Current status and recent research for the official approval and use of the dioxin bioassays in Japan

Go Suzuki

National Institute for Environmental Studies
Center for Material Cycles and Waste Management Research &
Japan Environment and Children's Study (Jecs) Programme Office

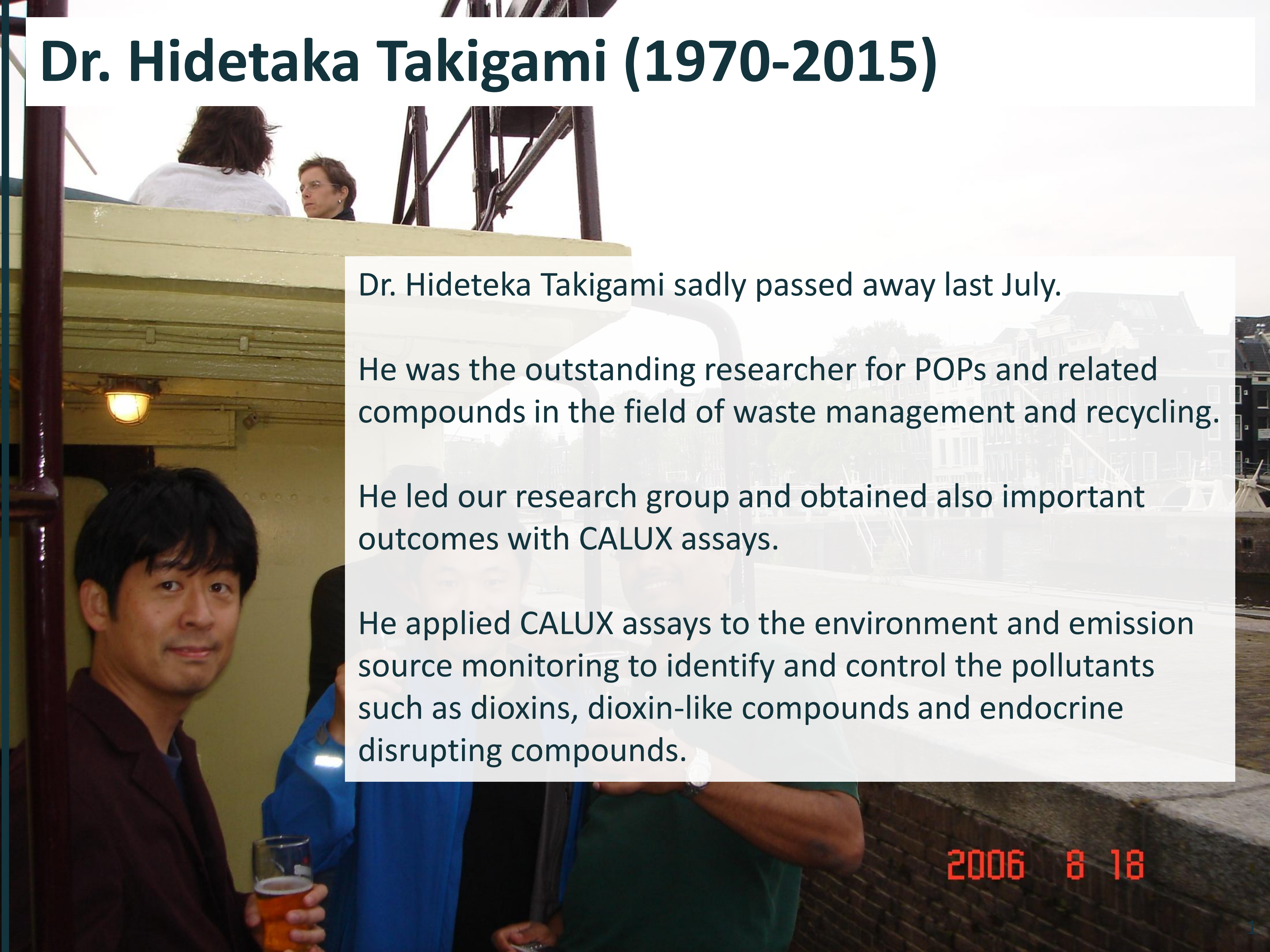
Dr. Hidetaka Takigami (1970-2015)

Dr. Hideteka Takigami sadly passed away last July.

He was the outstanding researcher for POPs and related compounds in the field of waste management and recycling.

He led our research group and obtained also important outcomes with CALUX assays.

He applied CALUX assays to the environment and emission source monitoring to identify and control the pollutants such as dioxins, dioxin-like compounds and endocrine disrupting compounds.



The official approval and use of the dioxin bioassay in Japan

Ministry of the Environment, Japan

In 2005 in Japan, the Government allowed the use of bioassay methods including DR-CALUX assay, which gave acceptable results through official evaluation, for measuring dioxins in emission gas from small scale waste incinerators and incineration ash from all the waste incinerators.

Nakano, T., Muroyoshi, H., **Takigami, H.,** Sakai, S., Morita, M. et al. *Organohalogen Compd* (2006)

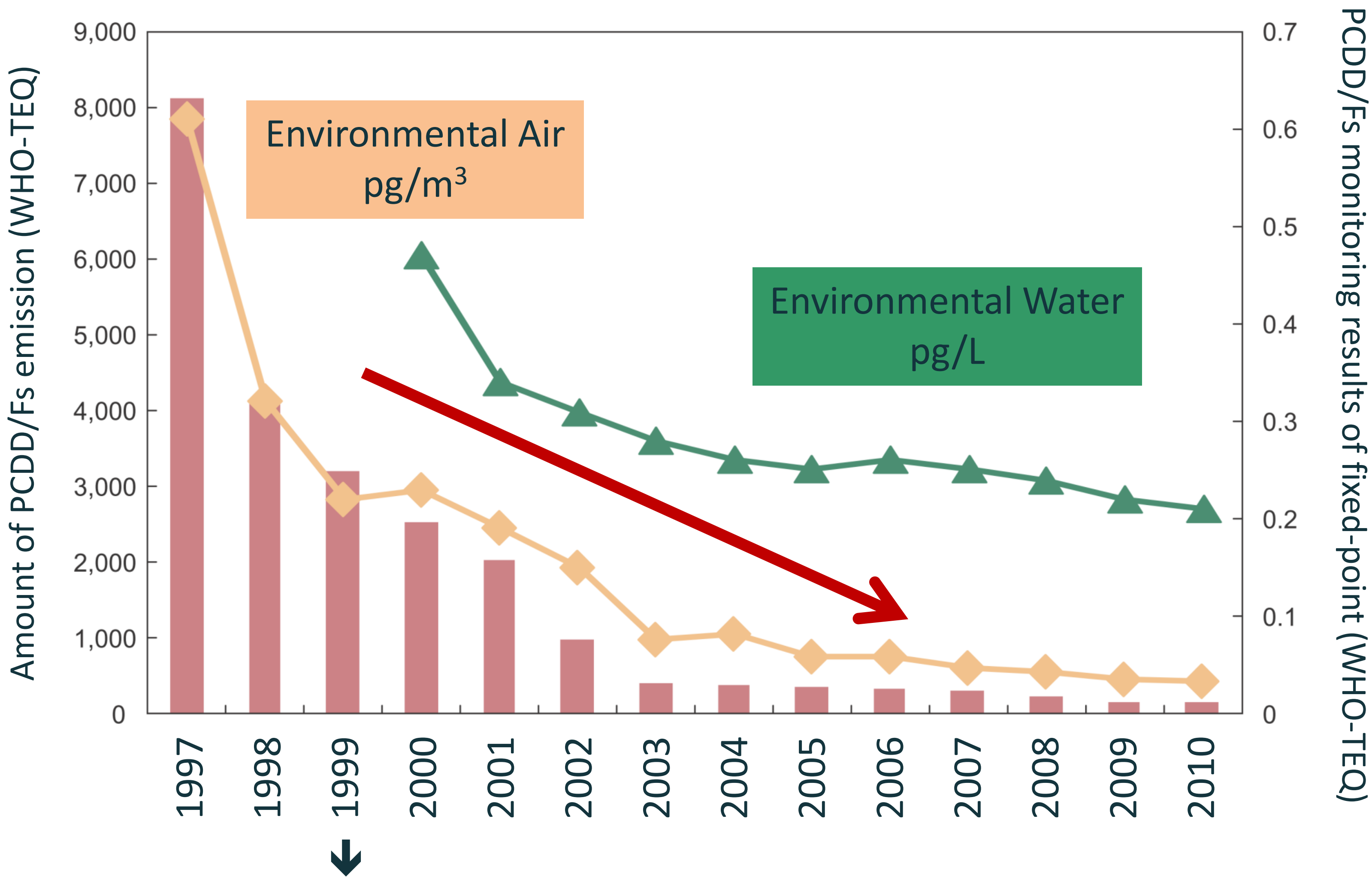
Topics for presentation

Current status for the official approval and use of the dioxin bioassays in Japan

Recent research with the dioxin bioassay

Current status for the official approval and use of the dioxin bioassays in Japan

Dioxin emissions and environmental levels in Japan



Enforcement of the Act on Special Measures concerning Countermeasures against Dioxins

Japanese Ministry of Environment (2012)

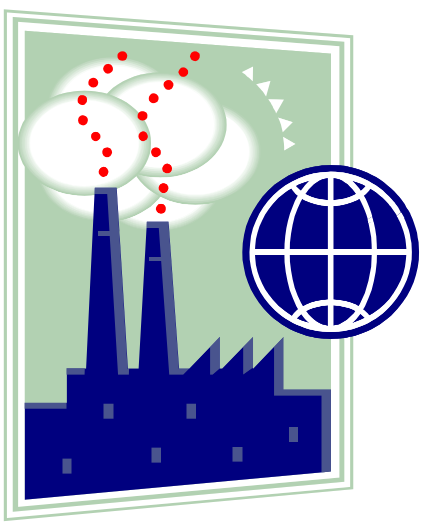
Regulations for Dioxins in emission gas

Type of Facilities		Flue Gas Emission limits (ng WHO-TEQ/m^3N)		Ash Treatment Standards (ng WHO-TEQ/g)
		New Facility	Existing Facility	
Waste incinerators	4 ton/h <	0.1	1	3
	2 – 4 ton/h	1	5	3
	< 2 ton/h	5	10	3
Sintering facilities for steel industry		0.1	1	-
Electric steel-making furnaces		0.5	5	-
Facilities for collecting zinc		1	10	-
Facilities for manufacturing aluminum base alloy		1	5	-

The Act on Special Measures concerning Countermeasures against Dioxins in Japan (1999)

Obligation to monitor dioxins

Business entities which own and manage specified emission sources such as an incinerator are also **obligated** under the law **to measure dioxins emission** at least once a year.



The Act on Special Measures concerning Countermeasures against Dioxins in Japan (1999)

Measurement of dioxins

GC-HRMS

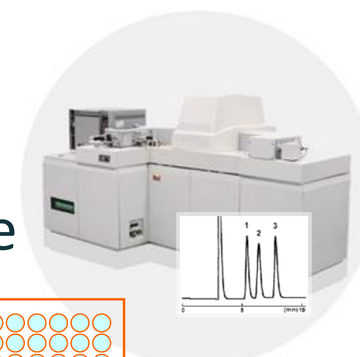
Selective, accurate and precise method
but a high cost of the instrument and maintenance

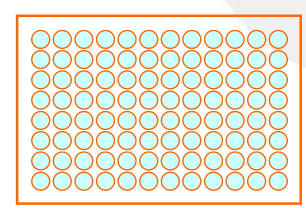
➔ ¥150,000 (€1,200) / 1 sample

In vitro bioassay detecting dioxins

High-throughput and cost-effective method
but no information about isomers

➔ ¥30,000 (€240) / 1 sample





Procedure for measurement of dioxins established in 2004

排出ガス、ばいじん及び燃え殻の
ダイオキシン類に係る簡易測定法マニュアル
（生物検定法）

平成２２年３月

環境省水・大気環境局総務課ダイオキシン対策室

Procedure for measurement of dioxins in incinerator flue gas, fly ash and bottom ash
(In vitro bioassays)

Revised in March 2009
(Established in September 2004)

Approved by Japanese Ministry of Environment

Measurement of dioxins by *in vitro* bioassays for incinerator flue gas, fly ash and bottom ash

Flue gas

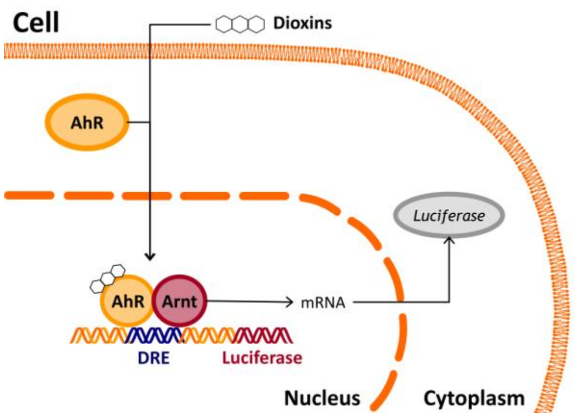
Fly ash

Bottom ash

➔ More than 4,000 samples per year for *in vitro* bioassay

In vitro bioassays selected by Japanese MOE

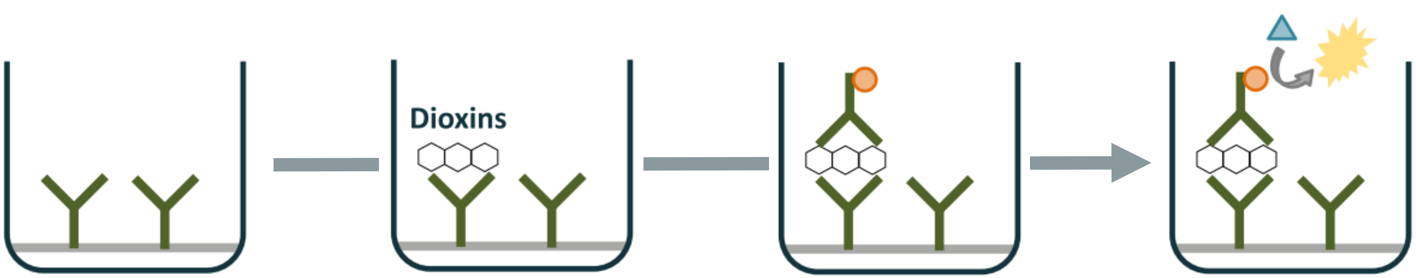
1. AhR-binding assay



→ AhR reporter gene assay

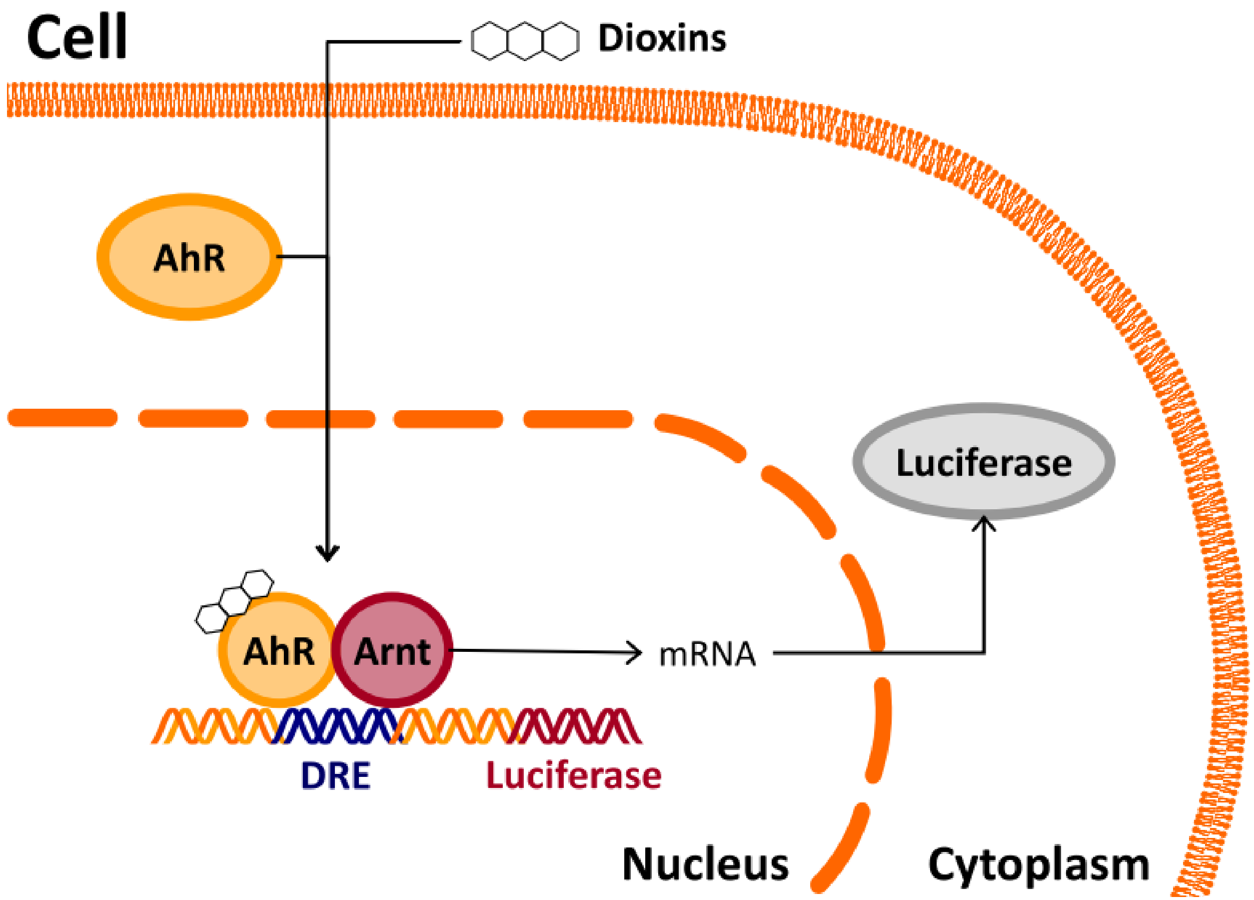
→ Polyclonal anti-body based immunoassay

2. Monoclonal anti-body based immunoassay



In vitro bioassays selected by Japanese MOE

1. AhR-binding assay

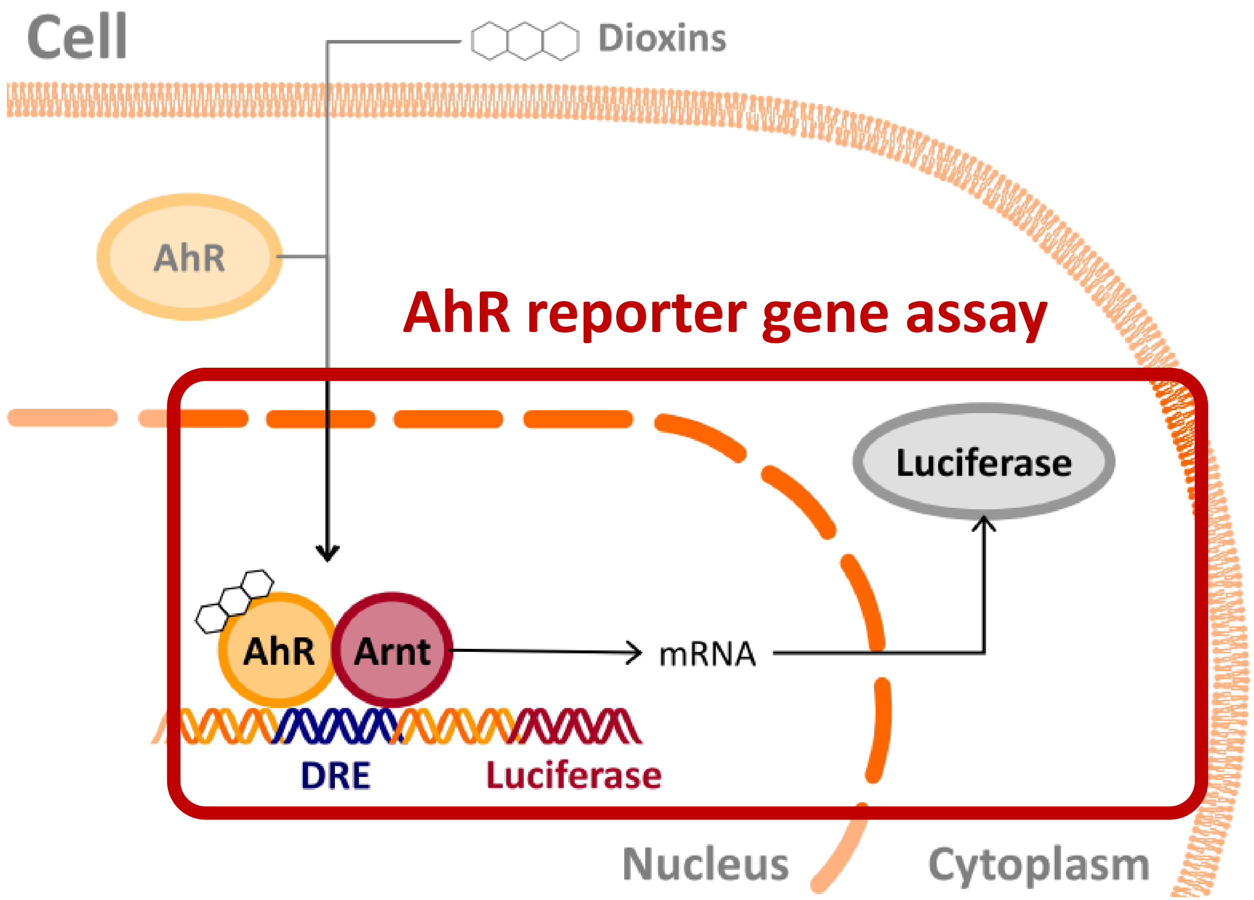


AhR activation leads to toxic effects of dioxins

van den Berg et al. (1998)

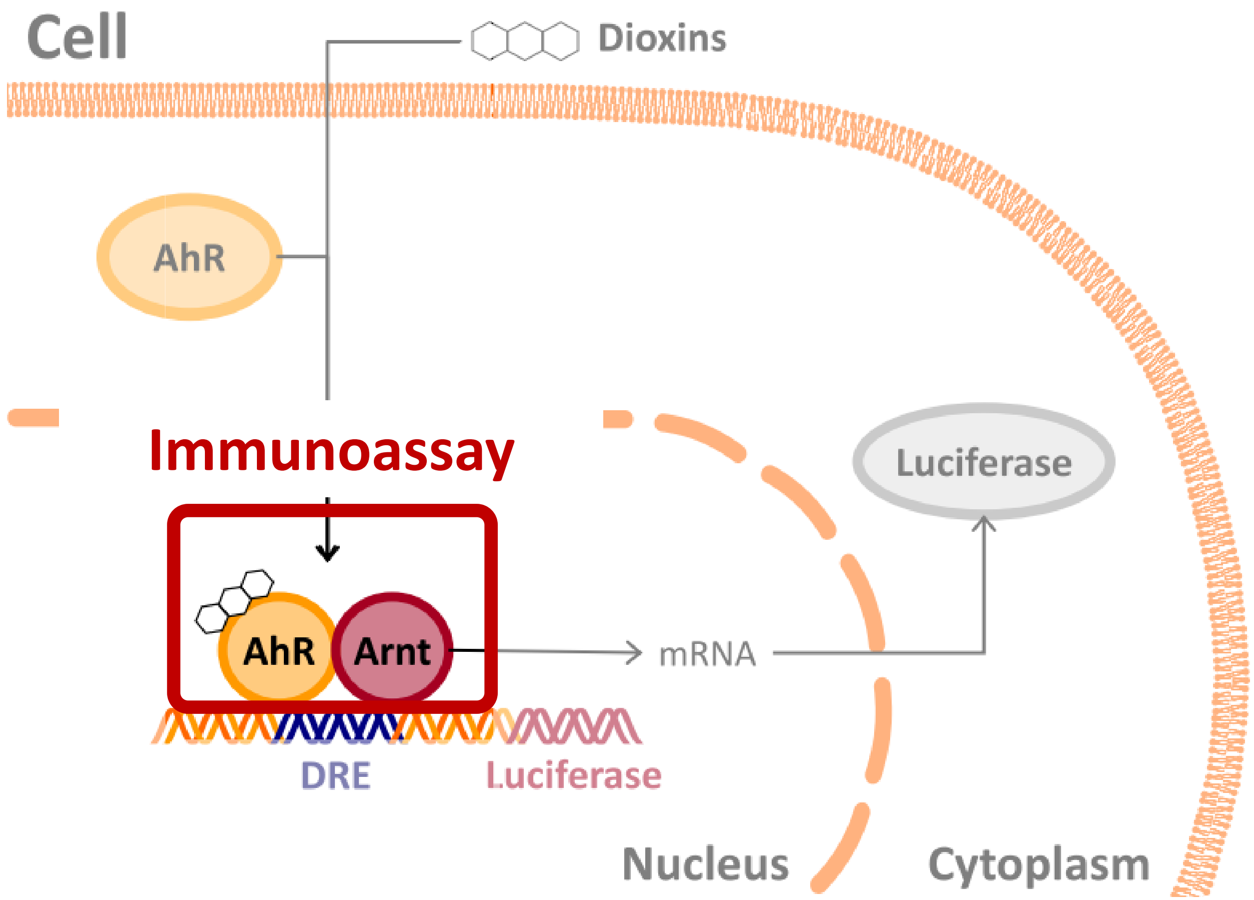
In vitro bioassays selected by Japanese MOE

1. AhR-binding assay



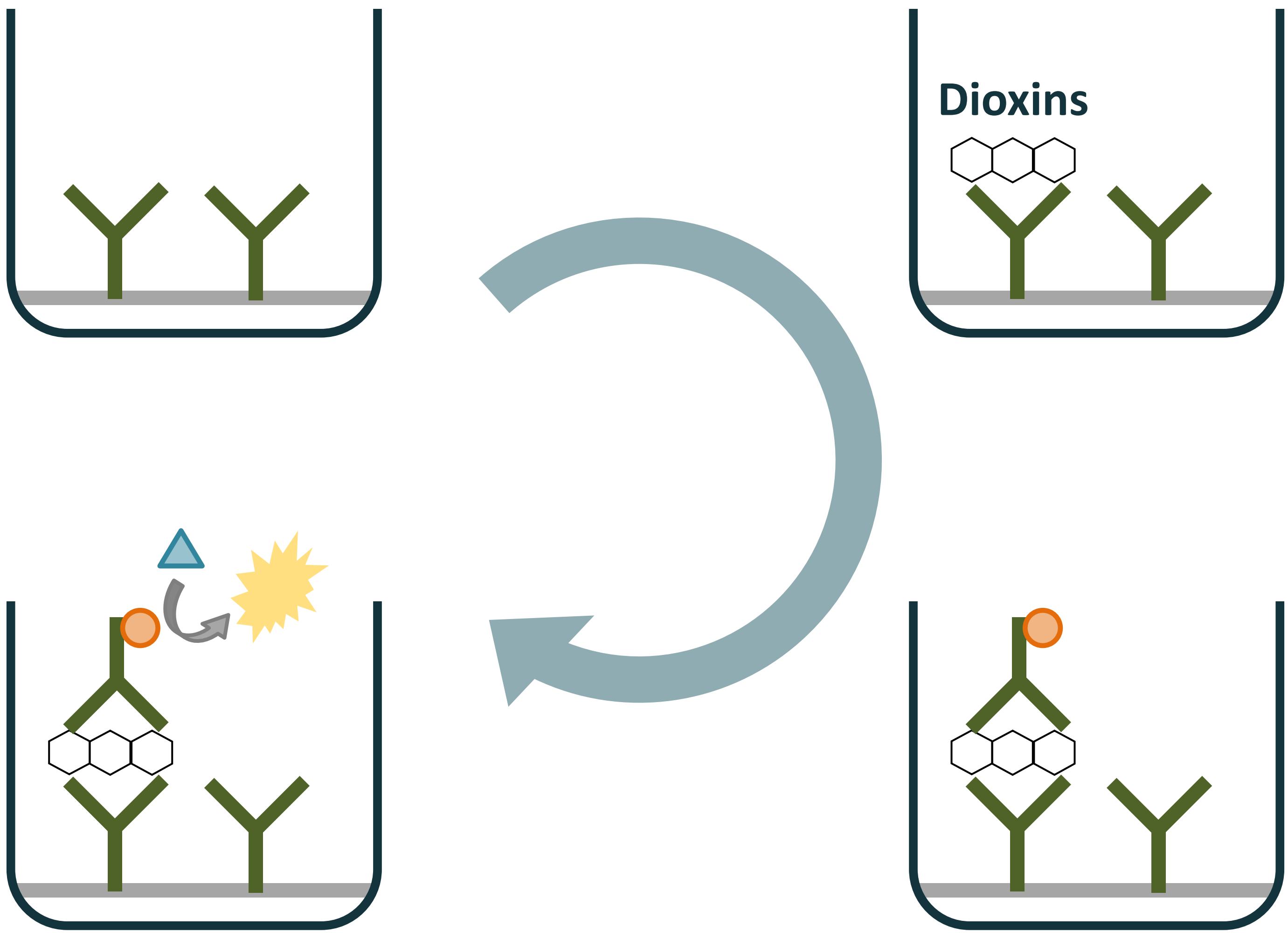
In vitro bioassays selected by Japanese MOE

1. AhR-binding assay

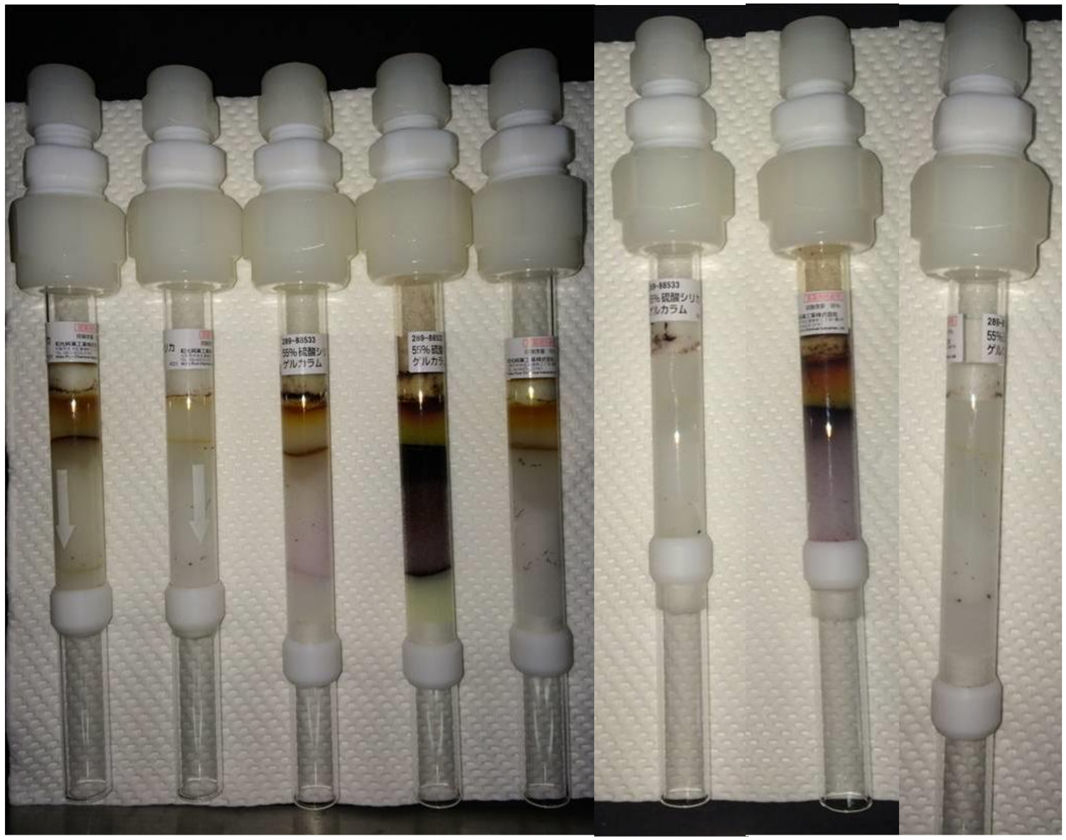


In vitro bioassays selected by Japanese MOE

2. Monoclonal antibody based immunoassay



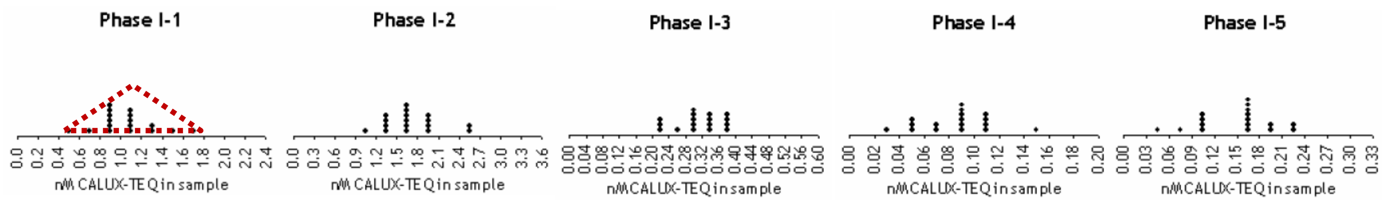
Importance of clean-up process for the dioxin bioassay



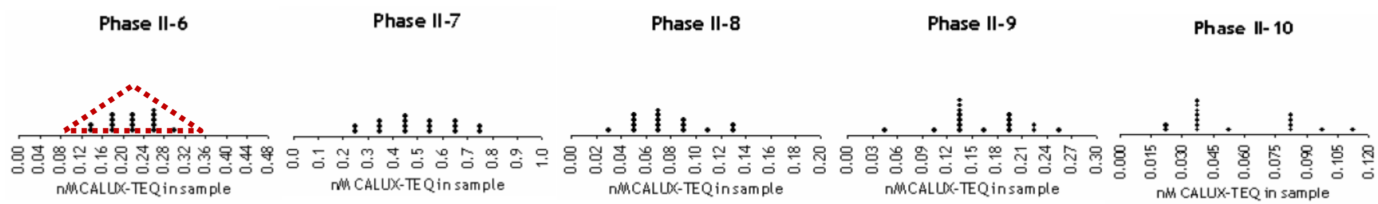
- ➔ To remove labile AhR ligands such as PAHs
- ➔ To extract dioxins from sample matrix without internal standards

Frequency distribution of *in vitro* bioassay data obtained in the interlaboratory calibration study

Phase I samples: Standard mixtures (PCDD/DFs, DL-PCBs)



Phase II samples: Clean-up extracts (sediment, food/feed)



Phase III samples: Original samples (3 fish oil, 3 feedstuff)

Phase III-11 Phase III-12 Phase III-13 Phase III-14 Phase III-15 Phase III-16

➔ Simple clean-up method with high recover rates of dioxins is an important for the detection of dioxins with *in vitro* bioassay!

Charted by Suzuki (2007) based on BICS 2005 report (2006)

In vitro bioassays combined with clean-up approved by Japanese Ministry of the Environment

1. AhR-binding assay

No.	Categories	Clean-up methods	Detection
1-1	AhR reporter gene assay	Sulfuric acid silicagel column + Carbon column	**XDS-CALUX assay** with mouse H1L6.1c2
1-2		Sulfuric acid silicagel column + Carbon column	**P450 HRGS assay** with human 101L
1-3		Multilayer silicagel column	**Ah-luciferase assay** with mouse HeB5
1-4		Sulfuric acid silicagel reflux treatment	**DR-CALUX assay** with rat H4IIE-luc
1-5		Multilayer silicagel column + Aluminum column	**DR-EcoScreen assay** with mouse hepatoma cell
1-6	Polyclonal antibody based immunoassay	Sulfuric acid + Multilayer silicagel column	Anti-Arnt polyclonal antibody **Ah immunoassay**

In vitro bioassays combined with clean-up approved by Japanese Ministry of the Environment

2. Immunoassay

No.	Categories	Clean-up methods	Detection
2-1	Monoclonal antibody based immunoassay	Multilayer silicagel column + carbon column	Anti-dioxin monoclonal antibody (specific to 2,3,7,8-substituted PeCDFs) **Dio-quicker**
2-2		Multilayer silicagel column + Carbon column	Anti-dioxin monoclonal antibody (specific to 2,3,7,8-substituted Pe&HxCDD/Fs) **EA-Test for dioxins**
2-3		Multilayer silicagel column + Aluminum column	Anti-dioxin monoclonal antibody (specific to ?) **Dioxin ELISA kit TK**
2-4		Multilayer silicagel column + Aluminum column	Anti-dioxin monoclonal antibody (specific to 2,3,4,7,8-PeCDF) **KinExA®**

Scheme of the accreditation for *in vitro* bioassay-laboratory in Japan (Since 2006)

環境省 **Ministry of the Environment**
Government of Japan

Document Assessment
Blind test
Give accreditation

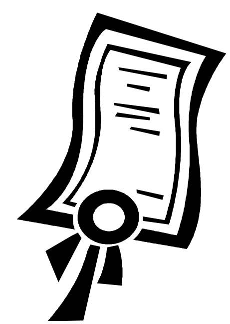
Accreditation ↓ ↑ Application

Specified *in vitro* bioassay Laboratory

Result report ↓ ↑ Analysis request

Users

Businesses
Local government



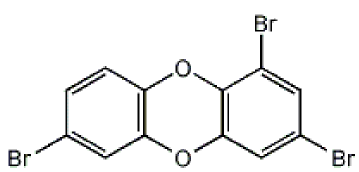
Recent research with the dioxin bioassay

TOXICOLOGICAL SCIENCES 133(2), 197–208 2013
doi:10.1093/toxsci/kft070
Advance Access publication March 14, 2013

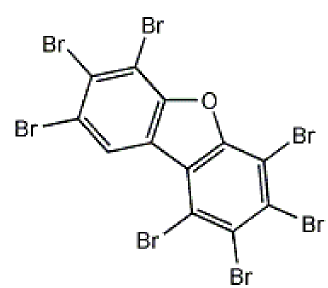
REVIEW

Polybrominated Dibenzo-*p*-Dioxins, Dibenzofurans, and Biphenyls: Inclusion in the Toxicity Equivalency Factor Concept for Dioxin-Like Compounds

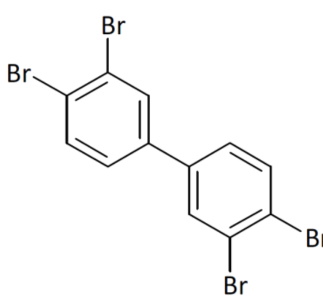
Martin van den Berg,[1,*] Michael S. Denison,[†] Linda S. Birnbaum,[‡] Michael J. DeVito,[‡] Heidelore Fiedler,[§] Jerzy Falandysz,[¶] Martin Rose,[||] Dieter Schrenk,[|||] Stephen Safe,[||||] Chiharu Tohyama,[#] Angelika Tritscher,[**] Mats Tysklind,[††] and Richard E. Peterson[‡‡]



PBDDs



PBDFs



dl-PBBs

Brominated dioxins as impurity of PBDE mixtures

Occurrence of Polybrominated Biphenyls, Polybrominated Dibenzo-*p*-dioxins, and Polybrominated Dibenzofurans as Impurities in Commercial Polybrominated Diphenyl Ether Mixtures

NOBUYASU HANARI,[†] KURUNTHACHALAM KANNAN,[*,‡] YUICHI MIYAKE,[†] TSUYOSHI OKAZAWA,[†] PRASADA RAO S. KODAVANTI,[§] KENNETH M. ALDOUS,[‡] AND NOBUYOSHI YAMASHITA[†]

National Institute of Advanced Industrial Science and Technology (AIST), 16-1 Onogawa, Tsukuba, Ibaraki 305-8569, Japan, Wadsworth Center, New York State Department of Health, and Department of Environmental Health Sciences, School of Public Health, State University of New York at Albany, Empire State Plaza, P.O. Box 509, Albany, New York 12201-0509, and Neurotoxicology Division, NHEERL/ORD, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711

TABLE 3. Concentrations of Polybrominated Dibenzofurans (ng/g) in Commercial PBDE Mixtures

PBDF congener	DE-71 (Lot 1550OI18A)	DE-79 (Lot 1525DD11A)	DE-79 (Lot 8525DG01A)	DE-83 (Lot 0480DL07B)	DE-83 (Lot 7480DL10C)
tetra-BDF	100	<100	<100	<100	<100
penta-BDF	157	315	231	<100	<100
hexa-BDF	<100	8506	4863	<100	<100
hepta-BDF	<200	4418	3657	1628	1242
octa-BDF	<200	5951	1718	47978	29540
total	257	19190	10469	49605	30783

TABLE 4. Global Demand for Commercial PBDE Mixtures (Tons) in 2001 and Estimated Content (kg) of PBBs and PBDFs in Commercial PBDE Mixtures

	total demand for PBDEs in 2001 (ton)[a]			
PBDE mixture	USA	EU	Asia	total
penta-BDE	7100	150	150	7400
octa-BDE	1500	610	1500	3610
deca-BDE	24500	7600	23000	55100
total	33100	8360	24650	66110
	PBBs and PBDFs in PBDEs (kg)			
PBBs	33	2.1	4.6	39.7
PBDFs	1009	315	947	2271

[a] Global PBDE demand data were from ref 2.

Hanari et al. *Environ Sci Technol* 2006

Unintentional production of brominated dioxins

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Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Review

Brominated flame retardants and the formation of dioxins and furans in fires and combustion

CrossMark

Mengmei Zhang[a], Alfons Buekens[a,b], Xiaodong Li[a,*]

[a] State key laboratory of clean energy utilisation, Institute for Thermal Power Engineering, Zhejiang University, Hangzhou, China

[b] Formerly with Chemical Engineering department, Vrije Universiteit Brussel, Brussels, Belgium

HIGHLIGHTS

- BFRs (PBDEs, HBCD and TBBP-A) are the main sources of PBDD/Fs in combustion process.
- Precursor formation is the most relevant pathway for PBDD/Fs formation.
- Adding bromine into combustion system can enhance the formation of PCDD/Fs.
- Primitive recycling of e-waste produces the largest amounts of PBDD/Fs.

Zhang et al. *J Hazar Mater* 2016

BFRs have been widely used in products in the world

Electronic

Car fabric

Compounds	Annual Sales (t)			
	2010	2011	2012	2013
Brominated flame retardants	**430,000**	**444,600**	**429,500**	**434,000**
Phosphorus flame retardants	188,000	190,000	187,000	190,000
Inorganic based-flame retardants	528,730	538,300	551,900	565,500

Fuji Chimera Research Institute, Inc. 2014

Environmentally sound management of brominated dioxins during lifecycle of product containing BFRs

Electronic

Car fabric

Manufacture ➔ Use ➔ Recycle ➔ Waste disposal

Brominated dioxins emission

E-waste open-burning area in developing countries

For retrieving Cu from e-waste

Investigation about e-waste recycling at January 2013

The first estimate of the worldwide volume of obsolete PCs

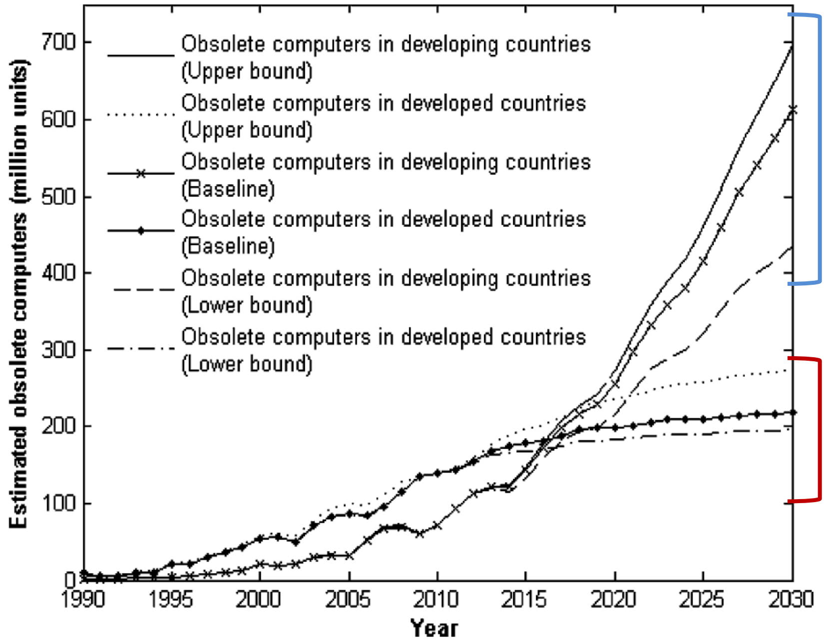


FIGURE 5. Forecasting of generation of obsolete computers in developed and developing world.

Developing countries:

Latin America, Eastern Eu, Ap, Middle East

Developed countries:

North America, Western Eu, Jpn/Au/NZ

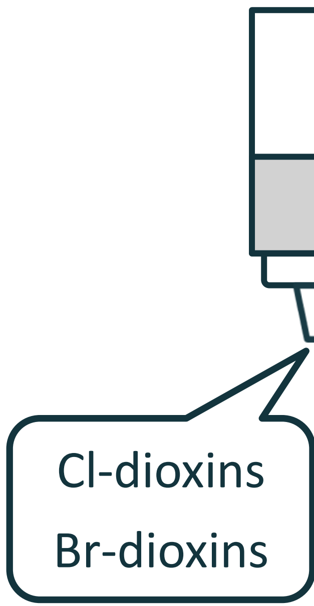
Developing countries will be disposing of more old computers than developed countries by 2018

Zhang et al. *Environ Sci Technol* 2016

➔ E-waste recycling will be conducting and expanding in various countries…

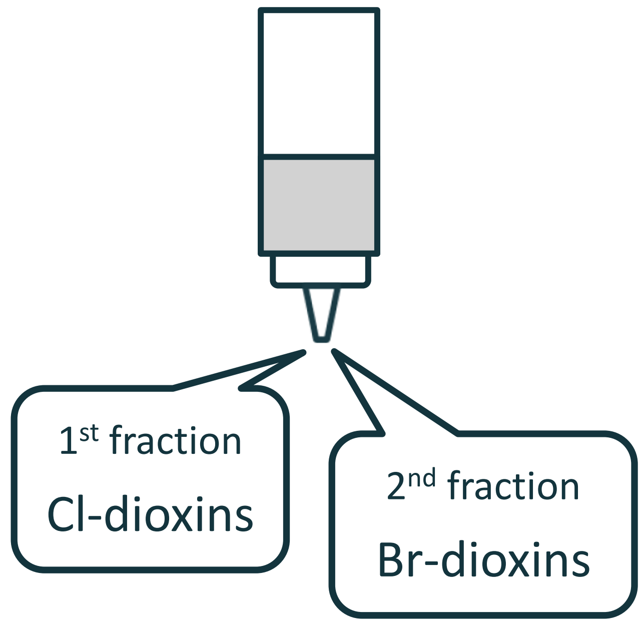
Development of the new clean-up methods

“Sulfuric acid column”



➔ Solvent-saving method
➔ Easy-to-use method

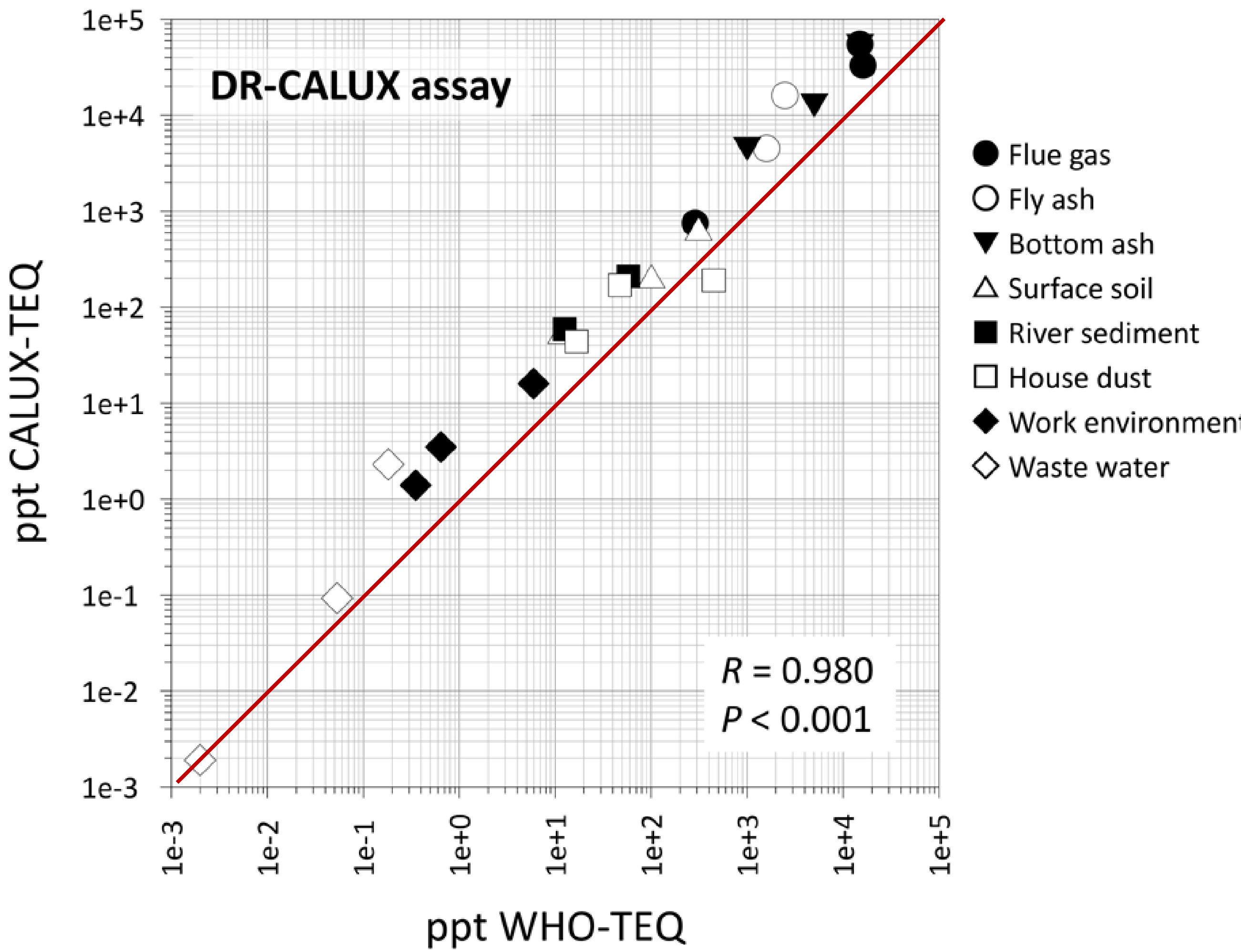
“Separation column”



➔ Separation of Br-dioxins from Cl-dioxins

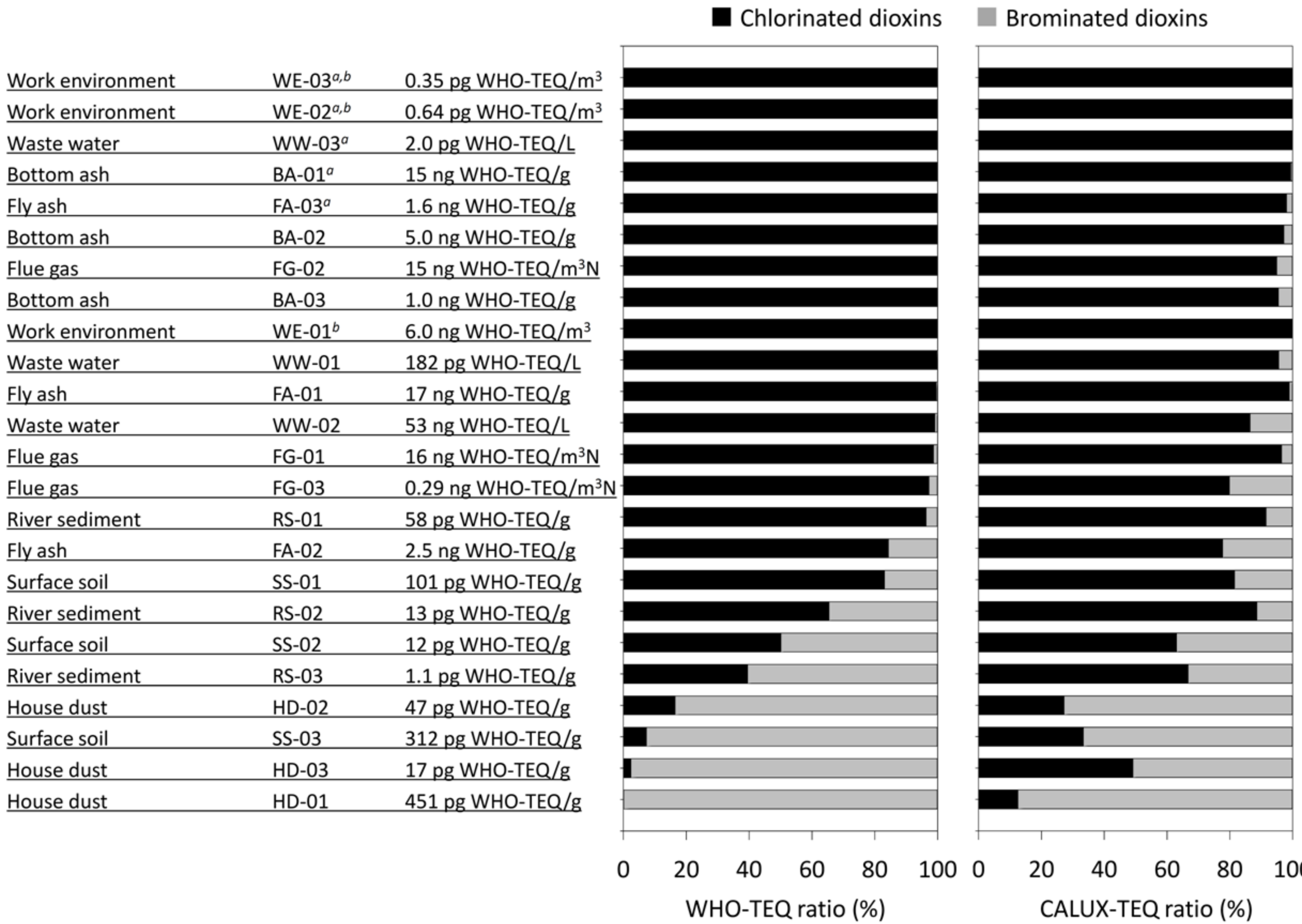
Environment Research and Technology Development Fund of the MOE, Japan (Go Suzuki, 3K133010)

The applicability of the new "sulfuric acid column"



Environment Research and Technology Development Fund of the MOE, Japan (Go Suzuki, 3K133010)

The applicability of the new "separation column"



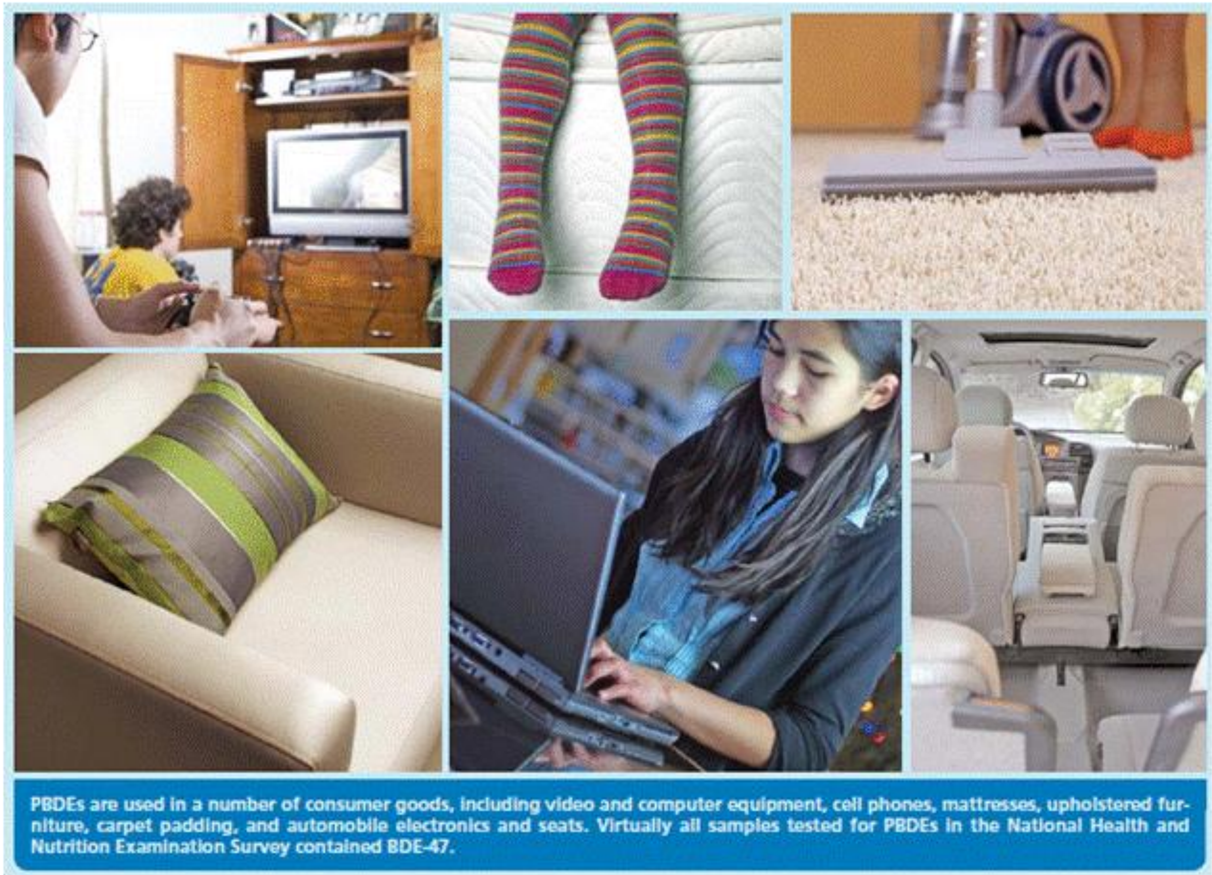
Environment Research and Technology Development Fund of the MOE, Japan (Go Suzuki, 3K133010)

Results of application study with developed method

Samples	Sampling year	Regulation WHO-TEQ	Origin and average CALUX-TEQ ratio from Br-DXN fraction of samples exceeding regulation
Flue gas (*n*=50)	2014-2015	10 ng/m^{3}N	Electronic manufacture, 90% (*n*=2)
Fly ash (*n*=22)	2014-2015	3 ng/g	Domestic waste disposal business, 9.5% (*n*=2) Industrial waste disposal business, 8.4% (*n*=3)
Bottom ash (*n*=19)	2014-2015	3 ng/g	Domestic waste disposal business, 21% (*n*=1) Construction industry, 15% (*n*=1)
Waste water (*n*=25)	2014-2016	10 pg/L	Flame-retarded textile factory, 67% (*n*=6) STP influent, 60% (*n*=2) Incinerator, 35% (*n*=5)
Soil (*n*=128)	2012-2014	1000 pg/g	E-waste open-burning area, 39% (*n*=9)
Sediment (*n*=35)	2004-2014	150 pg/g	E-waste processing area, 21% (*n*=7) Harbor, 10% (*n*=2)
Indoor air (*n*=18)	2014-2015	2.5 pg/m^3	Not detected
House dust (*n*=44)	2007-2008	(100 pg/g)	Hotel, 53% (*n*=3) Office, 43% (*n*=12) Household, 41% (*n*=2) Nursery school, 28% (*n*=3)

➔Brominated dioxins tended to be detected in samples related with brominated flame retardants (BFRs)

BFRs tend to be used in many kinds of household products



Betts *Environ Health Perspect* 2008

➔ Brominated dioxins should be managed for environmentally sound management of BFR-containing product

➔ Developed method will be useful screening tool for brominated dioxins

Thank you very much for kind attention!

Acknowledgments

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Mr. Akinori Hashimoto

Ministry of the Environment, Japan

Environment Research and Technology Development Fund
(no. 3K133010)