## *ELUCIDATION OF ENDOCRINE DISRUPTING MECHANISM OF DIOXIN AND RELATED COMPOUNDS FOR HEALTH RISK ASSESSMENT*

Core Research for Evolutionary Science and Technology Project

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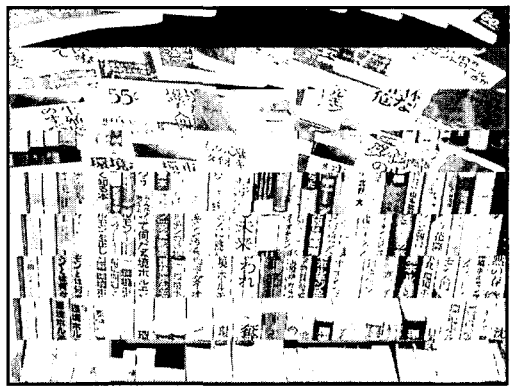
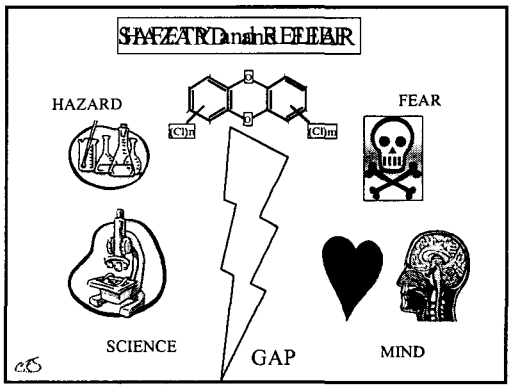
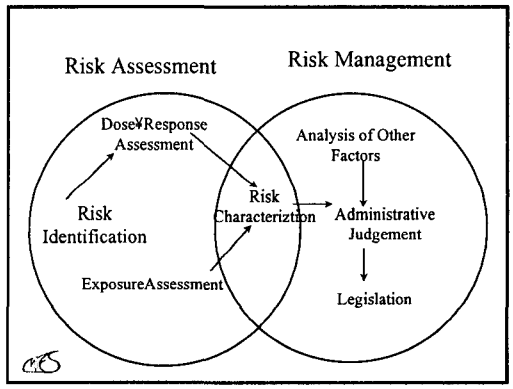
National Institute for Environmental Studies

and CREST, JST





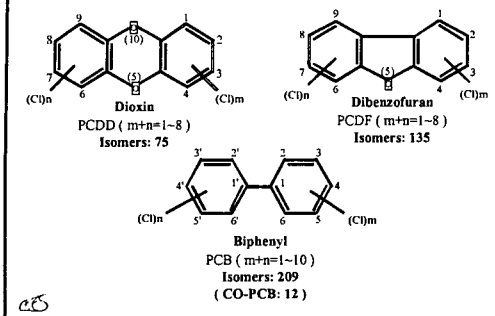

### Are Dioxins are the Strongest Toxicants on the Earth?

| Natural | LD 50 (μg / kg bw) | Man-made |
|---|---|---|
| Botulinus Toxin | $10^{-9}$ | |
| Tetanus Toxin | $10^{-8}$ | |
| | $10^{-7}$ | |
| | $10^{-6}$ | 2,3,7,8-TCDD (Guinea Pig) |
| Schigella Toxin | $10^{-5}$ | 2,3,7,8- TCDF |
| Tetradotoxin | $10^{-4}$ | Sarin |
| | $10^{-3}$ | 2,3,7,8-TCDD (Hamster) |
| | $10^{-2}$ | Mustard gas |
| | | Potassium Cyanide |
| Nicotine | $10^{-1}$ | |
| Caffeine | 1 | DDT |

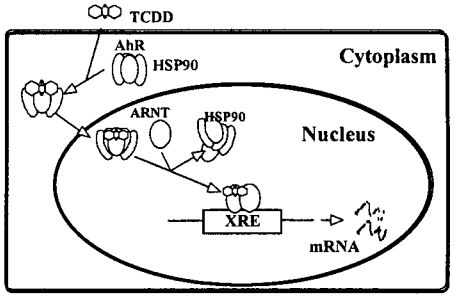
### Does Exposure to Dioxins in the Environment Cause Any Adverse Health Effects?

1. Cancer
2. Reproductive/Developmental Toxicity
   - Congenital Anormalies, Teratogenicity
   - Sex Ratio Deviated to Girls
   - Spermatogenesis, Endometriosis
3. Neurobehavioral Toxicity
   - A Decrease in Learning/Cognitive Ability
4. Immunotoxicity
   - Reduced Registance to Viral Infection, Atopy
   - Disruption in the Thyroid hormone homeostasis

## Dioxin and Related Compounds




## Mechanism of Transcription Activation Mediated by AhR



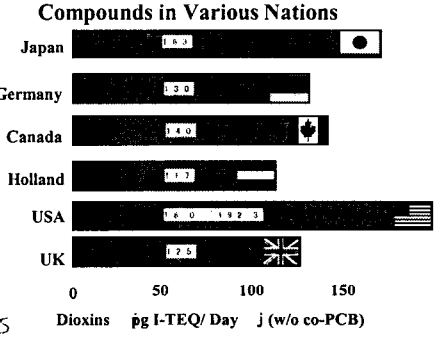

(Lees and Whitelaw. MCB,1999)

## *Project Background (TDI Values)*

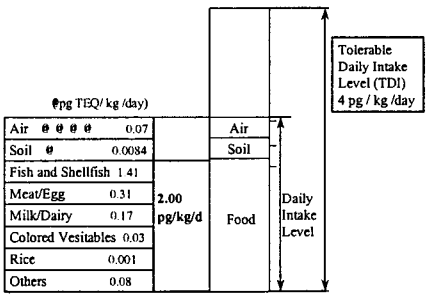
The Current TDI Values on Dioxins in Various Countries:
Based upon carcinogenicity and reproductive toxicity
(Endometriosis)
TDI values : 10 pg I-TEQ/kg/day (WHO; 1990)

The Re-evaluation of TDI Value of Dioxins by WHO (1998)
Based upon epidemiological and experimental findings
on endocrine disrupting mechanisms, such as reproductive,
neurobehavioral and immunological toxicity
TDI values 1 - 4 pg TEQ/kg/day

## Intake Levels of Dioxin and Related Compounds in Various Nations




## Daily Intake Level of Dioxins in Japan




Environment Agency, 1999

## *Message from Dioxin Issue*

The Current Exposure Level of
Dioxins in the Environment
May Cause Not Only
the Present Human Populations
but also the Future Generations Possibly
by Endocrine Disrupting Mechanisms

Dioxin---What is the Problem?

**Exposure Level in the General Environment**

Adults (2~6 pg TEQ/ kg bw/day)

Newborns Milk) ( 60 pg TEQ/ kg bw/day) >

TDI : 4 pg TEQ/ kg bw/ day

**Margin of Exposure**

$$\frac{\text{LOAEL}}{\text{Body Burden (~5 ng TEQ / kg Tissue)}} = <10$$

Uncertainty Factor

**Outline of CREST Project**

TEF
PCDD: 2,3,7,8-TCDD 1
1,2,3,7,8-PnCDD 1
PCDF: 2,3,4,7,8-PnCDF 0.5
co-PCBs: PCB126 0.1

Dioxin Congeners

Exposure Sensitive Stages

Sexual Differentiation

Fertilized egg

Birth

Adult

Rat / Mouse

AhR-KO
Arom-KO
TTR-KO
Ne/Nga (atopy)
Human AhR-KI

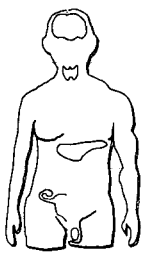
* P Reproduction
I. In Vitro Fertilization System: Sexual Differentiation; Sex Ratio
II. Organ Culture; Germ cell line
III. Embryo Placenta

2. Brain Functions & Behavior
I. Sexual differentiation; Sexual-behavior
II. Thyroid hormone system
III. Signal Transduction

3. Immune system
T-cell differentiation; Regulation of immunologic reactions
Atopy

4. Risk Assessment

Dose-Response Analysis

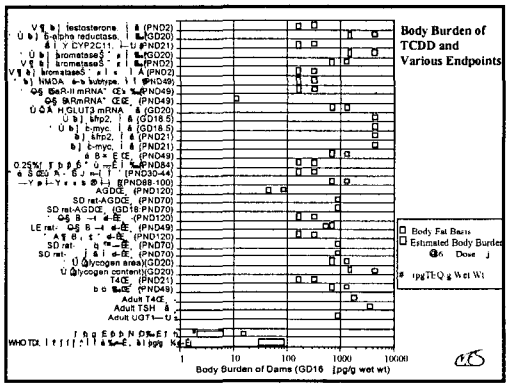


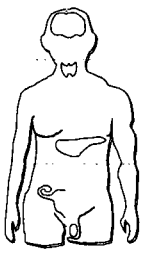
Neuron N

Immune I

Endocrine E

System S



Neuron N

Immune I

Endocrine E

System S

***Experimental protocol***

**TCDD** (0, 12.5, 50, 200, 800 ng/kg bw; oral)

**Holtzman rats**

Dam

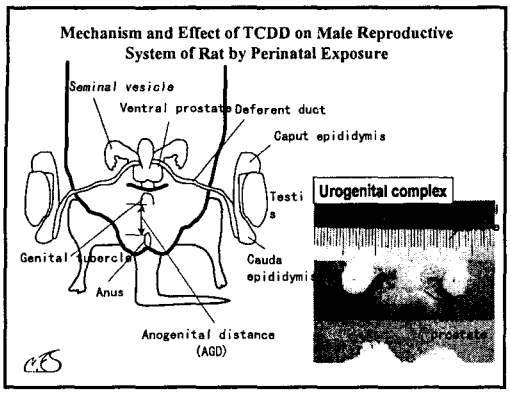
Birth

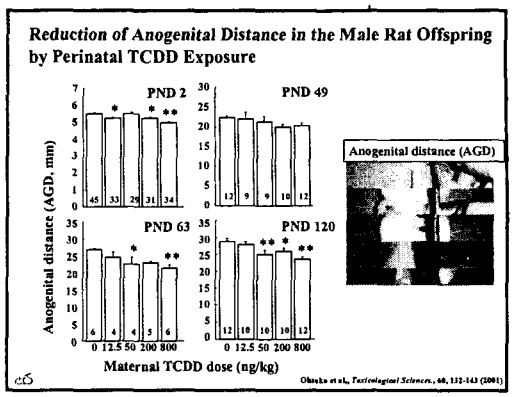
GD15

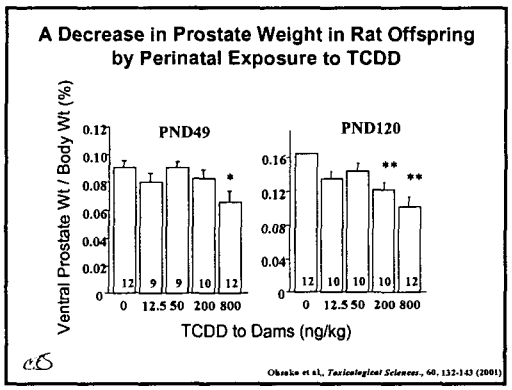
Sampling

PND2 PND49 PND63 PND120

Ohsako et al., *Toxicological Sciences*., 60, 132-143 (2001)



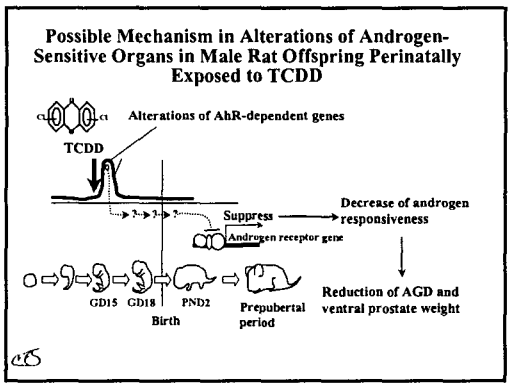



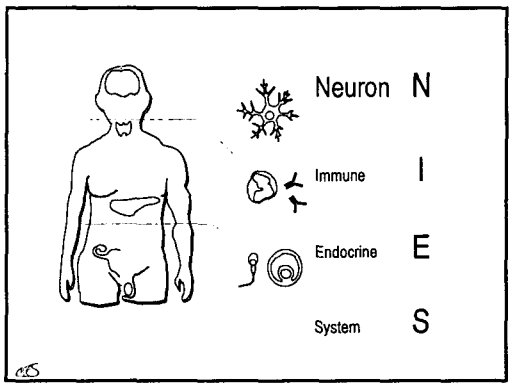

Joint-Expert Committee of Food Additives and Contaminants (JECFA)

▪ s▪ ▪ ▪ .▪.'▪ Tolerable Monthly Intake of Dioxin and Related Compounds @2001)

@ @ @0 pgTEQ/kg b.w. /day

- Adopted data of Ohsako @*et al.*, @oxicol. Sci. @001
  - @ Exposure of TCDD to Pregnant Holzman Rats on GD15 @
  - @ @ @ @ E Anogenital distance
  - @ @ E Gene Expression in the prostate F @
  - @ @ @ @ @ @ @ @ ▪ -Reductase and AR



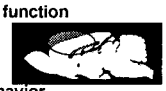
**Maternal Exposure to Dioxin Causes Permanent or Semi-permanent Dysfunction in the Frontal Cortex of Offspring at Behavioral and Molecular Levels**

Kakeyama M. et al., NeuroToxicology, 24: 207-217 (2003)

## Preceding Studies on Behavioral Effects of Dioxin

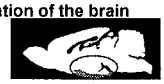
**Dioxins affect performance of learning behavior**
(Schantz et. al. 1996, Schantz & Bowman 1989, Seo et al. 2000)

**Effects on the advanced brain function (neocortical axis)**

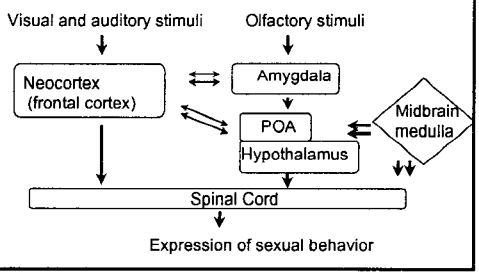


**Dioxins inhibit masculine sexual behavior**
(Mably et al. 1992; Bjerke et al. 1994, Gray et al. 1995)

**Effects of the sexual differentiation of the brain (POA-hypothalamic axis)**



## Brain Areas Controlling Masculine Sexual Behavior

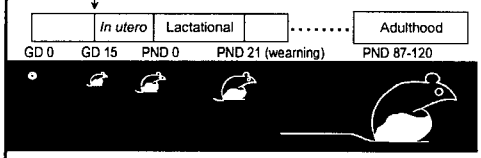




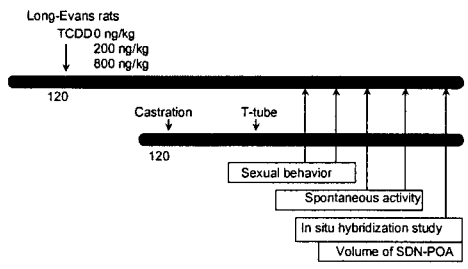
2,3,7,8-tetrachlodibenzo-p-dioxin (TCDD)

A single oral administration of TCDD to dams

Masculine sexual behavior
Activity-dependent mRNAs expression




## Experimental Protocol



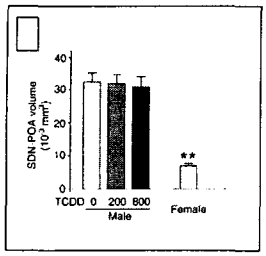

## Effects of Maternal Exposure to TCDD on Man ifestation of Masculine Sexual Behavior

| TCDD (ng/kg) | Non-Cast 0 | Non-Cast 200 | Non-Cast 800 | CAST+T 0 | CAST+T 200 | CAST+T 800 |
|---|---|---|---|---|---|---|
| Mount Latency (sec)[b] | 23 (11-26) | 21 (11-31) | 25 (5-28) | 63 (25-91) | 71 (45-100) | 61 (33-87) |
| Intromission Latency (sec)[b] | 30 (1[illegible]) | 27 (19-54) | 30 (6-60) | 82 (50-112) | 81 (51-215) | 88 (44-101) |
| Ejaculation Latency (sec)[b] | 450 (388-542) | 471 (365-540) | 441 (377 551) | 814 (745-822) | 915 (756-1524) | 822 (784-914) |
| Number of Mounts[c] | 16.6 ±1.5 | 14.0 ±2.1 | 8.4 ±1.0[d] | 18.2 ±1.4 | 17.5 ±1.4 | 10.2 ±1.0[d] |
| Number of Intromissions[c] | 12.1 ±1.8 | 11.0 ±1.5 | 8.2 ±1.0[d] | 14.1 ±1.9 | 14.0 ±1.8 | 7.2 ±0.9[d] |
| Post Ejaculatory Interval (sec)[b] | 295 (251-314) | 305 (286-322) | 301 (254-330) | 298 (286-314) | 301 (244-320) | 311 (277-330) |

a Sexual behavior was assessed on PNDs 87 (first test) and 97 (second test), and the data obtained on PND 97 are shown (n=21 in Non-CAST males and n=14 in CAST+T males).
b Mount Latency, Intromission Latency, Ejaculation Latency, Post Ejaculatory Interval are represented as median (min-max). Statistical difference among each-exposed group was not found by the Mann-Whitney U-test (P > .05).
c Number of Mounts, Number of Intromissions are represented as mean ±S.E
d Significant (p<0.01) difference between vehicle- and 800 ng TCDD/kg- exposed groups

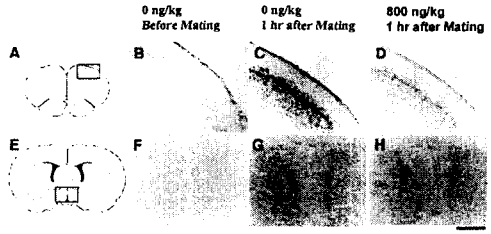
Kakeyama M. et al., NeuroToxicology, 24: 207-217 (2003)

## Perinatal Exposure to TCDD Did Not Affect the Volume of SDN-POA in the Male Offspring




Kakeyama M. et al., NeuroToxicology, 24: 207-217 (2003)

## Effect of TCDD on the activity-dependent mRNAs expressions induced by the mating stimulus




Kakeyama M. et al., NeuroToxicology, 24: 207-217 (2003)

## Conclusion

**The frontal cortex is sensitive to dioxin.**

## CONCLUSIONS

A perinatal exposure of pregnant rats to a low dose TCDD affected developing offspring with a wide variety of toxicities.

The effects of TCDD were found in development of reproductive organs, such as anogenital distance and prostate, male sexual behavior, brain development, immunologic functions and so on.

The body burden (internal dose) of TCDD that may cause these effects is consistent with the accumulated data used so far to derive the current Tolerable Intake levels.