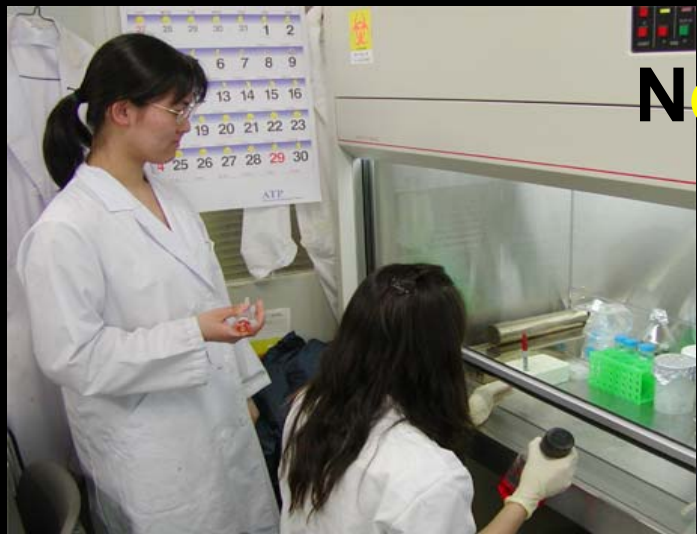


Current trends and perspectives for *in vivo/in vitro* researches of environmental chemicals.

*In vivo/in vitro*試験系における試験研究の現況と
将来への提言

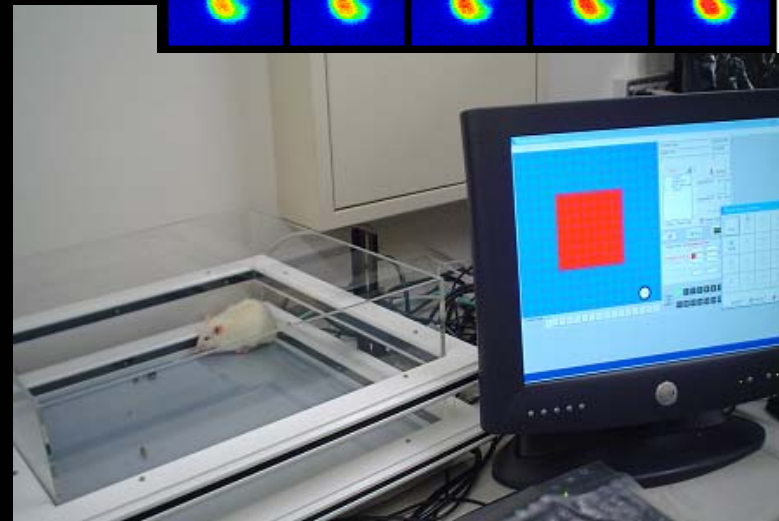
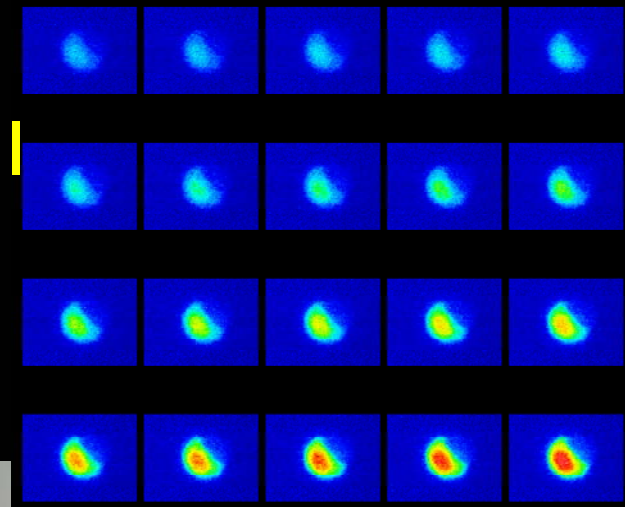


Noriyuki KOIBUCHI

鯉淵典之

Gunma University

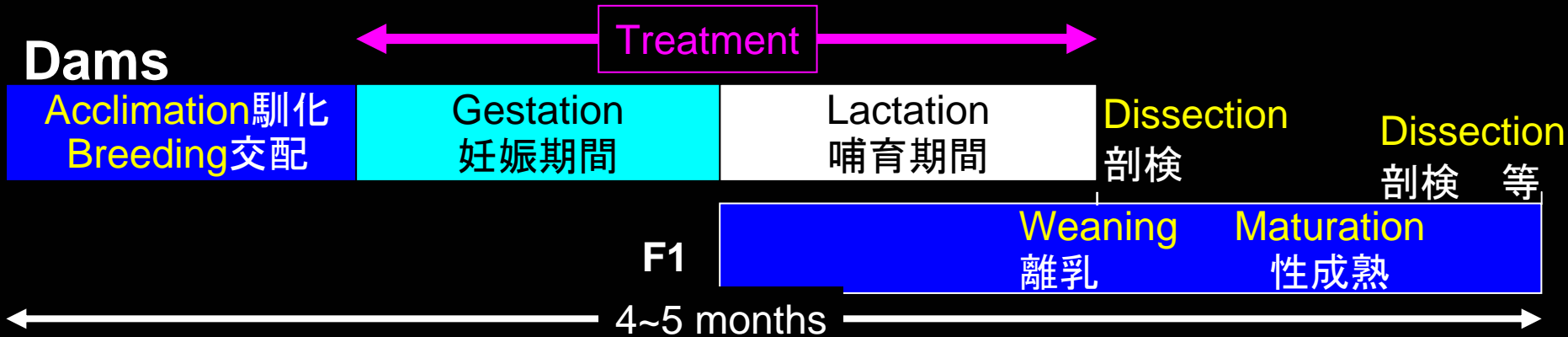
群馬大学



Research projects in SPEED'98

Enhanced 1-generation reproductive toxicity study

Speed'98におけるラット改良一世代試験



Studies were done at weaning (day 21) or after sexual maturation (10~12 weeks). 離乳期または性成熟後に試験実施。

Body & Organ weights & histology

Developmental parameters (sexual development)

Expression of mRNAs

Functional tests (from other projects)

Uterotrophic assay (estrogen or anti-estrogen)

Hershberger test (androgen or anti-androgen)

Research projects in SPEED'98

In vitro studies

1. Estrogen-like actions

- 1) ER α and β receptor binding assays (受容体への結合能)
- 2) Proliferation test (E-screen test) using breast cancer-derived clonal cell (MCF-7 cells). (乳がん細胞増殖能)
- 3) Yeast two-hybrid tests (酵母内でのER-共役因子結合)
(ER ligand binding domain-coactivator)

2. Androgen-like actions

- 1) AR receptor binding assay(受容体への結合能)
- 2) Reporter gene assay using breast cancer-derived clonal cell (MCF-7 cells) (レポーターアッセイ)

3. Thyroid hormone-like actions

- 1) Yeast two-hybrid tests (酵母内でのTR-共役因子結合)
(TR α or β ligand binding domain-coactivator)

Results of SPEED'98 rat studies

Of 22 environmental chemicals studied,

“No clear endocrine disrupting action was observed within an estimated exposure doses”

“ヒト推定暴露量を考慮した用量での明らかな内分泌かく乱作用は認められなかった”

Does this really mean that
all chemicals are safe?

Studies were mainly focused on reproductive functions.

生殖機能に対する作用の解析に重心

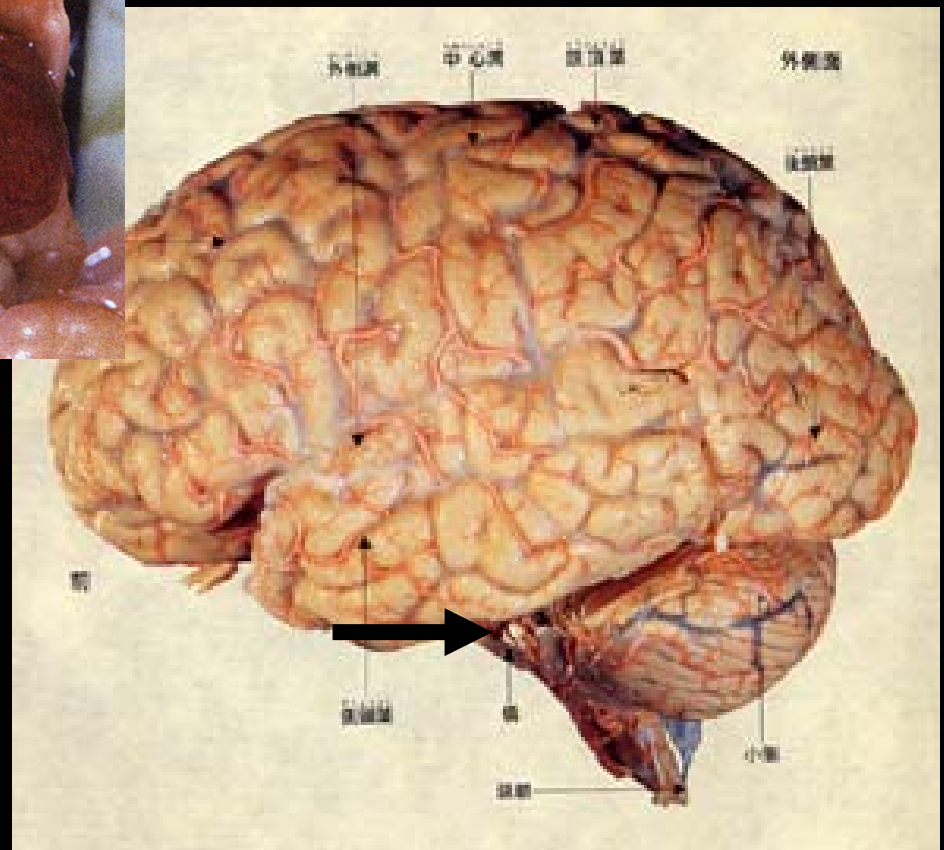
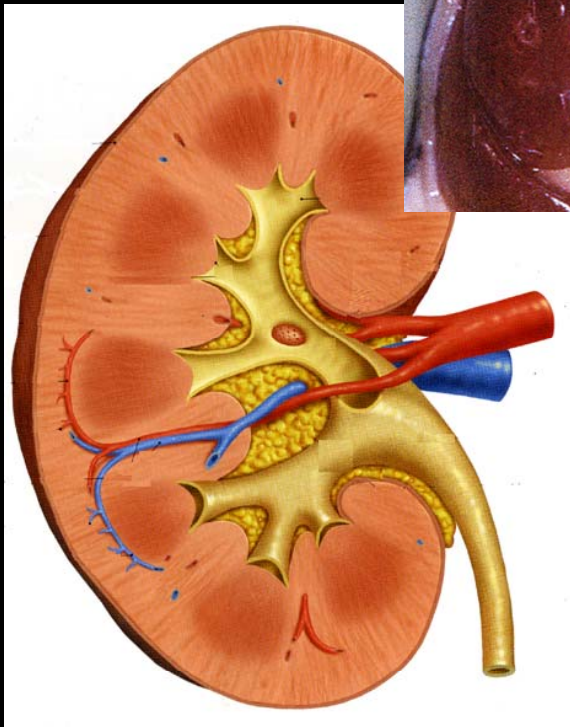
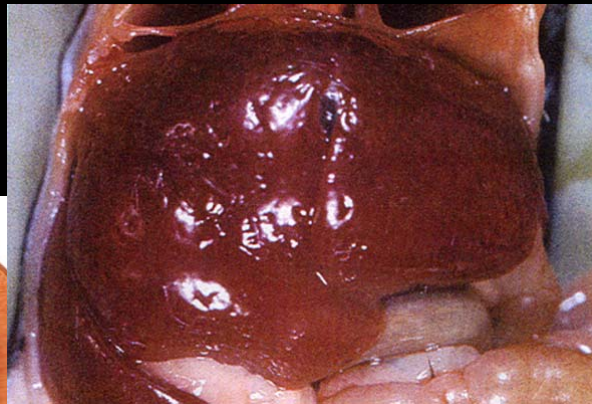
Since a great concern was initially expressed in reproductive organs, the testing strategy may be reasonable for the beginning.

当時、環境化学物質は生殖系への影響が最も懸念されており、試験開始当初の内容は妥当なものである。

Limitations of SPEED'98 rat study

Mass screening using the common method cannot fully cover the organ-specific (cell specific) actions.

画一的試験法で器官(細胞)特異的作用を網羅する事は不可能

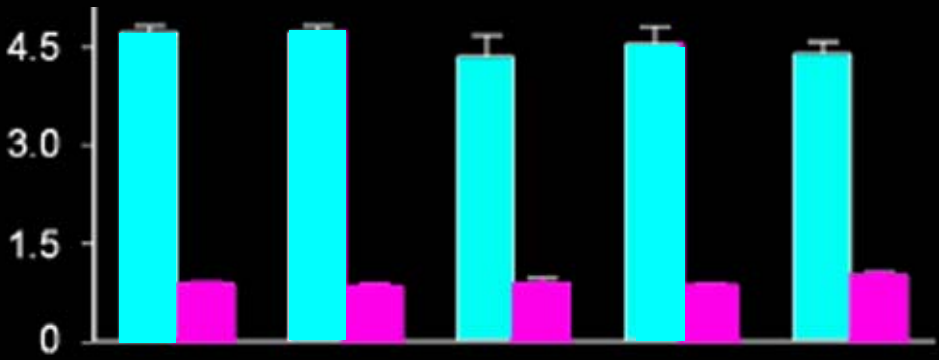


Brain area - dependent effects of environmental chemicals

脳領域による環境化学物質の作用の違い

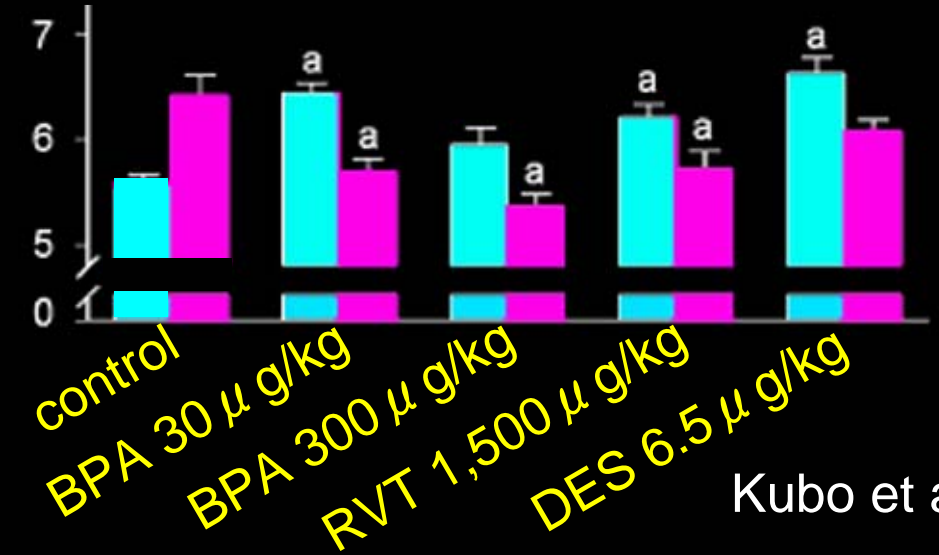
Size of sexual dimorphic nucleus

脳の性差に関与する神経核の大きさ

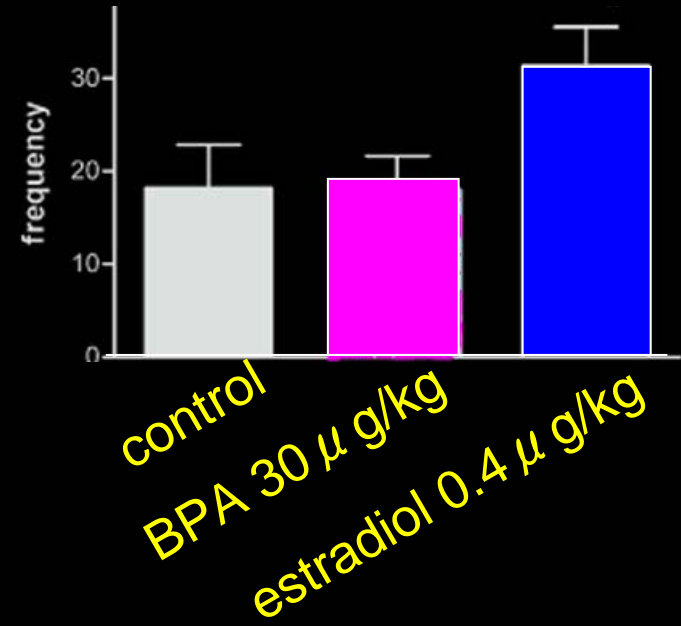


Size of the locus ceruleus

青斑核(Noradrenergic neurons)の大きさ



40 μ g/kg BPA does not alter male sexual behavior (intromission)

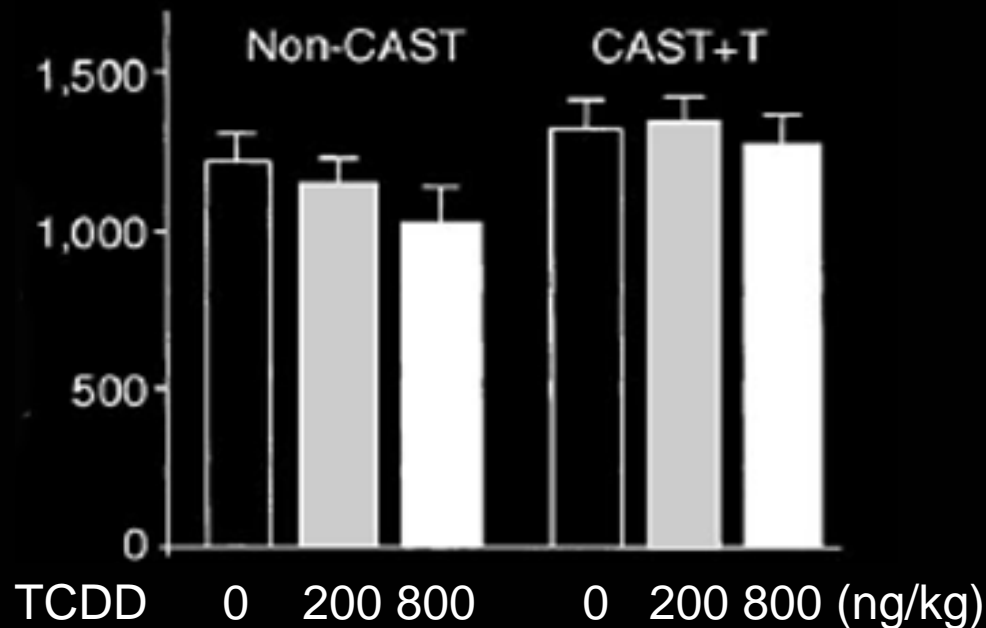


Seta et al. Horm Behav 50: 301, 2006

Kubo et al. Neurosci Res 45: 345, 2003

Perinatal dioxin treatment does not alter the locomotion

周産期のダイオキシン投与はラット行動量には影響しない

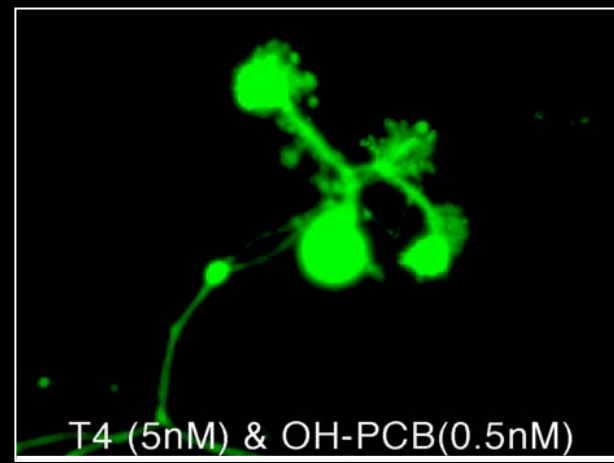
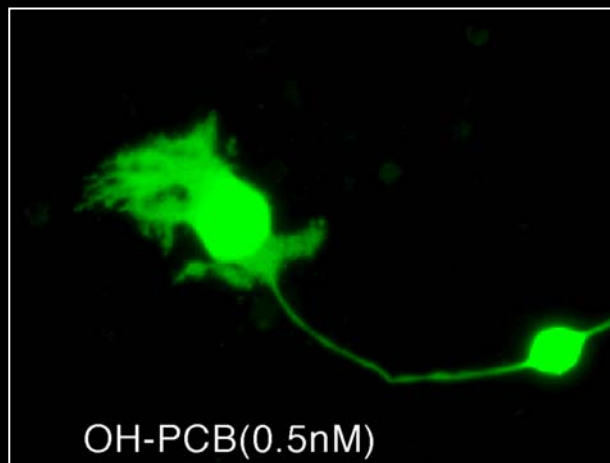
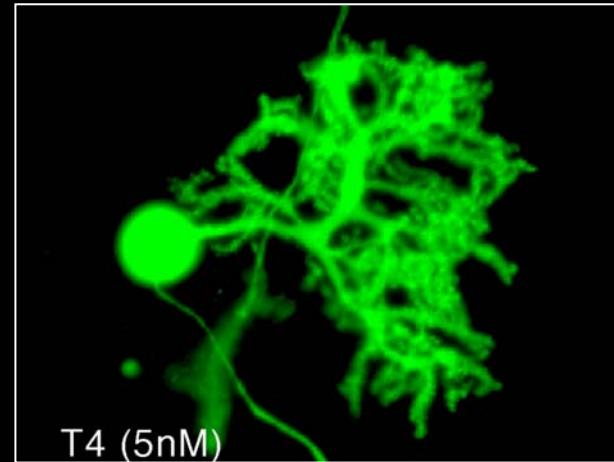
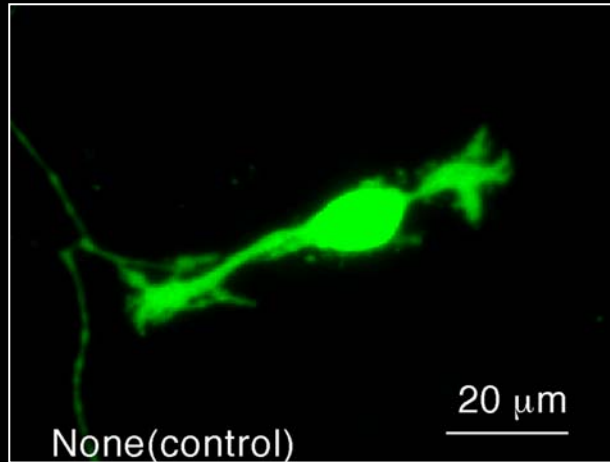


Many data have shown that Dioxin does not alter the locomotion both in open field and in home cage.

低用量ダイオキシンはラット行動量（自発およびopen field）には影響しないという報告がほとんど。

In vitro studies: Inhibition of neuronal development by PCBs (PCBによる神経発達抑制作用)

OH-PCB:4-OH-2',3,3',4',5'-pentachlorobiphenyl

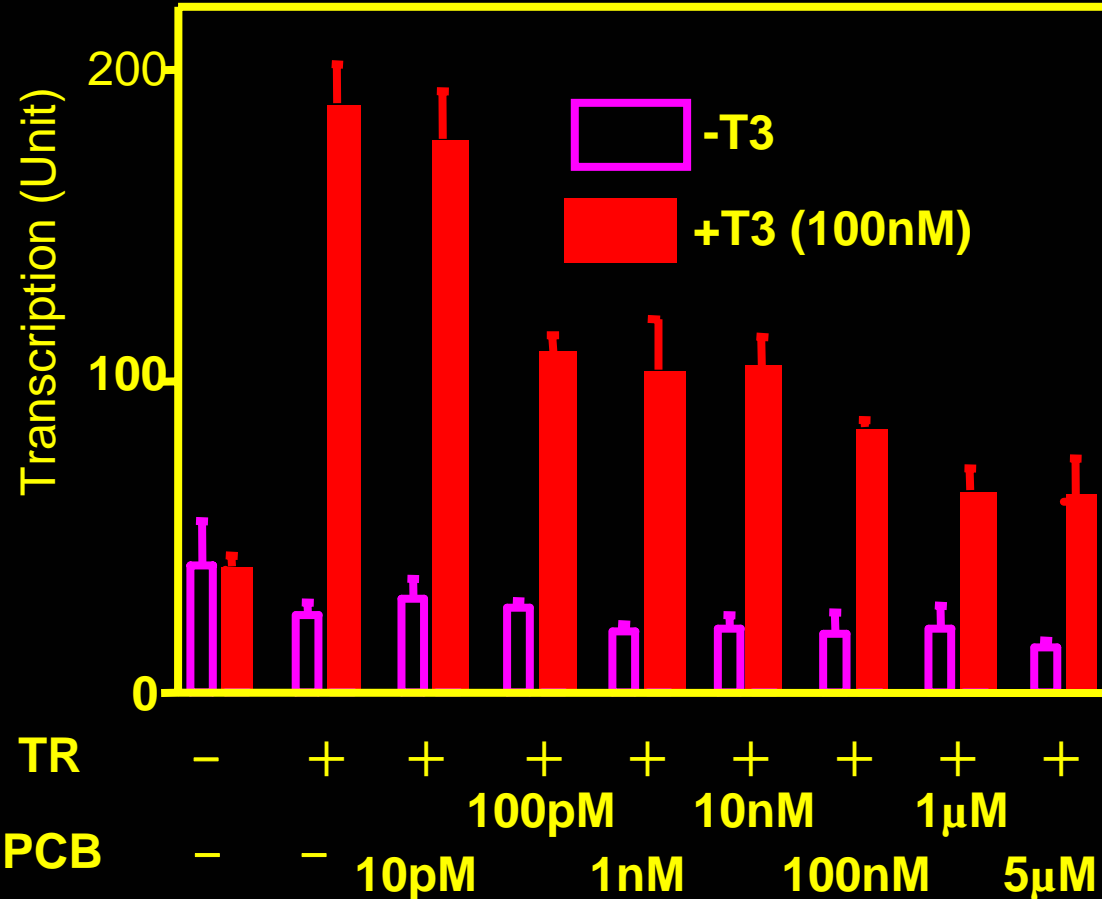


Kimura-Kuroda J, et al. Dev Brain Res. 154: 259, 2005

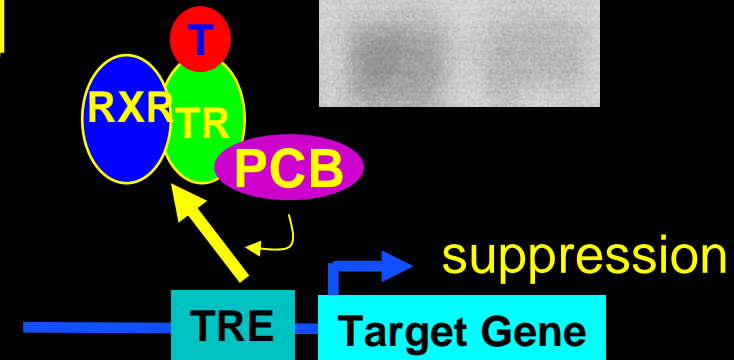
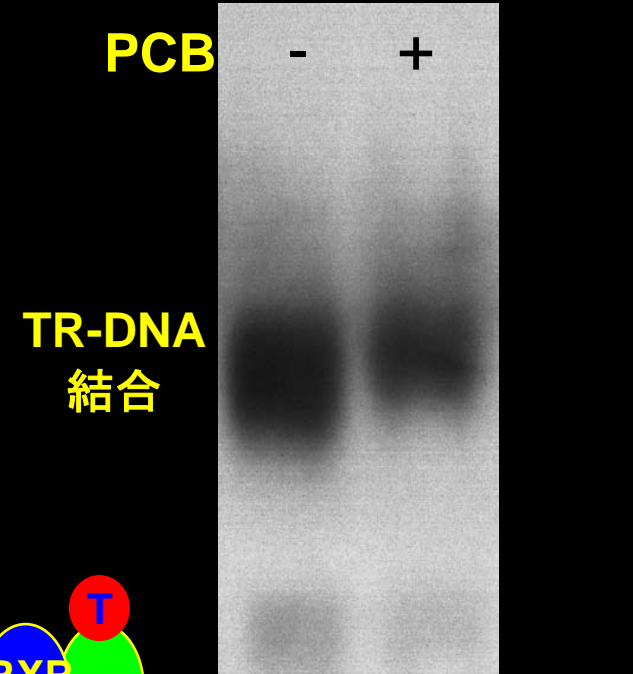
Suppression of TR action by low dose OH-PCB

低用量水酸化PCBによるTR作用の抑制

Suppression of TR-mediated transcription by hydroxylated PCB (水酸化PCBによる転写抑制)



Dissociation of TR-DNA by hydroxylated PCB (TRのDNAからの解離)



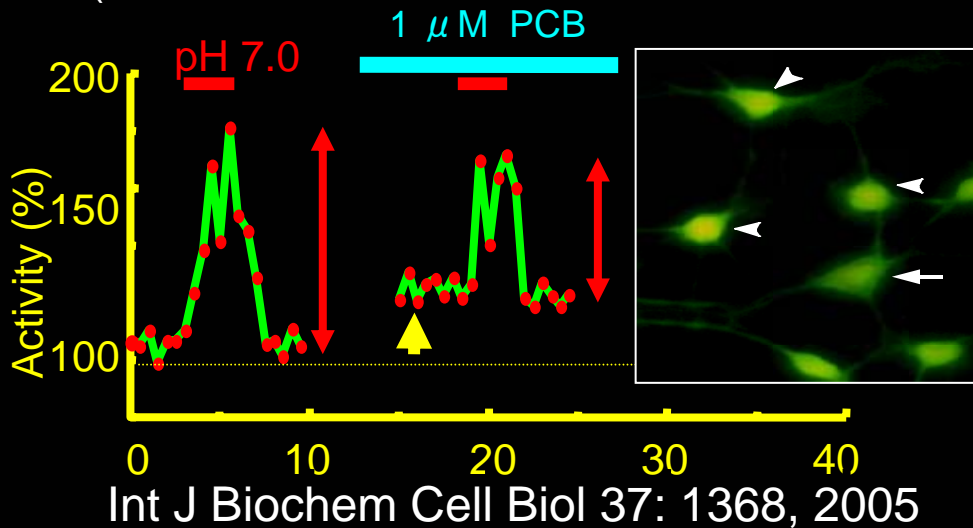
BBRC 299: 384, 2002

J Biol Chem 279: 18195, 2004

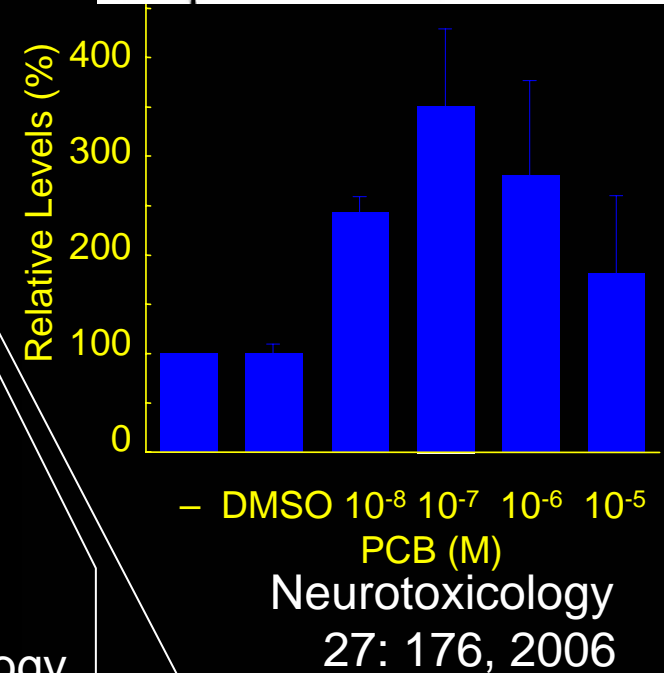
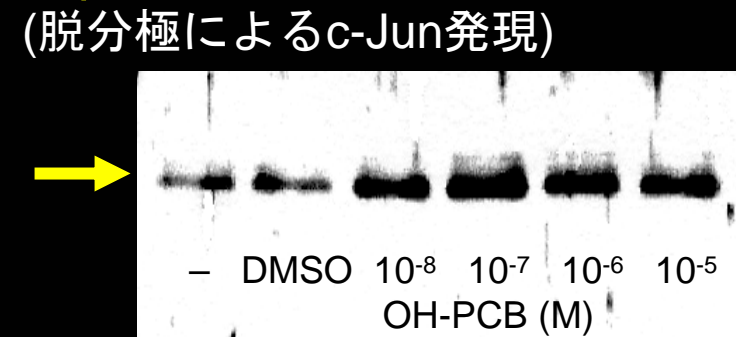
Acute effects of PCB on neurons

PCBによる神経細胞への急性作用

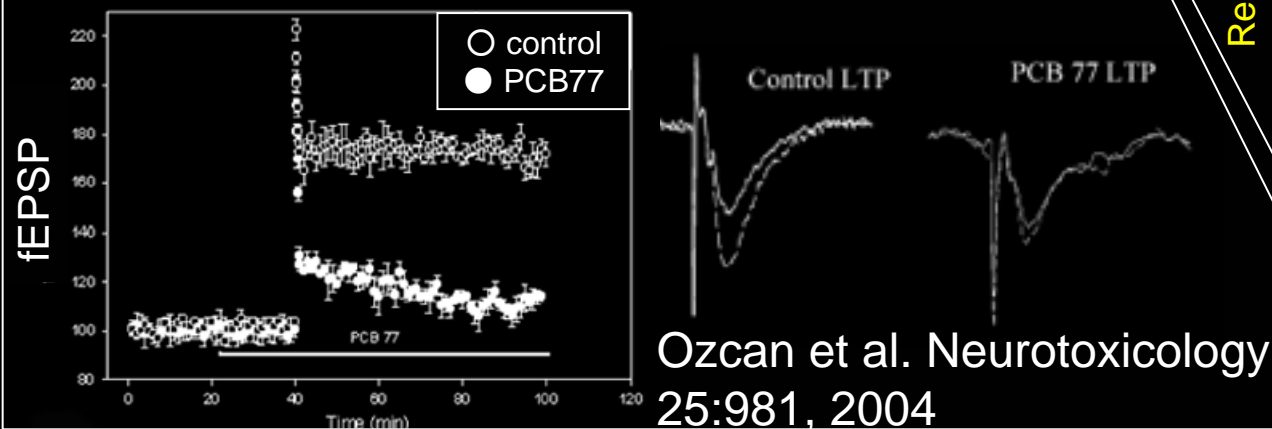
Aroclor 1254-induced depolarization (A1254による脱分極)



Depolarization-induced c-Jun expression (脱分極によるc-Jun発現)



Reduction of long-term potentiation by PCB 77 (ニューロン活動長期増強作用の抑制)



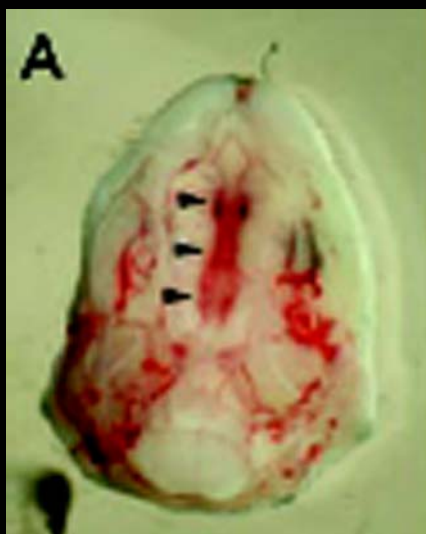
Difficulty in implementation:

Species-difference in dioxin action

データ外挿が時に困難：ダイオキシン作用の種差

Studies using AhR humanized mouse

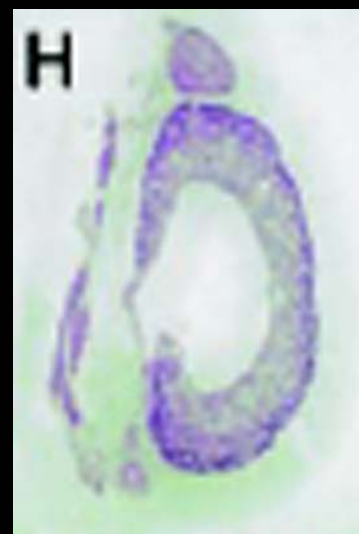
Genotype	TCDD dose	Fetuses(n)	Fetuses with	
			Cleft palate (口蓋裂) %	Hydronephrosis (水腎症) %
Mouse AhR	40 μ g/kg	9	100	100.0
Hetero hAhR	40 μ g/kg	25	48	88.0
Homo hAhR	40 μ g/kg	12	0	66.6



Mouse AhR



Homo hAhR



Mouse AhR



Homo hAhR

Problems on environmental chemical research

1. Receptor-Ligand concepts cannot simply apply for environmental chemicals.

受容体-リガンドの一般的概念は必ずしも当てはまらない

High affinity	→	Varied affinity
High specificity		Low specificity

Multiple site of action 複数の作用部位

(Binding site may not be the same as natural ligand)

(内因性リガンドと結合部位が同一である必然性なし)

Great species difference 大きな種差

2. Mechanisms of endogenous hormone action are still not clear. 内因性ホルモン作用機構自体に不明点がまだ多い

Generation of critical period 臨界期の形成機構

Organ difference of action 作用の器官特異性

etc.

How can we establish effective and innovative screening system?

効果的で革新的な試験系を確立するには？

1. Definition of specific “end point” 特異的エンドポイントの設定
Which function, which organ, and which cell are to be studied? 対象となる機能, 器官, 細胞の的確な選択
2. Identification of the target molecule. 標的分子の同定
Multiple targets need to be considered.
複数の標的にわたる可能性を考慮
3. Collaboration with other researchers.
他分野の研究者との共同研究
Endocrinologists, Neuroscientists, Molecular biologists, etc.

Researchers' interests are not always consistent with administrative needs.

研究者の興味と行政の要求は必ずしも一致しない

Science and Policy Conundrum:

Regulatory vs Academic Science, different constraints

科学と政策の難問:

“規制のための科学”と“学究のための科学”の異なる制約

http://sciencepolicy.colorado.edu/klamathbasin/science_policy/regulatory_vs_academic.htm

	Regulatory Science	Academic Science
Institution	Government/ Industry	Universities (Academia)
Goals	Provide reliable information for regulation. 規制のための信頼性の高いデータの供給	Expand understanding of the natural world. 自然科学の知見を広める独創的研究
Role of Uncertainty 不確実性の取り扱い	Predictive certainty is required for political process. 政治的措置のため不確実性を予測で補う必要あり	Uncertainty is expected. 不確実性を許容 (次の実験へ)
Products	Monitoring data & regulatory documents. 監視データや規制文書	Published papers and presentations. 論文や学会発表

Science and Policy Conundrum:

Regulatory vs Academic Science, different constraints

科学と政策の難問:

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http://sciencepolicy.colorado.edu/klamathbasin/science_policy/regulatory_vs_academic.htm

	Regulatory Science	Academic Science
Time-frame	Determined by the political process. 政治的理由で設定	Open-ended. 無期限
Political Influence	Directly influenced by politics. 政策が直接影響	Indirectly influenced by the researcher's political philosophy. 研究者の研究(政治)哲学が間接的に影響
Accountability 説明責任	Legislatures, courts and public. 議会, 法廷, 市民	Professional peers. 専門家による検証
Incentives	Compliance with legal requirements. 法の準拠	Professional recognition. 研究者としての地位向上

Conclusion

For research on environmental chemicals, all who are involved in this process should be aware that two completely different scientific philosophies have been applied to solve this issue.

Collaboration of Academia, Government and Company is absolutely required. One cannot force to follow his/her own philosophy. Mutual understanding of each standpoint is important.