

The background of the slide is a photograph of three zebrafish swimming in clear, green-tinted water. The fish are positioned diagonally across the frame, with one at the top, one in the middle, and one at the bottom. The text is overlaid on this image.

**Evaluation for the wildlife in the effects
of endocrine disrupting substances
with using *in vivo* bioassay**

***in vivo*試験による内分泌かく乱化学物質の
野生生物に対する影響評価**

Norihisa TATARAZAKO
National Institute for Environmental Studies

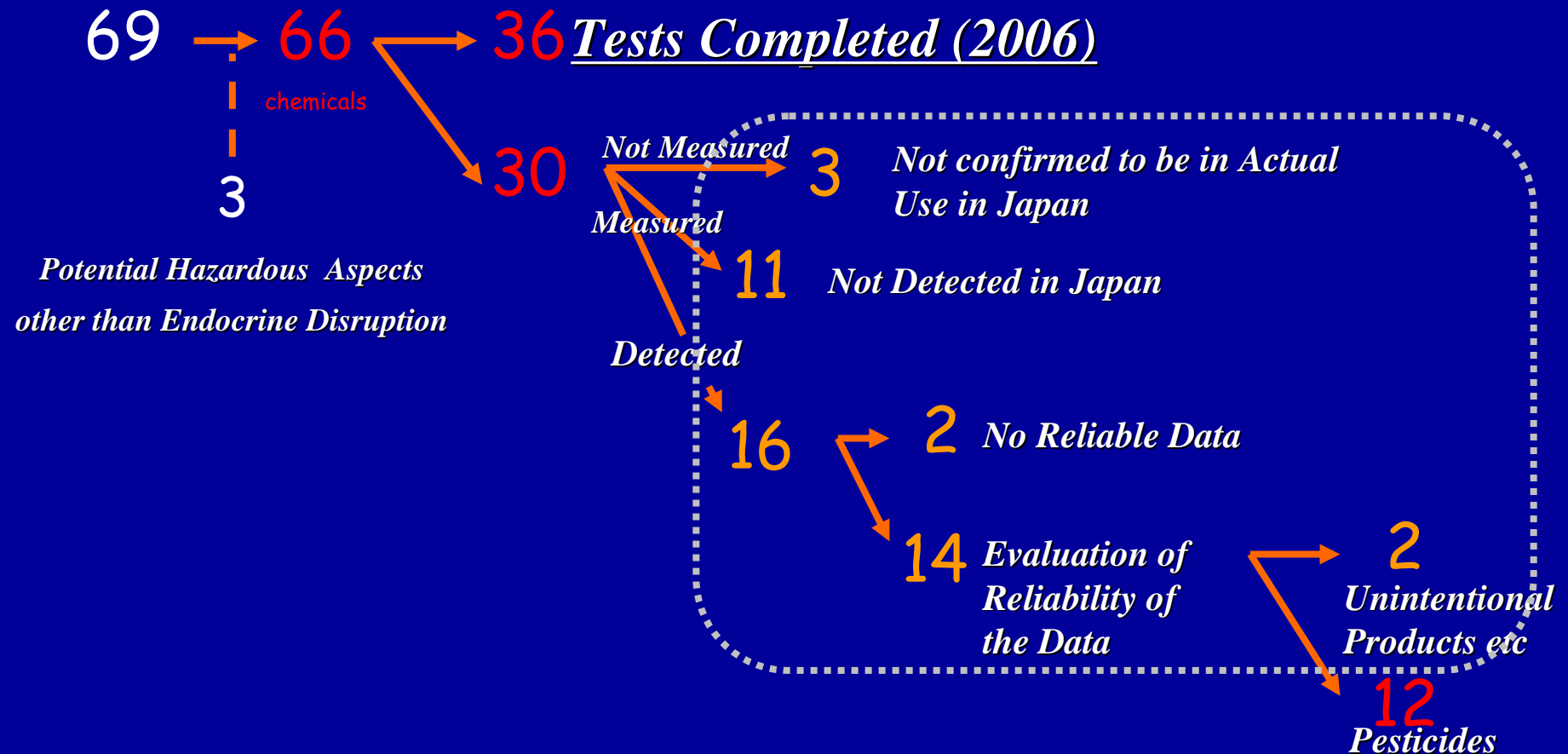
History of EDCs Issues in Japan

- Jan 1997 The Interagency Liaison Committee on Endocrine Disrupters was established
- May 1998 The then Environment Agency announced Strategic Programs on Environmental Endocrine Disrupters '98 (SPEED '98) and began research of endocrine disrupters
- Apr 2000 The Government started 'Millennium Project' (Assessment of chemicals)
- Oct 2003 Ministry of the Environment (MOE) established a working group to discuss the revision of SPEED'98
- Mar 2005 Start the new program "ExTEND2005"

SPEED'98 < May 1998 ~ >

Strategic Programs on Environmental Endocrine Disruptors

Evaluation of 69 Chemicals in the list Under the Framework of SPEED'98



Evaluated chemicals

Material	VTG	PLCT	FLCT	Results	Notes	
Tributyltin	CERI	CERI				before2001
Octyl phenol	CERI	CERI	CERI	Testis-ova		2001
Nonyl phenol	CERI	CERI	CERI	Testis-ova		2002
Di-n-butyl phthalate	CERI	CERI	CERI	Testis-ova		2003
Octachlorostyrene	METO	CERI				2004
Benzophenone	METO	CERI				
Dicyclohexyl phthalate	METO	CERI				
Di-(2-ethylhexyl)phthalate	METO	CERI				
Triphenyltin	NIES	NIES				
Butyl benzyl phthalate	NIES	NIES				
Diethyl phthalate	NIES	NIES				
Diethylhexyl adipate	NIES	CERI				
Pentyl phenol	CERI	CERI	CERI			
Pentachlorophenol	NIES	NIES				
Amitrole	NIES	NIES				
Bisphenol A	METO	NIES	NIES	Testis-ova		
2,4-Dichlorophenol	NIES	NIES				
4-Nitrotoluene	METO	CERI				
Dipentyl phthalate	METO	CERI				
Dihexyl phthalate	METO	CERI				
Dipropyl phthalate	METO	CERI				
Hexachlorobenzene (HCB)	CERI	CERI			POPs	
Hexachlorocyclohexane	METO	CERI				
Chlordane	METO	CERI			POPs	
trans-Nonachlor	METO	CERI				
o,p-DDT	CERI	CERI			POPs	
p,p-DDT	NIES	NIES			POPs	
p,p-DDE	NIES	NIES				
p,p-DDD	NIES	NIES				
Aldrin	NIES	NIES			POPs	
Endrin	NIES	NIES			POPs	
Dieldrin	NIES	NIES			POPs	
Heptachlor	METO	CERI			POPs	
Mirex	METO	CERI			POPs	
Kelthane	CERI	CERI				
Malathion	METO	CERI				
Permethrin	CERI	CERI				
17β-Estradiol		NIES	CERI	Testis-ova		
17α-Ethinyl Estradiol	CERI	NIES&CERI	NIES	Testis-ova		
Metyl testosterone	CERI		CERI			
Fultamide	CERI		CERI			

Framework for the risk assessment of EDCs in Japan

Screening

In vivo testing animal: Japanese medaka *Oryzias latipes*

Estrogen (Androgen) receptor binding assay



Selected priority materials



In vivo vitellogenin assay (VTG assay)



21 days: 4 months old, male

Partial life-cycle test (PLCT): Evaluation during 1 generation

Secondary sex character · Hepatic VTG · Gonad histopathology



70 days: Eggs to 2 months old, both sex

Final examination

Full life-cycle test (FLCT): Evaluation during 2 generation

Examining the same analysis as PLCT in both F0 and F1

Breeding test is examined between F0 and F1.

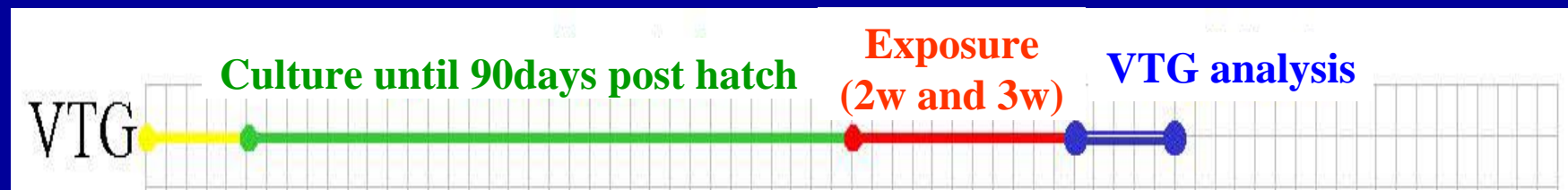
170 days: Eggs to 2nd generation →

Evaluation



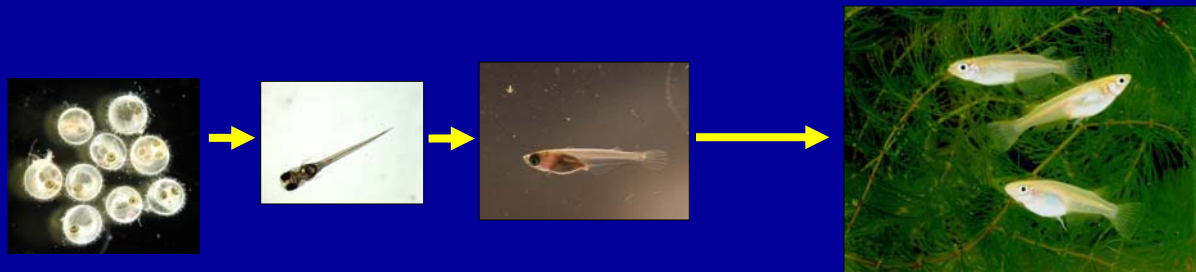
In vivo vitellogenin assay

- **Animals** : Japanese Medaka(*Oryzias Latipes*)
age:2~3month sex:male keep & breed without EDCs
- **Dose**:six concentrations including control
- **Measuring**: pH, DO, temperature (everyday) and actual concentration of chemicals (once a week)
- **Feeding** :altemia 3times per day, Gluttony
- **Exposing method**: Continuous-flow mini-diluter system
- **Periods** : 1,2,3 weeks, dissect 10 fish at every week
- **Endpoint**: measure amount of vitellogenin in liver by ELISA
- Calculate statistically and obtained no-effective-concentration(NOEC)

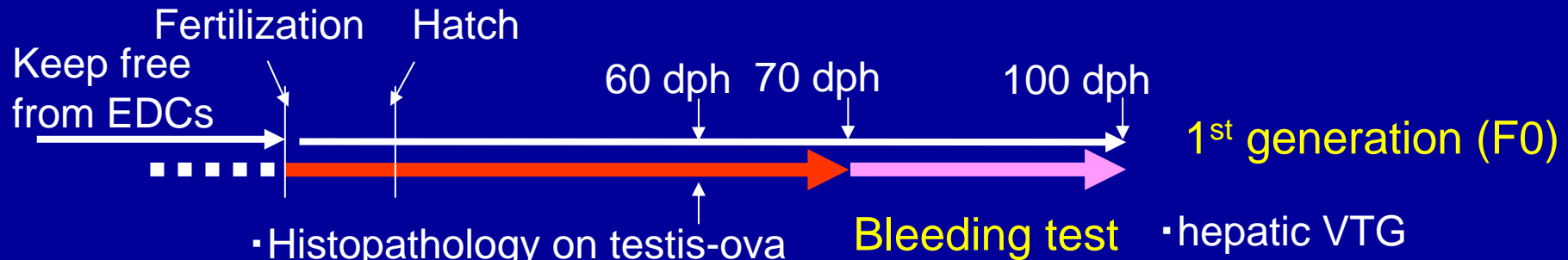


Partial life-cycle test

- **Eggs** : Japanese Medaka(*Oryzias Latipes*)
fertilized ovum within 4 hours after egg-laying
- **Dose**: six concentrations including control
- **Measuring**: pH, DO, temperature (everyday) and actual concentration of chemicals (once a week)
- **Feeding** : altemia 3times per day, Gluttony
- **Exposing method**: Continuous-flow mini-diluter system
- **Periods** : about 2 months,
(until the secondary sex characteristics appeared)
- **Endpoint**: measure amount of vitellogenin in liver by ELISA
microscopic observation of testis-ova development
delay or abnormal of secondary sex characteristics
check dystropy
- Calculate statistically and obtained no-effective-concentration(NOEC)



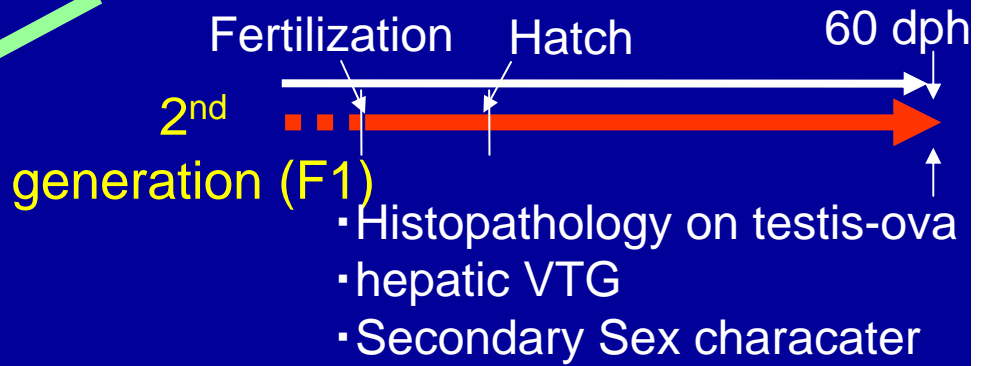
Full life-cycle test



- Histopathology on testis-ova
- Secondary Sex character
- Bleeding test**
- hepatic VTG



F1 eggs accept Maternal Effects.



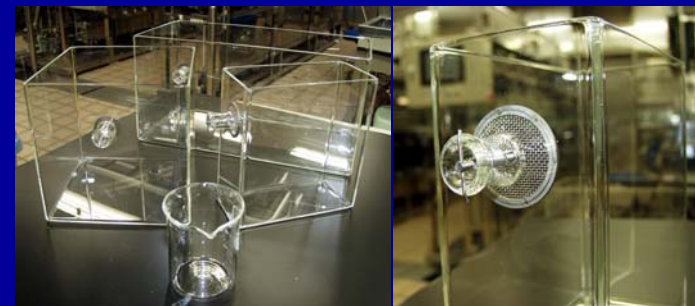
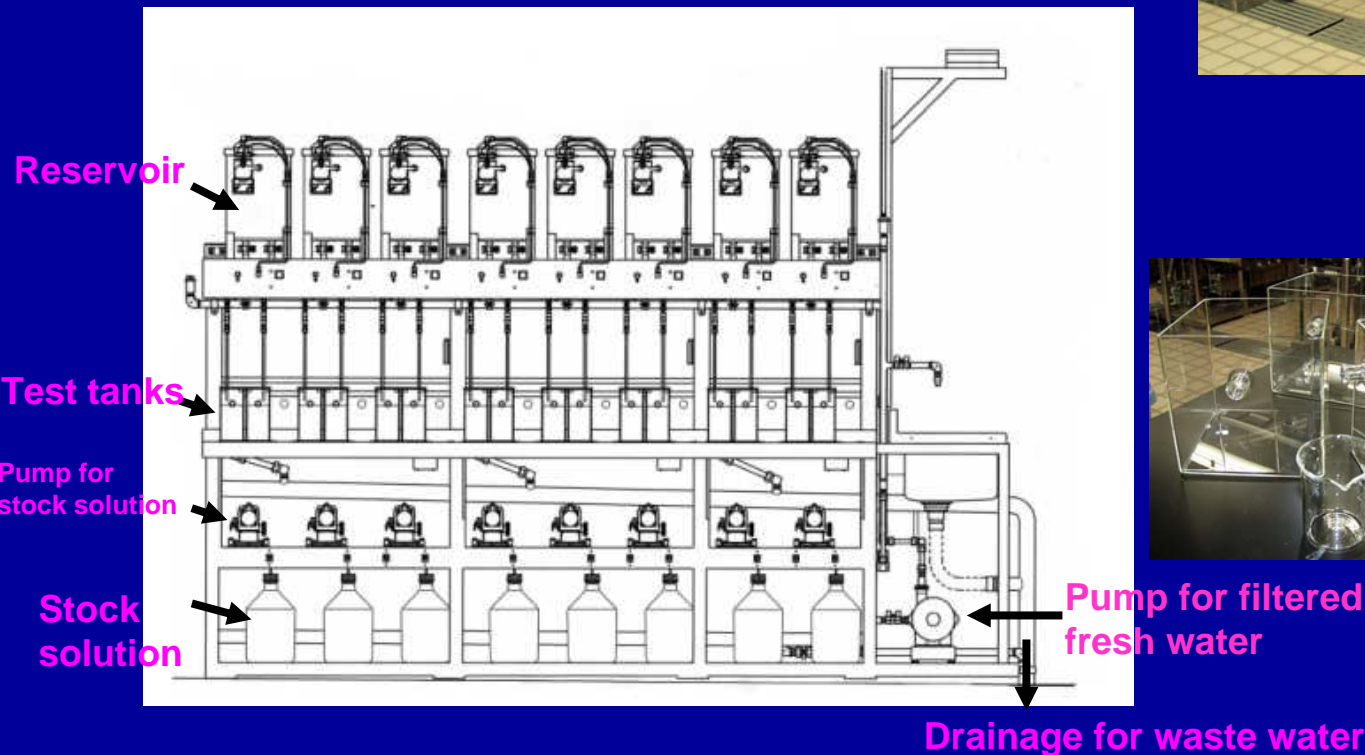
- Histopathology on testis-ova
- hepatic VTG
- Secondary Sex character



Facts of testing

Exposure system, facility

Continuous exposure using flow-through exposure system



Test tanks
(Use all glassware tank, and stainless steel mesh net to avoid the contamination of unknown chemicals from equipments.)

It is possible to keep the concentration of test chemical with constantly mixing of stock solution and fresh water.

Dissection

Medaka: A useful SMALL fish in Laboratory



Need high skills for dissection