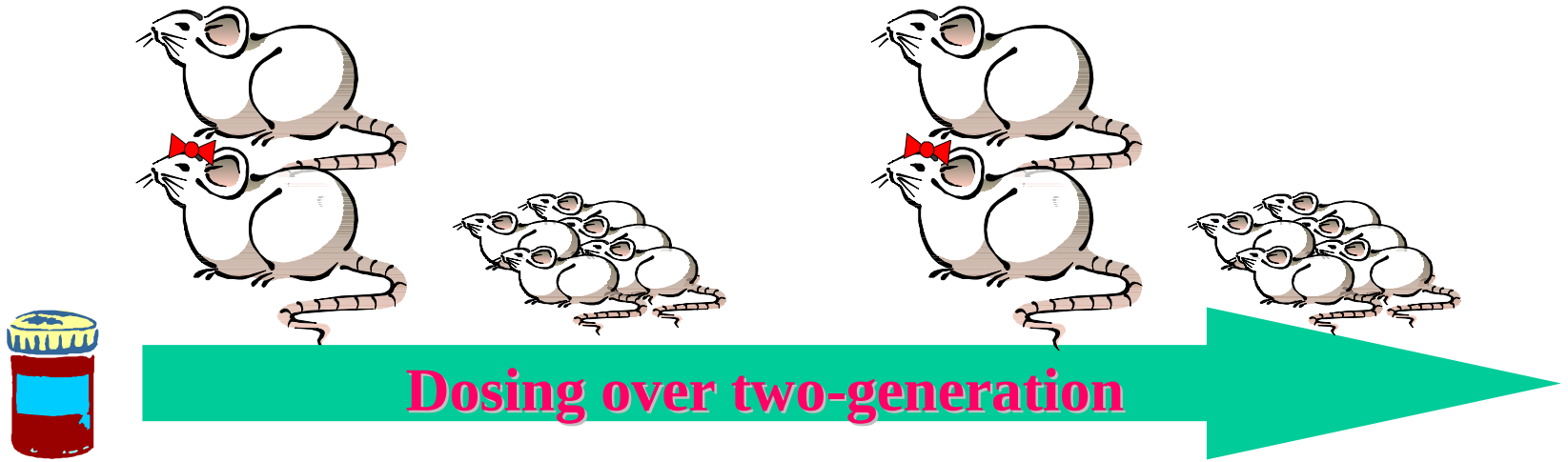
Outline of Two-generation reproduction study

- **A study for effects of the chemicals on the reproductive capacity of animals over two-generation**



Examine the effects of chemicals on ovum formation or spermatogenesis, reproductive capacity, process of sexual maturation etc.

Outline of *in utero* and lactationary exposure study

- **A simplified version of the one/two generation study**

Examine the systemic and reproductive effects of chemicals on offspring by dosing during pregnancy and lactationary period.

New approach to develop definitive testing procedures

- **Reproductive effects;**
  - †Effects on maturation and aging of female reproductive organs
  - † Effects on maturation and function of male reproductive organs
- **Immuno-toxicological effects;**
  - † Using autoimmune disease model
  - † Application of LLNA to detect immuno-toxicological effect
- **Neuro-toxicological/behavioral effects;**
  - † Effects on mouse operant behavior
  - † Effects on sexual differentiation of brain

Current status of the Level 5 testing

- Hazard assessment of 15 chemicals was completed in METI's EDS program, 7 of these chemicals were conducted with typical two generation study protocol as the definitive testing.

- No test method was recognized in OECD as definitive study

- Diverse trials for searching new endpoints and test methods to detect neuro (behavior)- and immuno-, toxicological effects as well as endocrinological effects are currently running.

OECD Conceptual Framework for the Testing and Assessment of Endocrine Disrupting Chemicals

Level 1 **Sorting & prioritization based upon existing information**	- physical & chemical properties/fate (MW, reactivity, volatility, persistence and bioacumulation, pH, Po/w - exposure information/models (production volume, release and use pattern, human and environ-mental monitoring data, etc.) - hazard information (e.g. QSAR, human data, available toxicological data)
Level 2 ***In vitro* assays providing mechanistic data**	- ER, AR, TR receptor binding affinity - transcriptional activation - aromatase and steroidogenesis inhibition *in vitro* - Aryl hydrocarbon receptor recognition/binding - QSARs - High Through Put Prescreens - Thyroid function - Fish hepatocyte VTG assay - Others (as appropriate)
Level 3 ***In vivo* assays providing data about single endocrine mechanisms**	- Uterotrophic assay (estrogenic related) - Hershberger assay (androgenic related) - Non –receptor mediated hormone function - Others (e.g. thyroid)
Level 4 ***In vivo* assays providing data about multiple endocrine mechanisms**	- enhanced OECD 407 (endpoints based on endocrine mechanisms) - male and female pubertal assays - adult intact male assay
Level 5 ***In vivo* assays providing adverse effects data from endocrine & other mechanisms for RA**	- 1-generation assay (TG405 enhanced)[1] - 2-generation assay (TG416 enhanced)[1] - reproductive screening test (TG421 enhanced) [1] - combined 28 day/reproduction screening test (TG 422 enhanced) [1] 1 Potential enhancements will be considered by VMG mamm

OECD Conceptual Framework for the Testing and Assessment of Endocrine Disrupting Chemicals

Level 1 **Sorting & prioritization based upon existing information**	- physical & chemical properties/fate (MW, reactivity, volatility, persistence and bioacumulation, pH, Po/w - exposure information/models (production volume, release and use pattern, human and environ-mental monitoring data, etc.) - hazard information (e.g. QSAR, human data, available toxicological data)
Level 2 ***In vitro* assays providing mechanistic data**	- ER, AR, TR receptor binding affinity - transcriptional activation - aromatase and steroidogenesis inhibition *in vitro* - Aryl hydrocarbon receptor recognition/binding - QSARs - High Through Put Prescreens - Thyroid function - Fish hepatocyte VTG assay - Others (as appropriate)
Level 3 ***In vivo* assays providing data about single endocrine mechanisms**	- Uterotrophic assay (estrogenic related) - Hershberger assay (androgenic related) - Non –receptor mediated hormone function - Others (e.g. thyroid)
Level 4 ***In vivo* assays providing data about multiple endocrine mechanisms**	- enhanced OECD 407 (endpoints based on endocrine mechanisms) - male and female pubertal assays - adult intact male assay
Level 5 ***In vivo* assays providing adverse effects data from endocrine & other mechanisms for RA**	- 1-generation assay (TG405 enhanced)[1] - 2-generation assay (TG416 enhanced)[1] - reproductive screening test (TG421 enhanced) [1] - combined 28 day/reproduction screening test (TG 422 enhanced) [1] 1 Potential enhancements will be considered by VMG mamm

Future direction

1. *Contribution for OECD validation works*

- *In vitro* testing: Participation of international validation work for ER binding assay, reporter gene assay and steroidgenesis assays.
- *in vivo* testing: Works of validation-protocol establishment and execution of validation studies on uterotrophic and Hershberger bioassays, and enhanced TG407 study.

2. *Information disclosure and data sharing*

- Information for previous works of METI's ED-related programs is available in METI's website.
  (http://www.meti.go.jp/english/report/data/g020205ae.html)
- Outline document on METI's latest 5 years (FY2000-FY2004) ED-related programs will be published as English version in near future.

3. *Additional approach to the unsolved problems with regard to the test procedures up to 5 years (FY2006-)*

Acknowledgements

- We deeply appreciate to METI and MHLW because most of our work with regard to the EDS programs have been supported by the grants of these authorities.

- Also thanks to all the CERI staffs engaged in the this program for their extensive efforts.