A Japanese View on Assessment of Endocrine Disrupting Chemicals

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Food Safety Committee of Japan (FSCJ)

FSCJ: 7 Commissioners chaired by Dr. Hiroshi Satoh

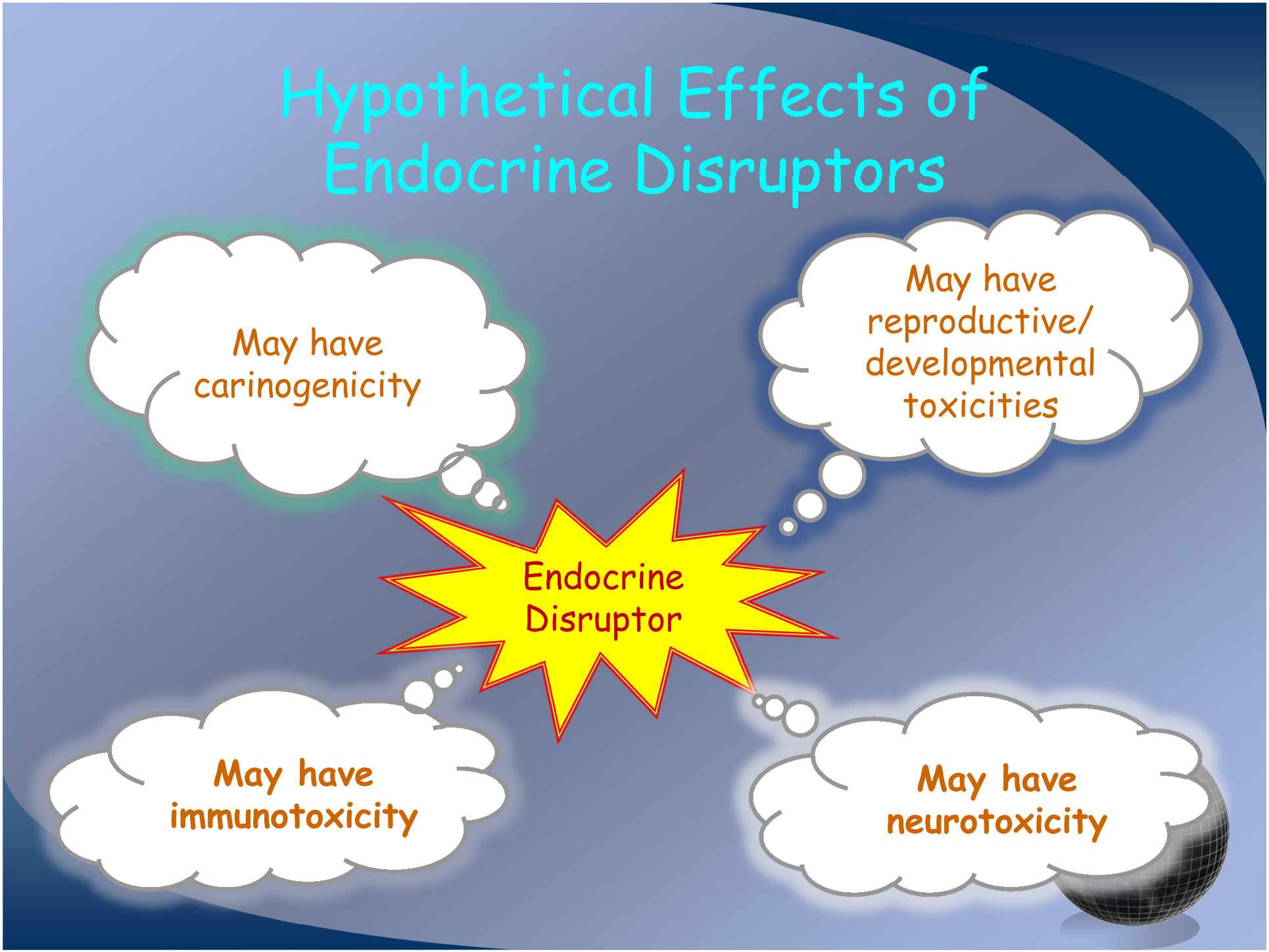
12 Expert Committees (218 experts,1 chair for each committee)

- ✓ Planning
- ✓ Food additives
- ✓ Pesticides
- ✓ Veterinary medical products
- ✓ Apparatus and containers/packages
- ✓ Chemical and contaminants
- ✓ Microorganisms and viruses
- ✓ Prions
- ✓ Natural toxins and mycotoxins
- ✓ Genetically modified foods
- ✓ Novel foods
- ✓ Feed, fertilizes

Secretariat: DG, DDG and 4 divisions (approx. 100 staffs)

Within a cabinet office

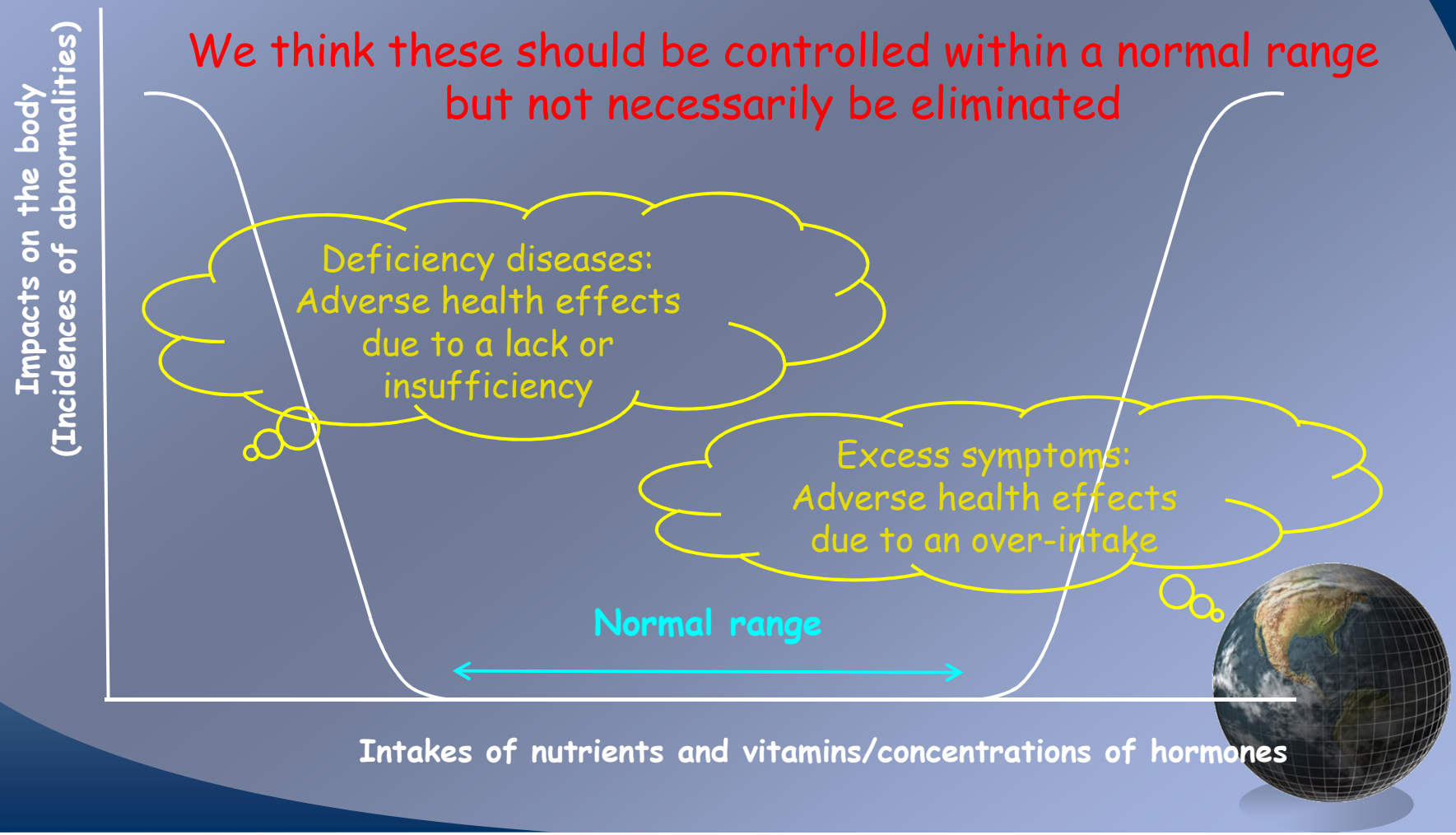
Hypothetical Effects of Endocrine Disruptors



Definition of Endocrine Disruptors: We accept the IPCS definition (2002)

An endocrine disruptor is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations.

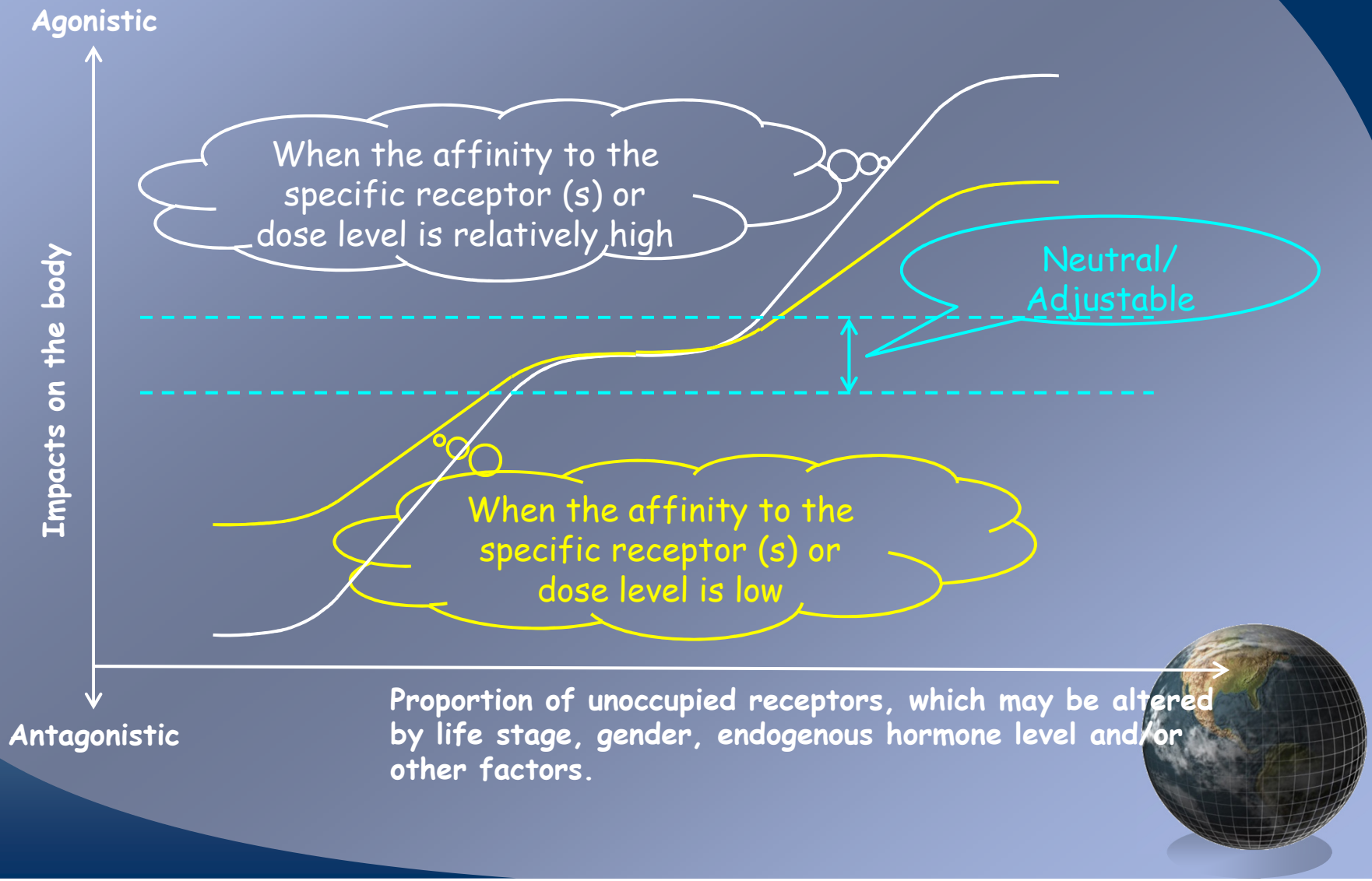
Our Basic Concept for Risk Assessments of Nutrients, Vitamins, Hormones and/or Hormonally Active Compounds in Our Foods



Our recognition of endocrine disruptions

- ✓ An endocrine disruption is one of the typical modes of action of toxicity, through which adverse health effects are induced. Toxicities mediated by altered endocrine status are not rare: e.g., even cleft palate induction by maternal restraint is known to be due to an increase in blood corticosterone level in mice (Barlow *et al.*, 1975; Hemm *et al.*, 1977).
- ✓ We think we can set ADI based on NOAEL obtained in a common set of toxicology studies.
- ✓ We sometimes request the applicant to conduct additional mechanism studies to make sure the specific mode of action(s) of manmade hormones and/or hormonally active compound for not overlooking possible adverse effects.

Speculated Mode of Action of Exogenous Weak Hormone-like Compounds (Environmental Hormones)



Low-Dose Effect Issue (Inverted U-shape Dose-response)

We are conscious of the issue but think that the evidences are still quite insufficient

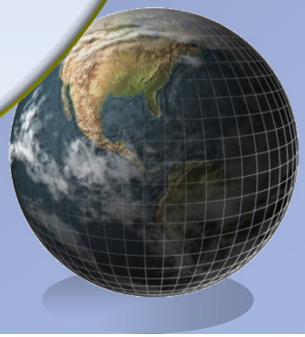
Our Doubt about Inverted U-shape Dose-response

- ✓ Most studies reporting inverted U-shape dose-responses used small numbers of outbred animals carrying genetic polymorphism(s). ⟹ Genetic variation(s) might be the cause of such phenomenon.
- ✓ Most studies reporting inverted U-shape dose-responses used phytoestrogen-rich diets. ⟹ Can we exclude the interference by phytoestrogens?
- ✓ Most studies reporting inverted U-shape dose-responses did not elucidate the basic mechanism(s) of such phenomenon.

Possible Genetic Variations (Polymorphisms) in Outbred Animals Contributable to NMDR

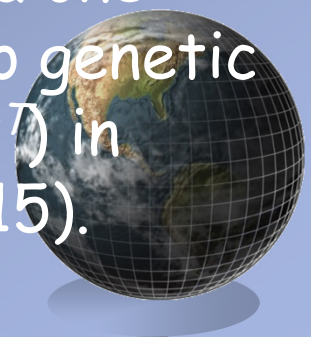
Cells of the liver, reproductive organs, endocrine systems, target organs and/or others

- ✓ Drug transporters
- ✓ Cytochrome P450s
- ✓ Peptide hormones/growth factors
- ✓ Nuclear/membrane receptors
- ✓ Downstream effectors



Examples of Genetic Polymorphisms in Outbred Animals Contributable to NMDR

- ✓ A mutation within Mdr1a (Abcb1a), a drug transporter, modulates the sensitivity to xenobiotics of mutant individuals in CF-1 mice (Umbenhauer *et al.*, 1997).
- ✓ A mutation within aryl hydrocarbon receptor (Ahr) modulates the sensitivities to xenobiotics of which toxicities are mediated by Ahr in SD rats (Okey *et al.*, 2005).
- ✓ We have reported that an apparent NMDR in a one-gen. repro-tox. study with Kelthane was due to genetic polymorphism of thyroglobulin gene ($Tg^{c.749\text{-}1G>T}$) in Wistar Hannover GALAS rats (Sato *et al.*, 2015).



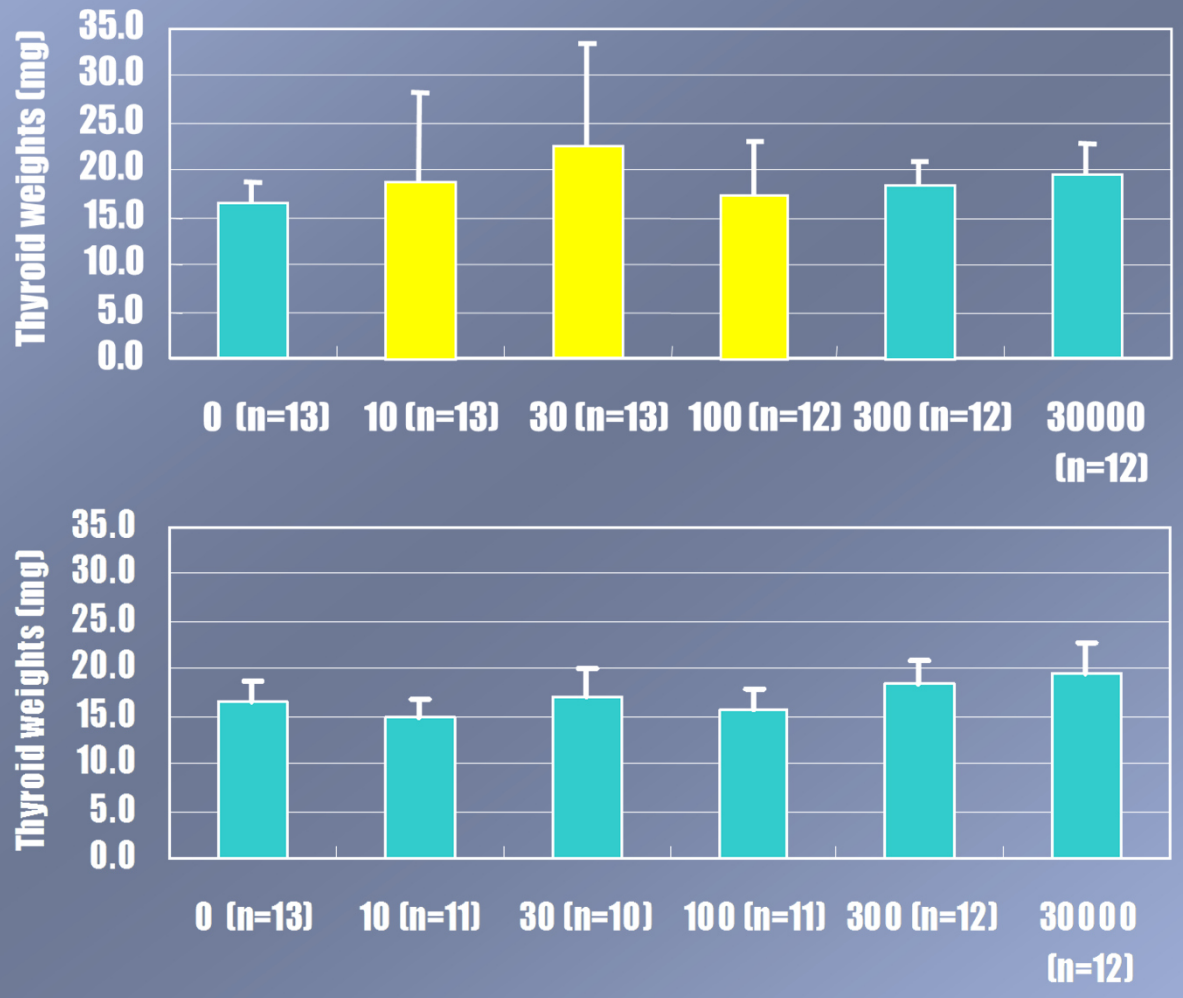
Dwarfism and Thyroidal Abnormalities Due to A Single Nucleotide Polymorphism (SNP) of *Tg*

Dwarf ($Tg^{c.749\text{-}1G>T}$/$Tg^{c.749\text{-}1G>T}$) and his phenotypically normal littermate ($Tg^{c.749\text{-}1G>T}$/+) with goiter

Electrophoresis of thyroglobulin gene (cDNA including exon 7)

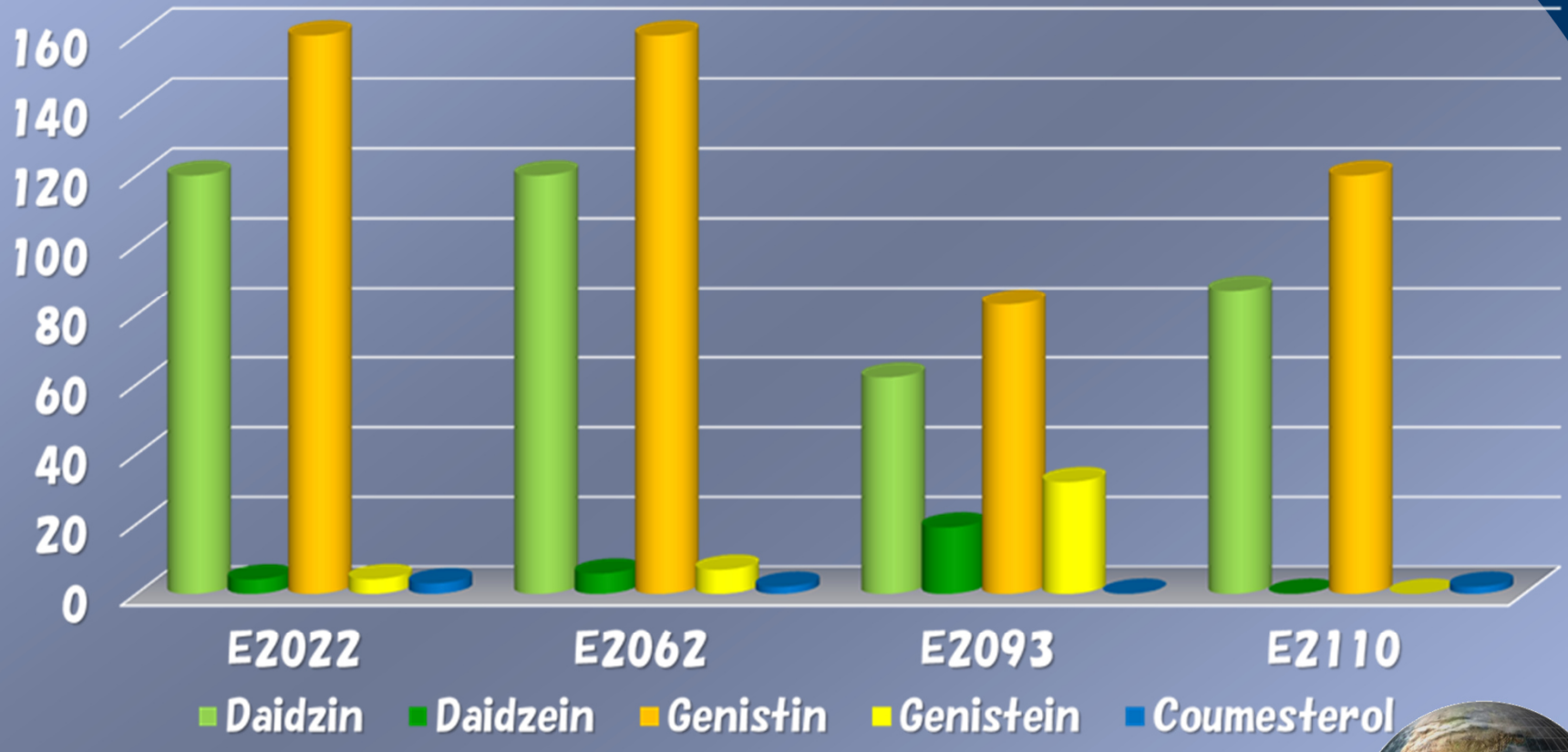
Sato *et al.*, 2014

Examples of Genetic Polymorphisms in Outbred Animals Contributable to NMDR



Sato *et al.*, 2014

Phytoestrogens in Basal Diets (Standard CE2 Diet)



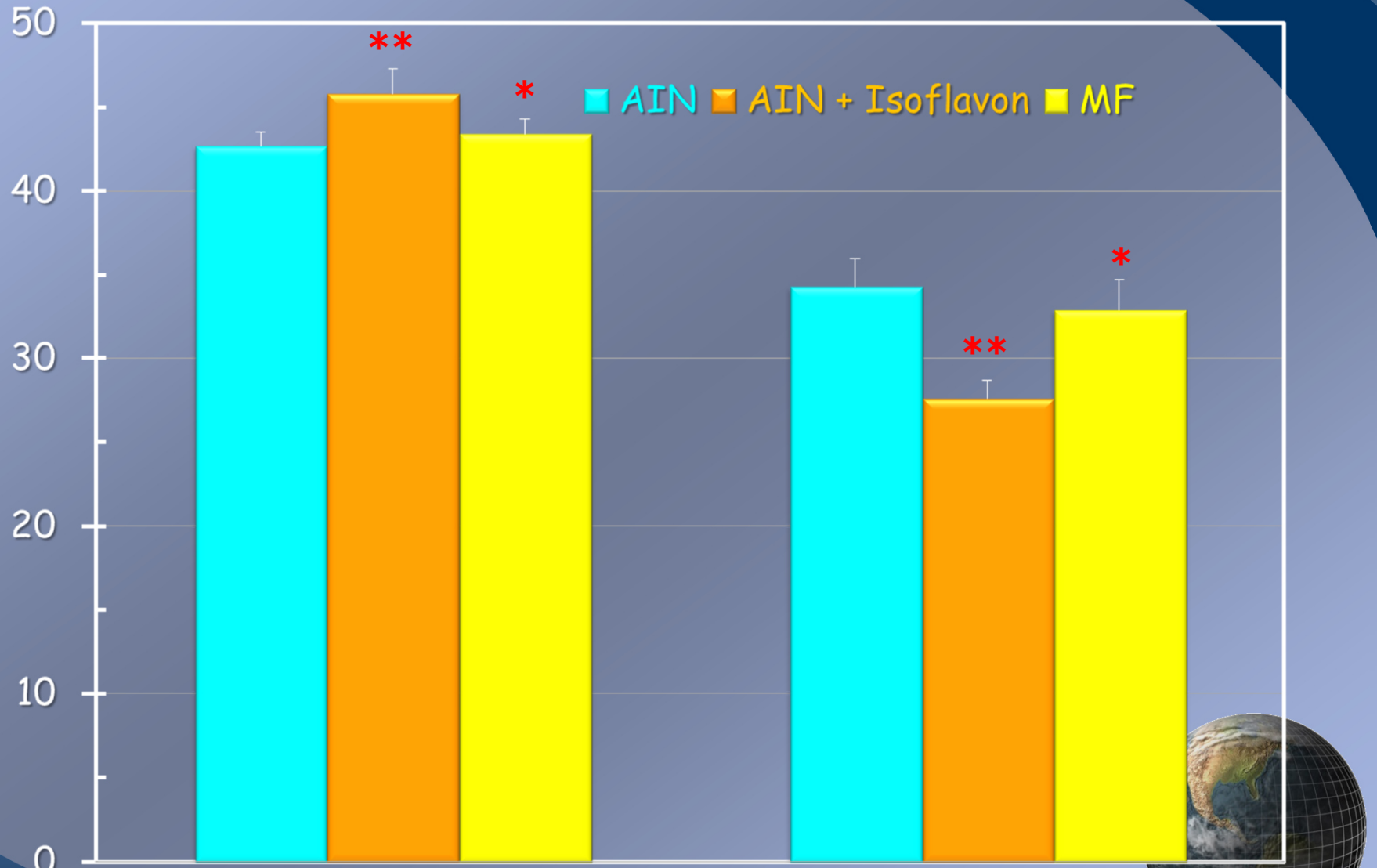
Concentrations of phytoestrogens vary widely among the lot of diet.

One-generation Reproduction Toxicity Study for Examining Effects of Phytoestrogens in The Diets (Unpublished Observations)

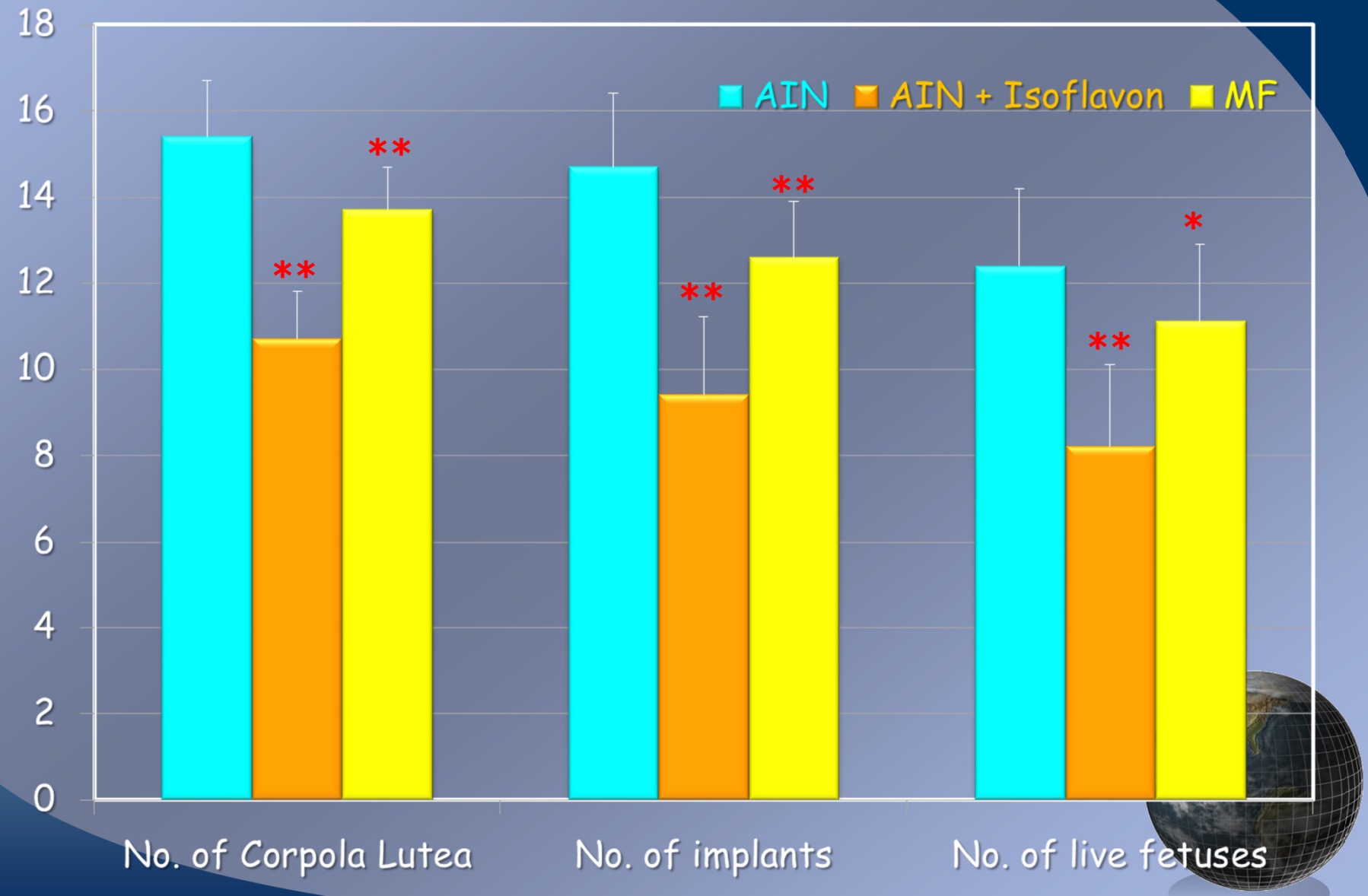
Concentrations of Isoflavones in Test Diets in One-gen. Repro-tox. Study

Isoflavones	Concentrations (ppm) in each Diet		
	AIN	AIN + IF	MF
Acetyl Glycitin	ND	90	40
Acetyl Genistin	ND	60	170
Acetyl Daidzin	ND	160	130
Malonyl Glycitin	ND	90	40
Malonyl Genistin	ND	60	180
Malonyl Daidzin	ND	180	140
Glycitin	ND	80	30
Glycitein	ND	ND	10
Genistin	ND	50	150
Genistein	ND	ND	20
Daidzin	ND	150	120
Daidzein	ND	90	20
Total	ND	1,010	1,050

Sexual Maturation (Days of Age) of F1 Weanlings



Observations at Cesarean Sectioning of F1 Females



Differences between AIN group and MF/AIN+Isoflavon groups

Type of parameters	Differences from the AIN group
Systemic:	Decreased reproductive organ weights in MF and AIN+Isoflavon males (prostates, seminal vesicles) and females (ovaries) Histopathology (prostates only in MF males)
Reproductive:	Decreased nos. of corpola lutea, implants and live fetuses in MF and AIN+Isoflavon females
Estrogenic Manifestations:	Increased juvenile uterine weights Accelerated sexual maturation in MF and AIN+Isoflavon females Delayed sexual maturation in MF and AIN+Isoflavon males

Our Proposal and Conclusions

- ✓ An endocrine disruption is one of the typical modes of action of toxicity, through which adverse health effects are induced.
- ✓ NMDR (or inverted U-shape dose response) should be carefully reconfirmed by using an inbred strain of rats or mice without any genetic variations and artificially synthesized phytoestrogen-free diet, if a common toxicology study revealed such phenomenon.
- ✓ We think we will be able to set ADI based on the results of a series of common toxicology studies and additional mechanistic studies for examining the mode of action.

Thank you for your attention