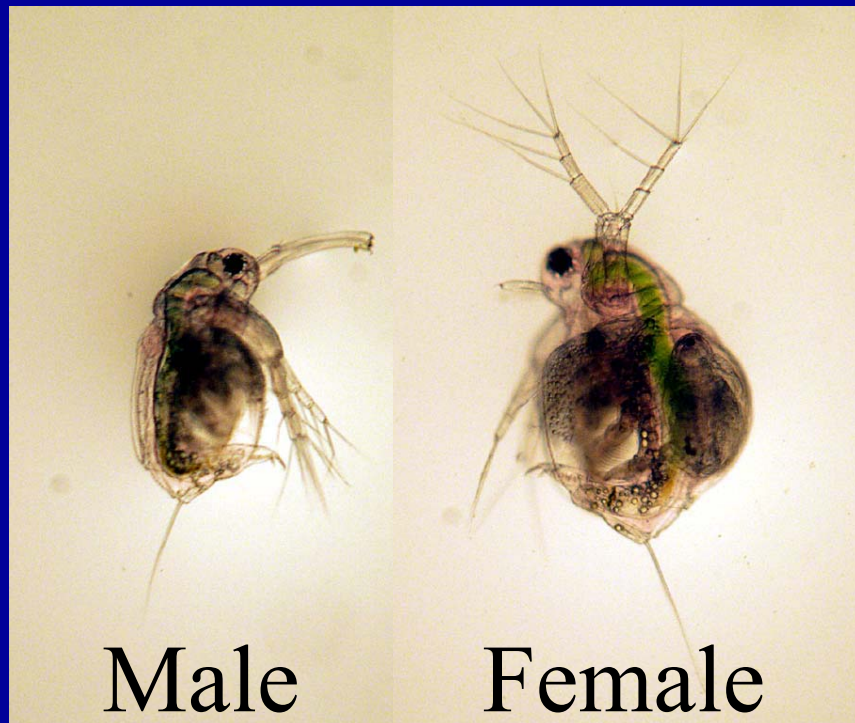


Change in Offspring Sex Ratio Exposed to Juvenoids



Other Daphnids in the Genus *Moina*

M. macrocopa



M. micrura



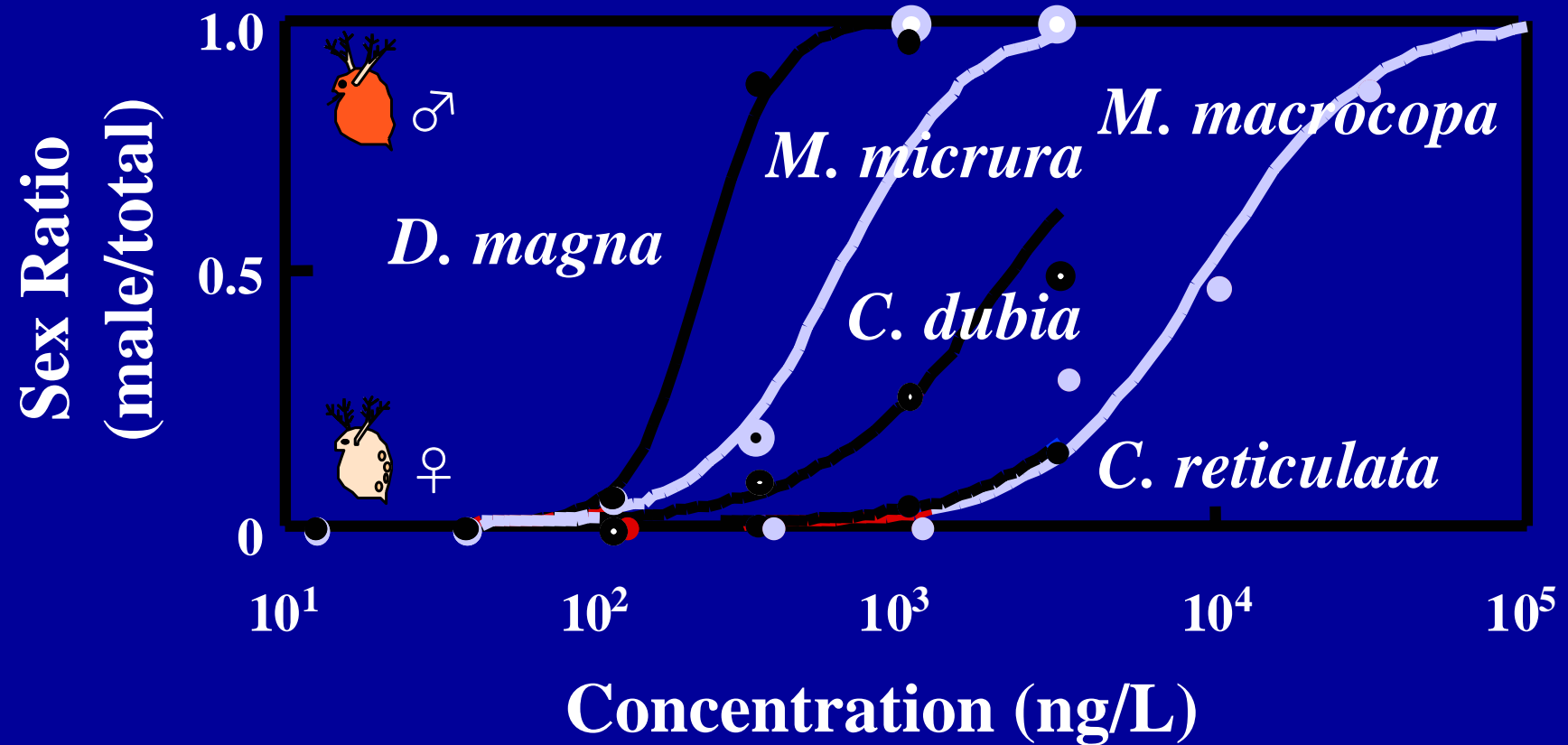
Other Daphnids in the Genus *Ceriodaphnia*

C. dubia

C. reticulata



Change in Offspring Sex Ratio Exposed to Fenoxycarb



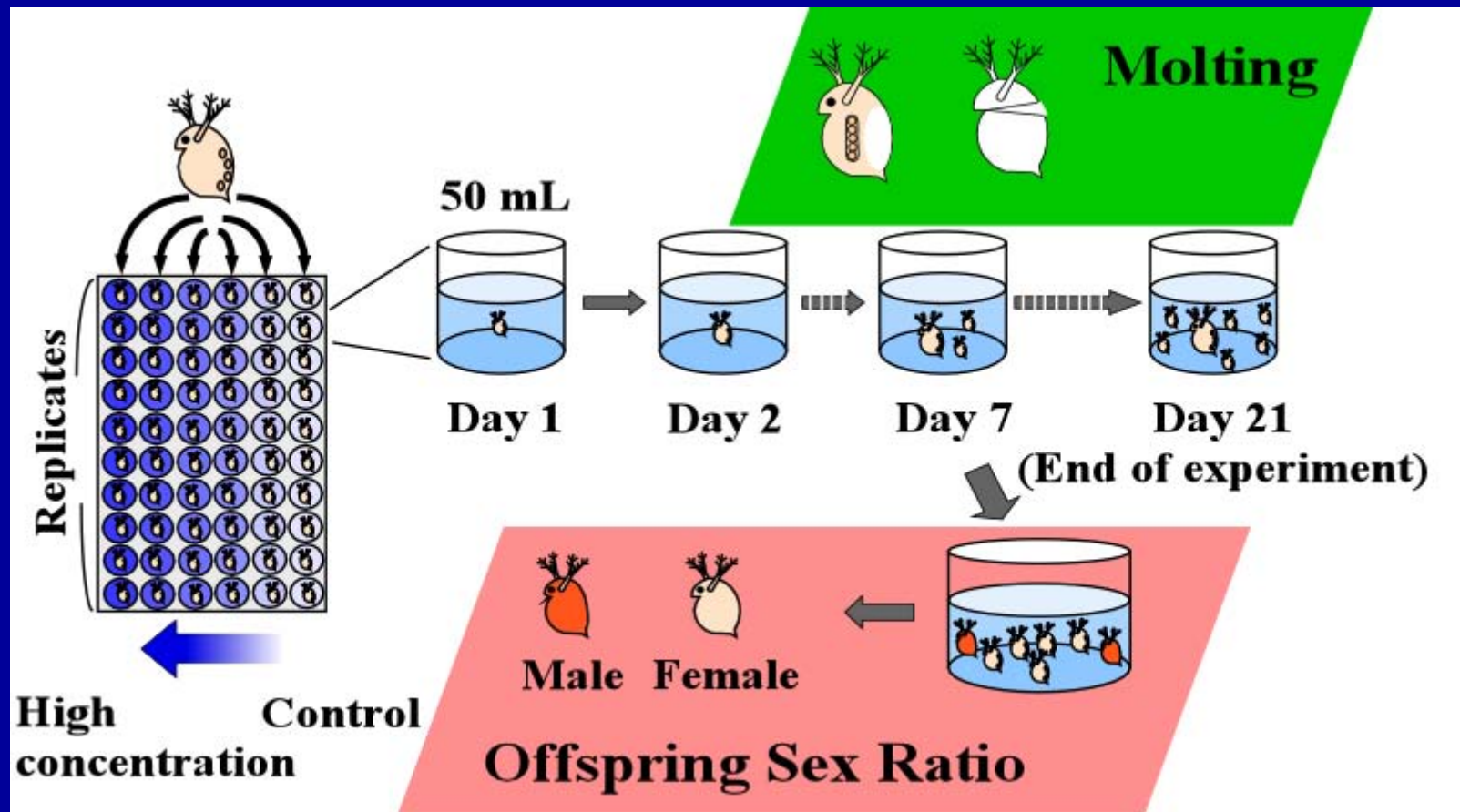
JH analogs increase male production
in cladoceran

Switching from asexual to sexual cycle
can be a marker for endocrine disruption

Experimental Design

Based on OECD TG 211

Daphnia magna reproduction test



List of Participants in the Ring Test

1. Finnish Environment Institute, Finland
2. French National Institute for Industrial Environment and Risks (INERIS), France
3. Laboratoire Ecotoxicité et Santé Environnementale (ESE) Equipe CNRS UMR, France
4. Aachen University, Germany
5. Bayer CropScience AG, Germany
6. Institute for Biological Analysis and Consulting (IBACON), Germany
7. UMWELTBUNDESAMT (UBA), Germany
8. Laboratory of Hydrobiology, Hungary
9. National Institute of Health, Italy
10. Agricultural Chemicals Inspection Station (ACIS), Japan
11. Kureha Special Laboratory, Co., Ltd., Japan
12. National Institute for Environmental Studies (NIES), Japan