

7TH INTERNATIONAL CONFERENCE ON SUSTAINABLE SOLID WASTE MANAGEMENT

26-29 June 2019, **AQUILA ATLANTIS HOTEL** Heraklion, Crete Island, Greece



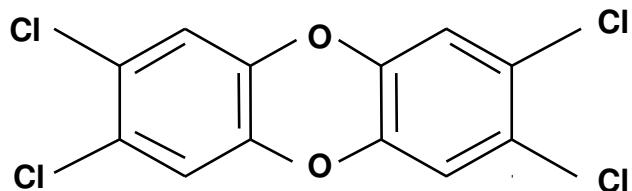
A NOVEL REPORTER GENE- BASED BIOASSAY SYSTEM FOR DIOXINS DETERMINATION IN SOLID

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06/28/2019

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Health Effects of Dioxins



**Dioxins &
Dioxin-like
Compounds**



- Immunotoxicity
- Hepatotoxicity
- Wasting Syndrome
- Dermal Toxicity
- Teratogenicity
- Lethality
- Endocrine Disruption
- Tumor Promotion
- Porphyria
- Induction of Gene Expression

Dioxin Emission Reduction

EUROPE

Emission standard

**Waste incineration
2000/76/EC**

Foodstuffs

2006/1881/EC

Industrial emissions

2010/75/EU

*EU Dioxin Emission
Inventory Stage I*

*EU Dioxin Emission
Inventory Stage II*

USA and JAPAN

Emission standard

1994 MSIWs 59 FR 48198

2000 HWIs 65 FR 47937

EPA 2005

*The Inventory
of Sources of Dioxin*

*Goverment of Japan
2000. Dioxin*

Emission standard
In Various fields

CHINA

Emission standard

MSIWs GB 18485-2001

HIWs GB 18484-2001

**Landfill of IWs
GB 16889-2008**

Steelmaking Plant
GB 28664-2012

Waste water
GB 3544-2008
GB 18918-2002

Detection Methods for Dioxins

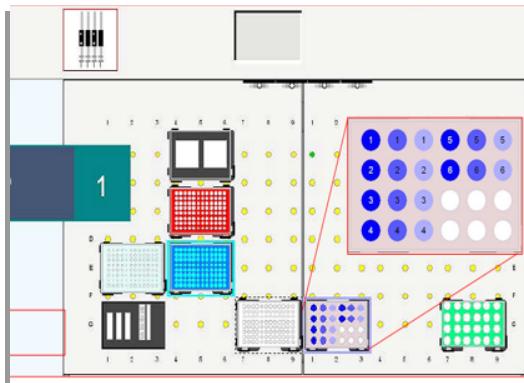


HRGC-HRMS

Concentration of congeners

TEF----TEQ

Expensive
Strict condition
Low throughput



BIOASSAY

EROD

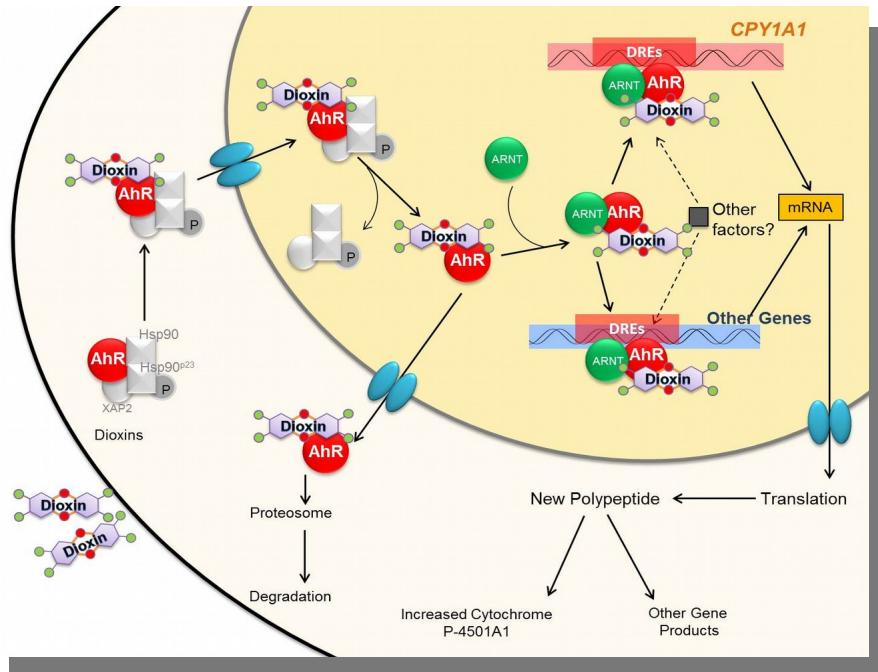
ELISA

Reporter gene

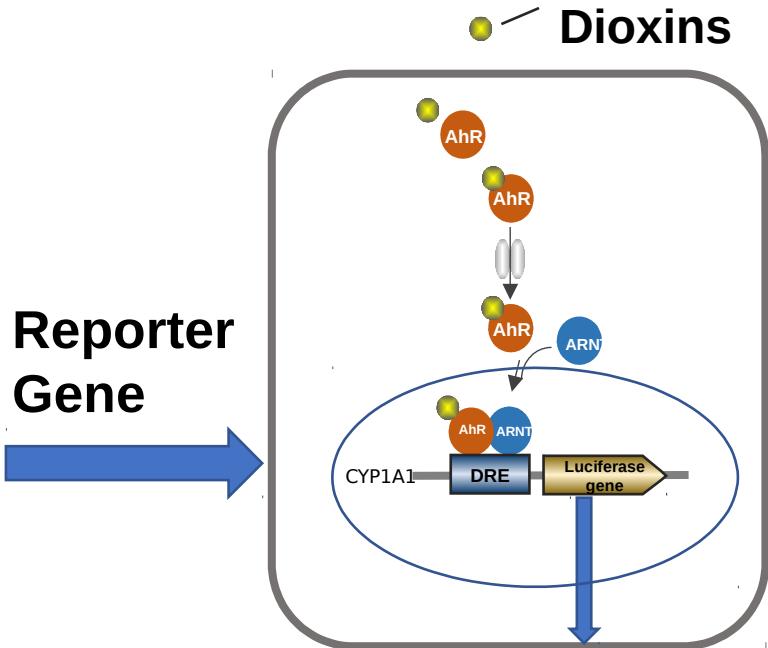
- Simple operation
- Efficiency
- High throughput

AhR-based Reporter Gene Bioassay

Toxicological mechanism of dioxins: AhR signaling pathway



Reporter
Gene



LUCIFERASE
+ Luciferin
Luminis
cence

CALUX
(Chemically Activated Luciferase gene eXpression)

Bioassay Development and Application

US EPA SW846-
4435, TOX 21

European Union

2005 Belgium NVRIP dioxins detection in feed and soil
2009 Revision EC No152/2009 DR-CALUX in feed
2011 EC No 252/2012 DR-CALUX in food

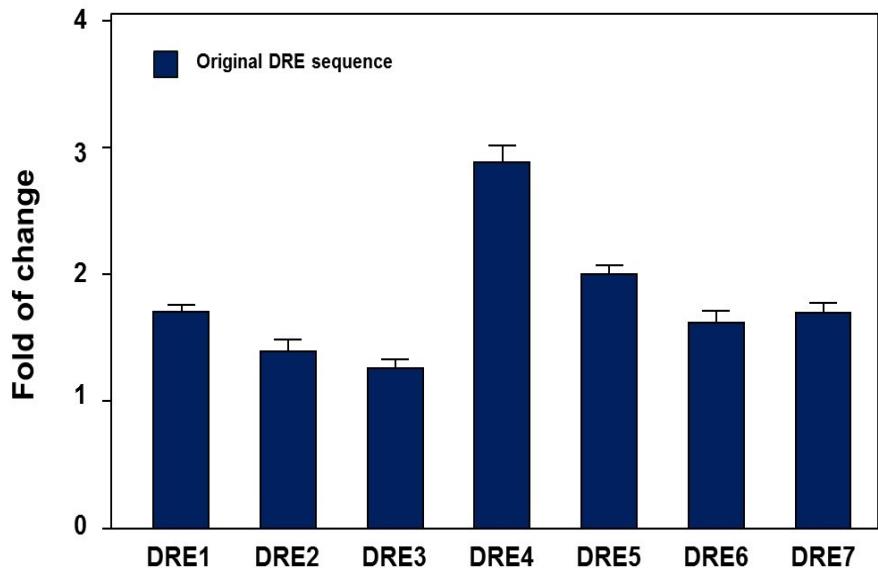
2018 China
Draft comments (HJ)
in solid waste

2013 Chile SAG
CALUX in birds

Japan JIS K 0463-2009
Environmental samples

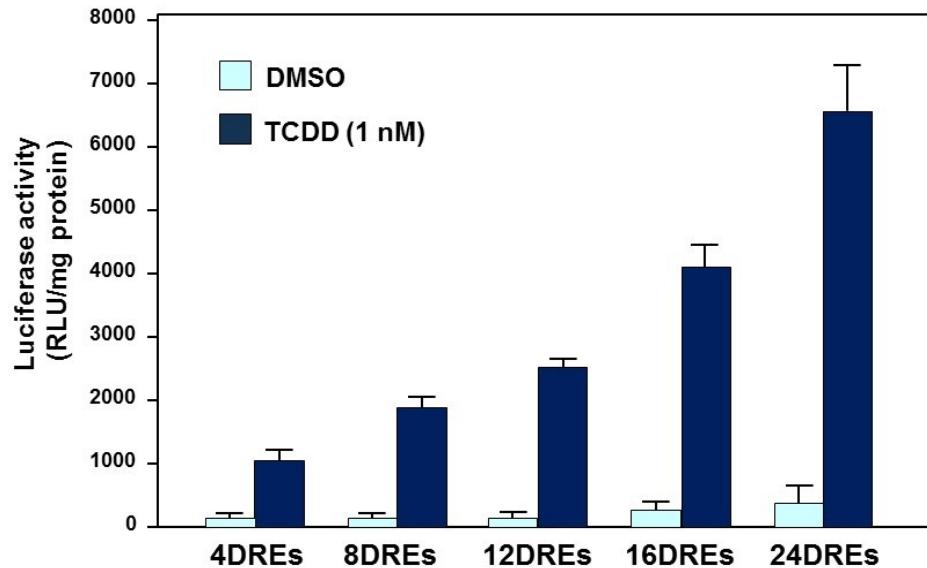
AU Queensland government
air pollution assessment

China: development of bioassay



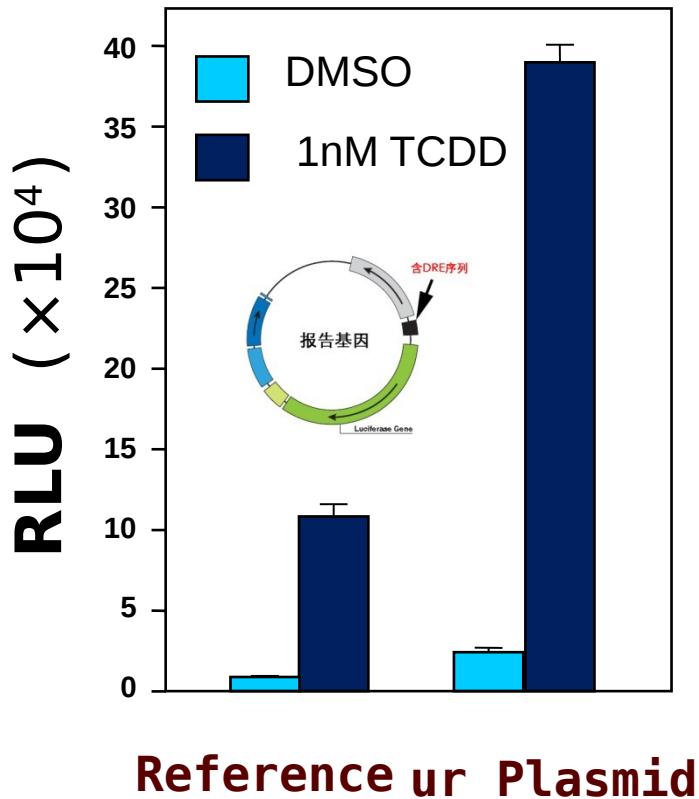
Sequence around DRE

Key : AHR-dependent transcription efficiency

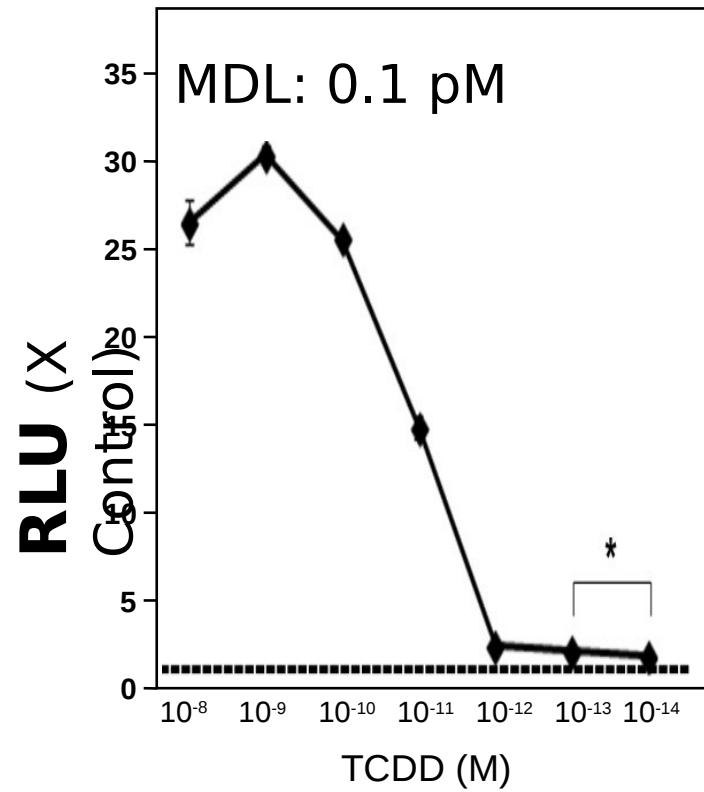


Frequency of DRE

Construction of Novel plasmid



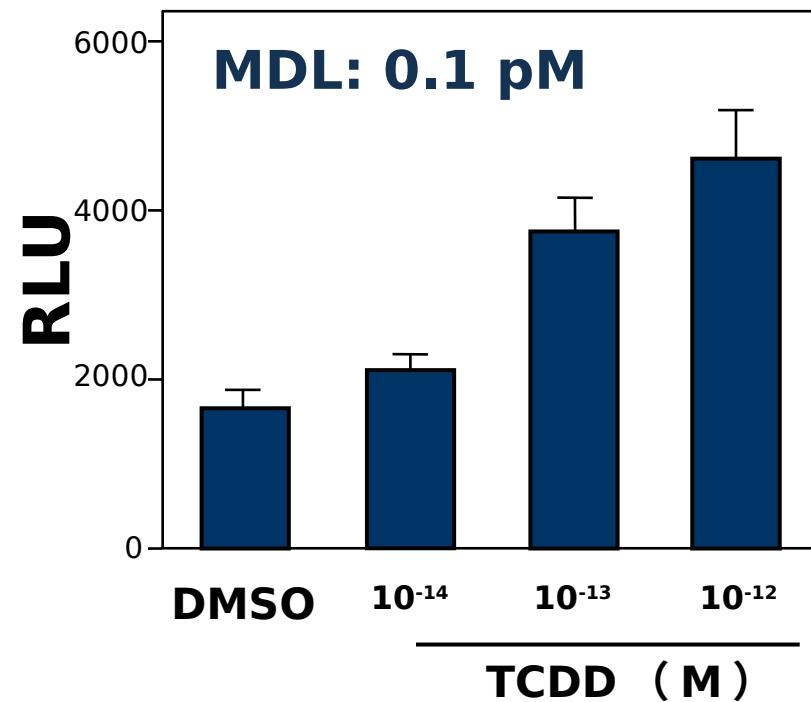
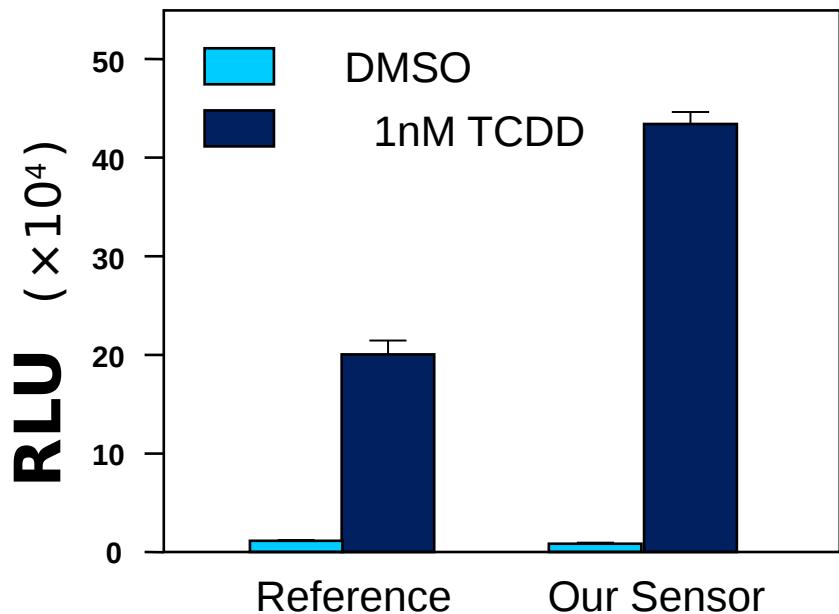
Reference ur Plasmid



pCL-CR2 : ultra-sensitivity

China Patent 201410099507.7

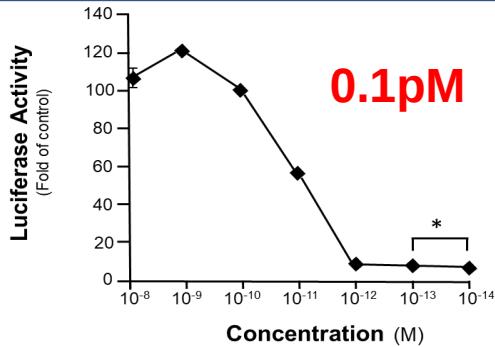
Novel cell-based biosensor



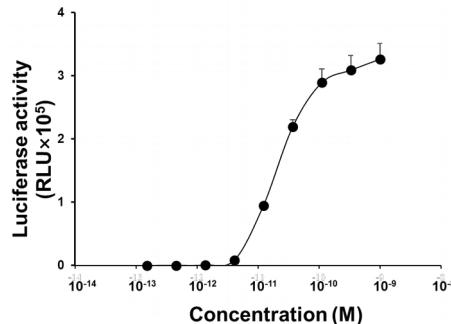
CBG2.8D
0.1 pM: 0.003 pg/assay

China Patent: 201410100654.1

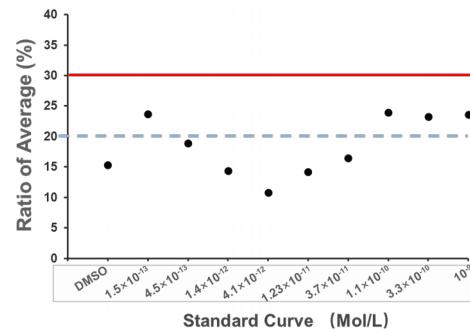
CBG2.8D Bioassay System



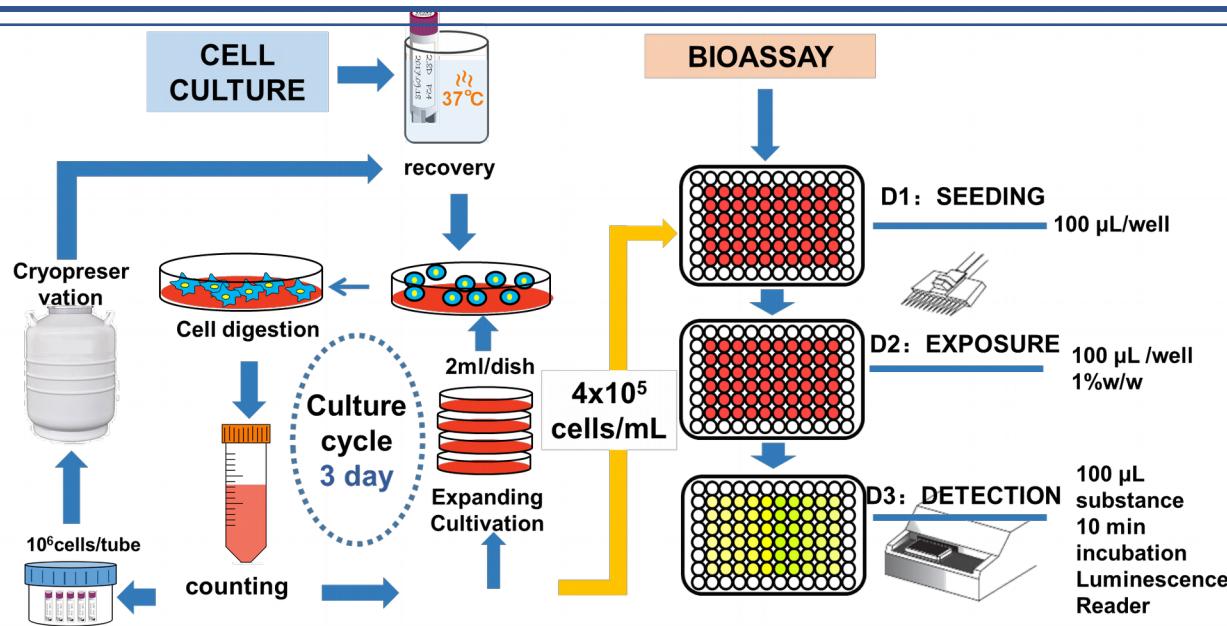
Minimum response to TCDD



standard curve of TCDD



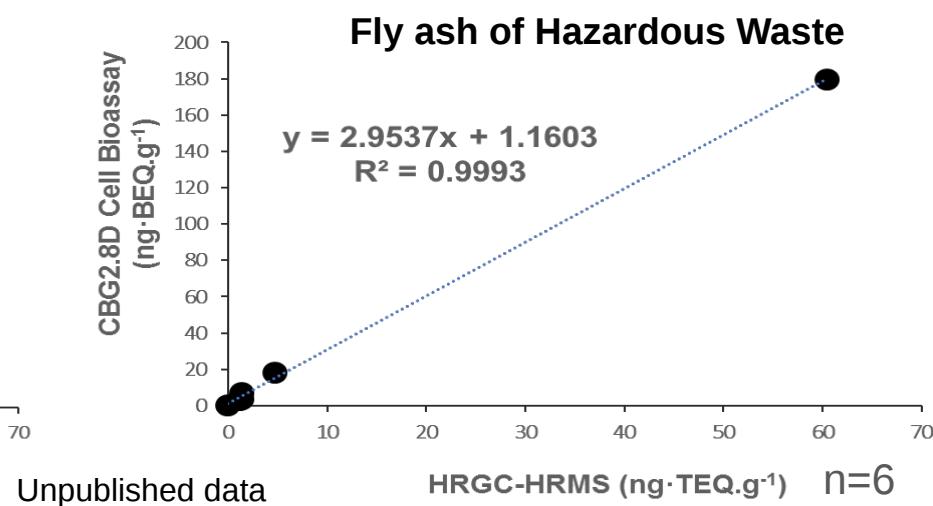
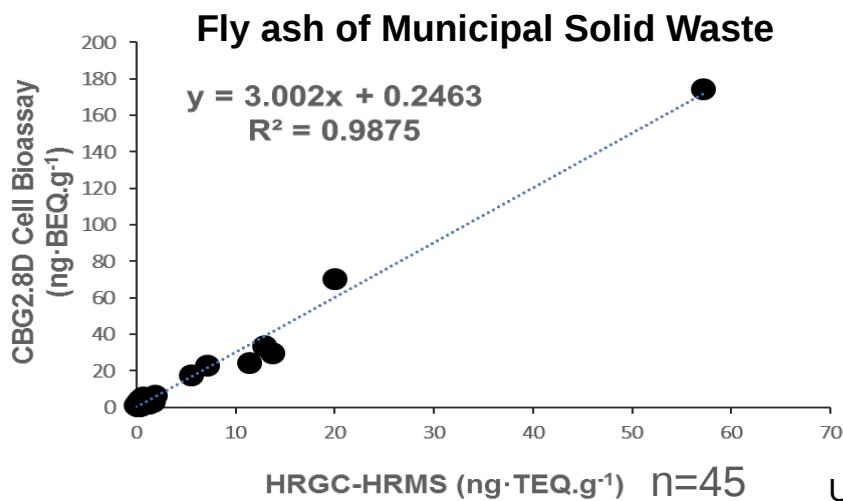
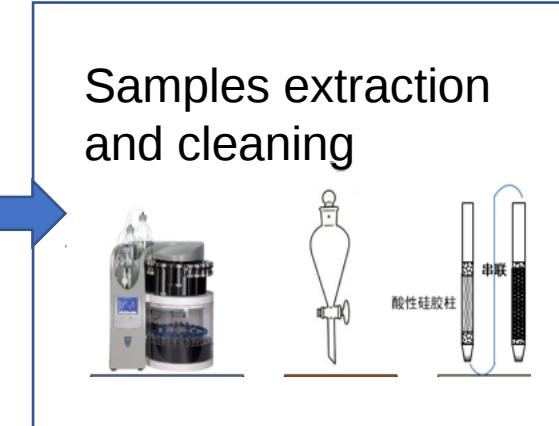
Stability of standard curve



- ✓ Stability
- ✓ Repeatability
- ✓ reproducibility

- S standard
- O operating
- P procedure

Application of CBG2.8D Bioassay System



Correlation between bioassay and HRGC-HRMS in Fly ash samples

China National Standard

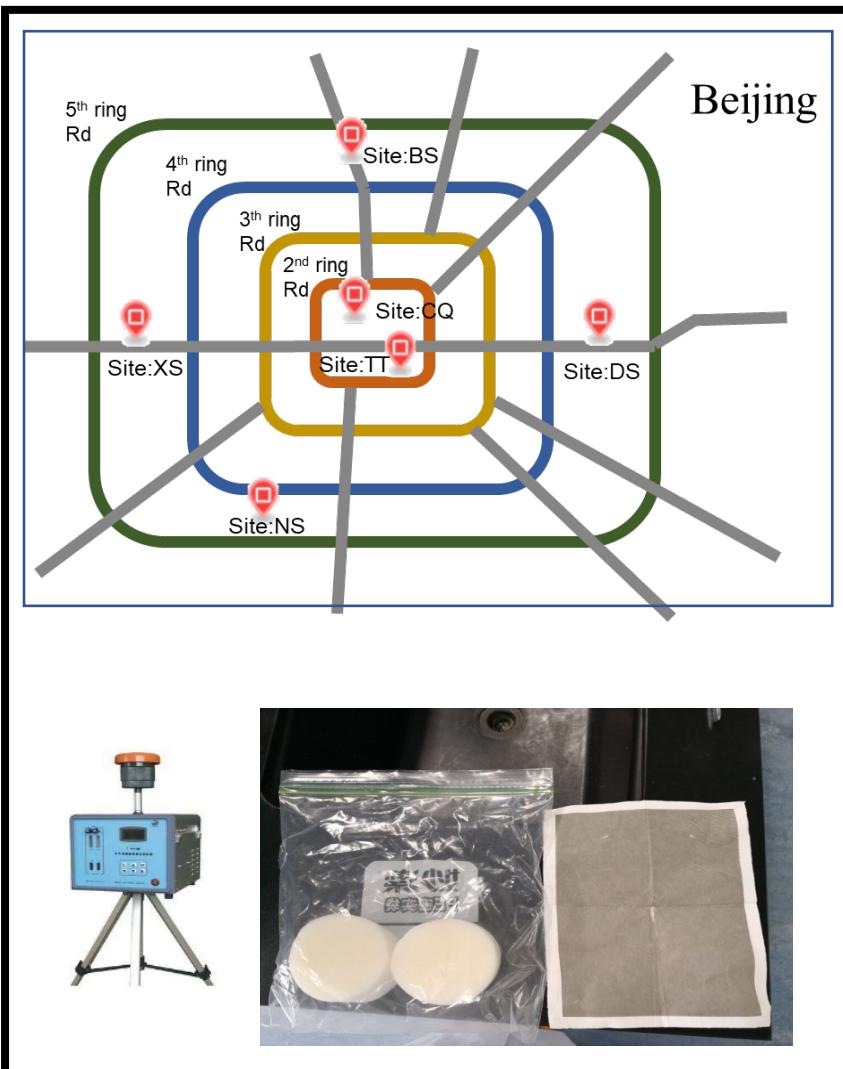


2015 年度国家环境保护标准计划项 目

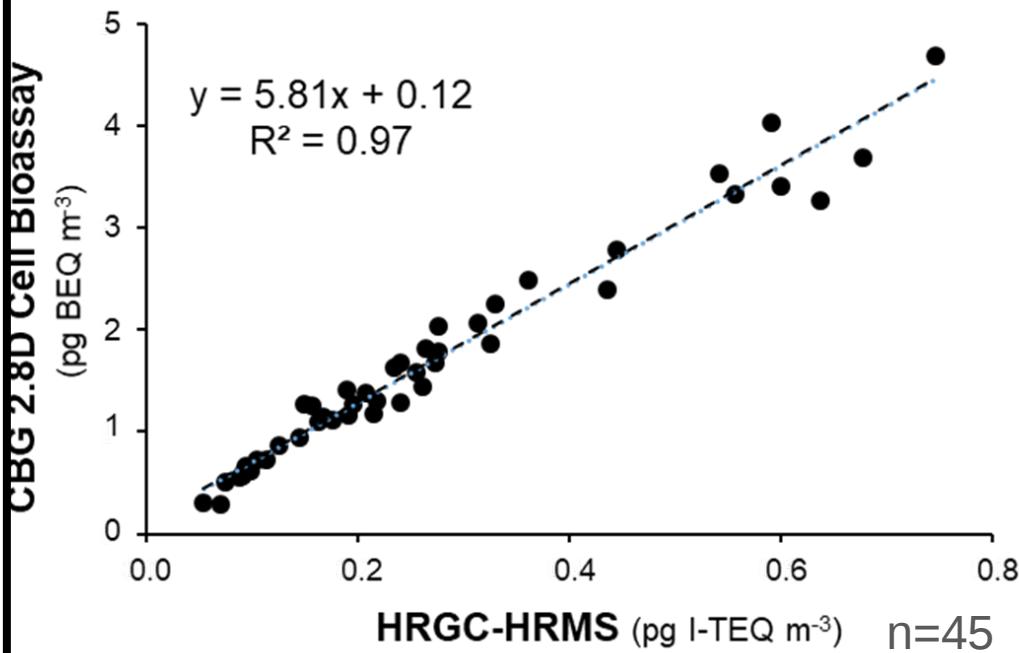
序号	项目统一 编号	项目名称	总经费 (万元)	年度经费 (万元)	完成时限 (年)	立项原因
一、标准制修订						
(二) 环境污染物监测方法标准及监测规范						
7		1 水质 急性毒性的测定 斑马鱼卵法			2016	配套排放标准
20		14 固体废物 二噁英类的筛查 报告基因法			2016	二噁英快速检测法
21		15 土壤和沉积物 故障的测定 气相色谱法			2016	GB 15618 修订配套
22		16 土壤和沉积物 代森锰锌的测定 顶空气相色谱法			2016	GB 15618 修订配套

Listed as China National Monitoring Method for Solid Waste Management

Application of CBG2.8D Bioassay System



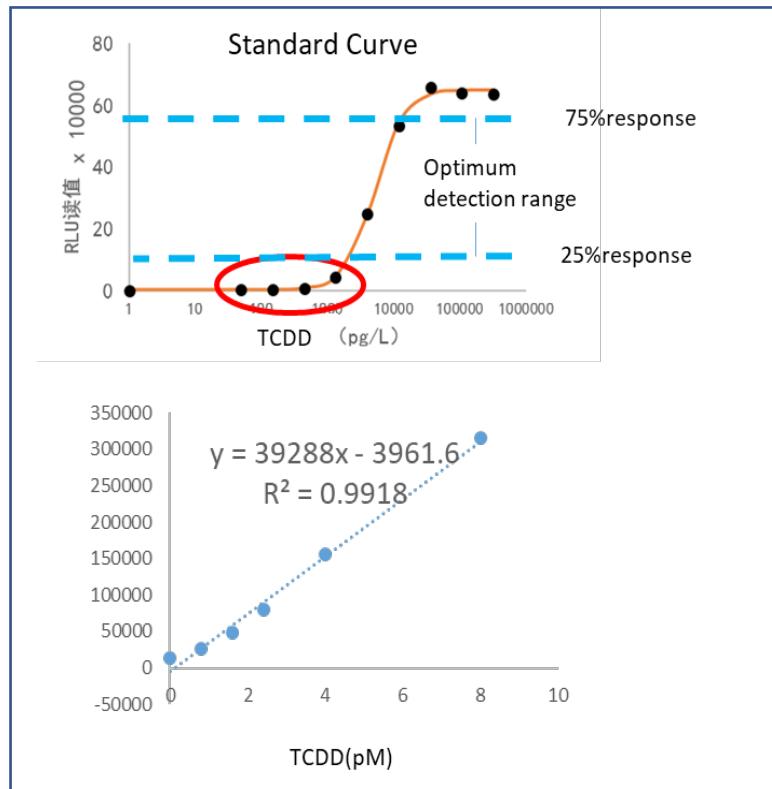
Detection of dioxins in Beijing atmospheric particulates samples



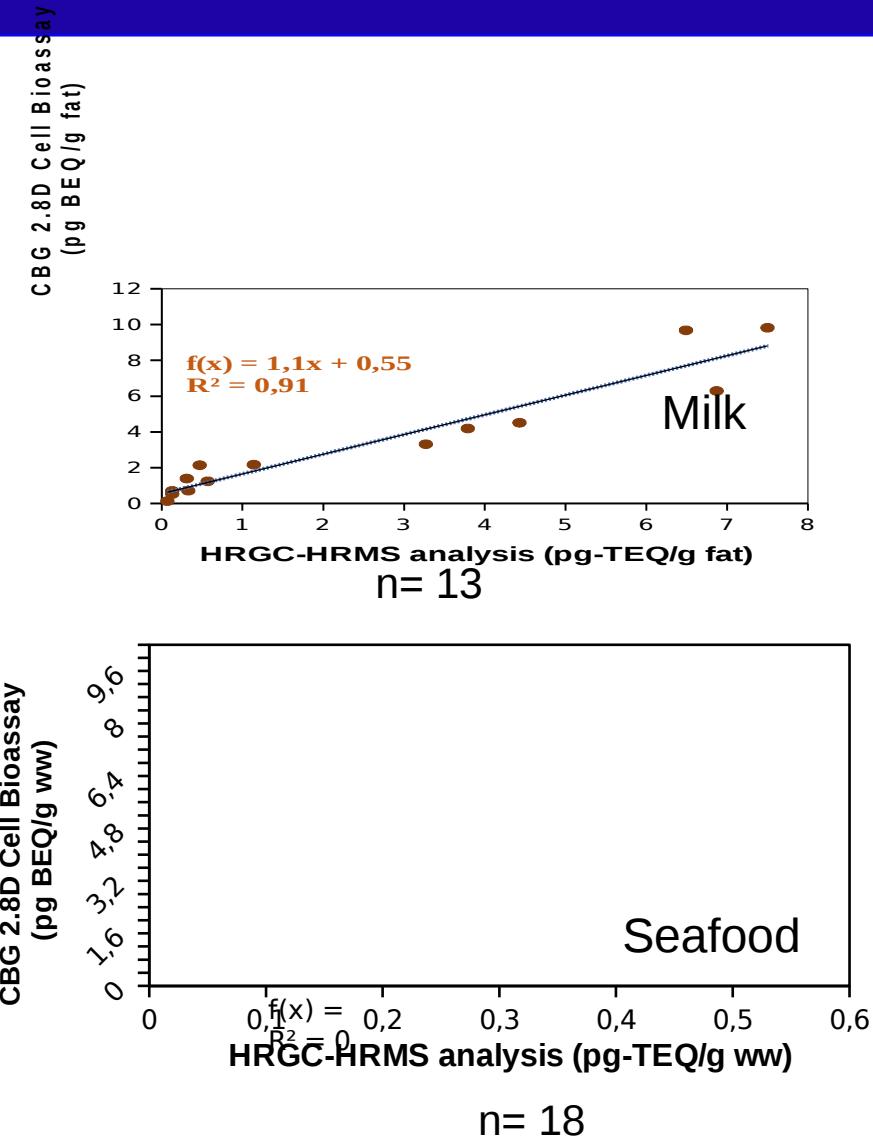
Zhang et al. *Environ. Sci. Technol.* 2018

Application of CBG2.8D Bioassay System

Detection of Dioxins in Food Samples

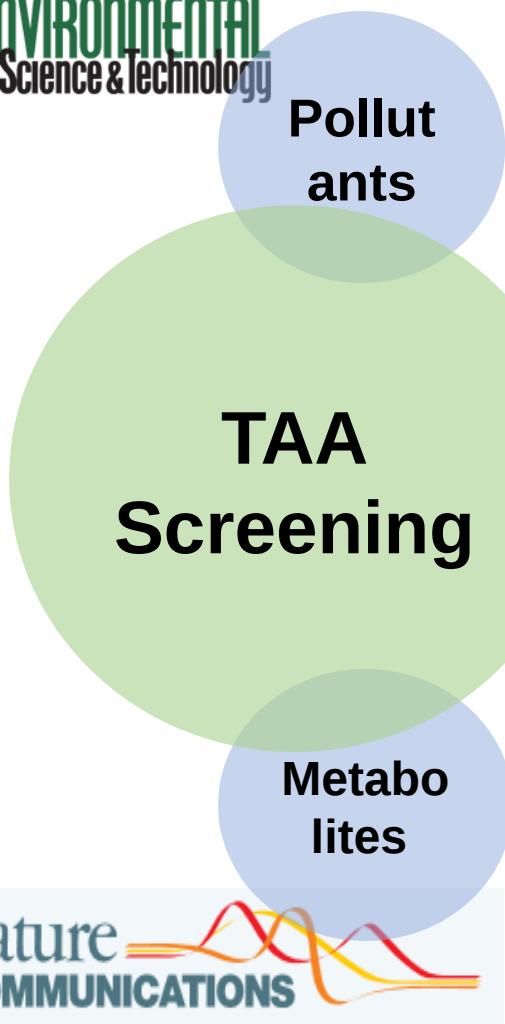


New standard curve



Application of CBG2.8D Bioassay System

ENVIRONMENTAL
Science & Technology

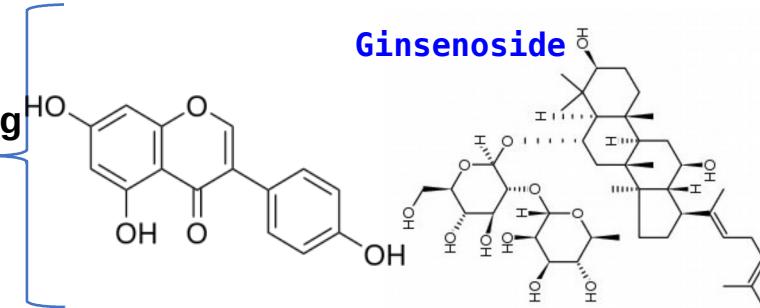


Toxicity Effect Assessment



Zhang et al. *Environ. Sci. Technol.* 2018

Activity Screening

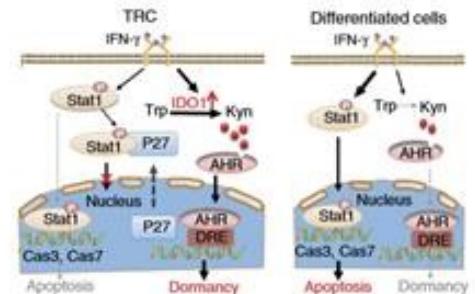


Health Risk Assessment

Tryptophan metabolites

Kyn

Indole compounds



Liu et al. *Nat. Commun.* 2017

nature
COMMUNICATIONS

Dioxin Research in China

Dioxin Emissions and Human Exposure in China: A Brief History of Policy and Research

EHP 2011 119, A112-113

Perspectives | Editorial

“Because of the historical focus of basic research on environmental pollutant levels rather than on environmental health. **It is important to further encourage and enhance basic environmental health research in China** in order to provide a scientific basis for environmental policies to protect the environment, ecological systems, and human health.”

COLLABORATIONS !!!

Acknowledgments



中国科学院大学
University of Chinese Academy of Sciences



