

Innovation is  
finding answers  
to questions  
no one has  
asked



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## Dioxin degraders and Sydney Harbour

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# 01

## Background of dioxin contamination



1949 – 1976 Union Carbide on Rhodes peninsula manufactured 2,4,5-T and 2,4,-D (used in Agent Orange). The process produces 2,3,7,8-TCDD.

Before 1970 process waste was landfilled in Homebush

Homebush Bay heavily industrialized, and sediments have high concentrations of heavy metals, OCPs PCBs and PAHs

Dioxins first investigated in late 1980s

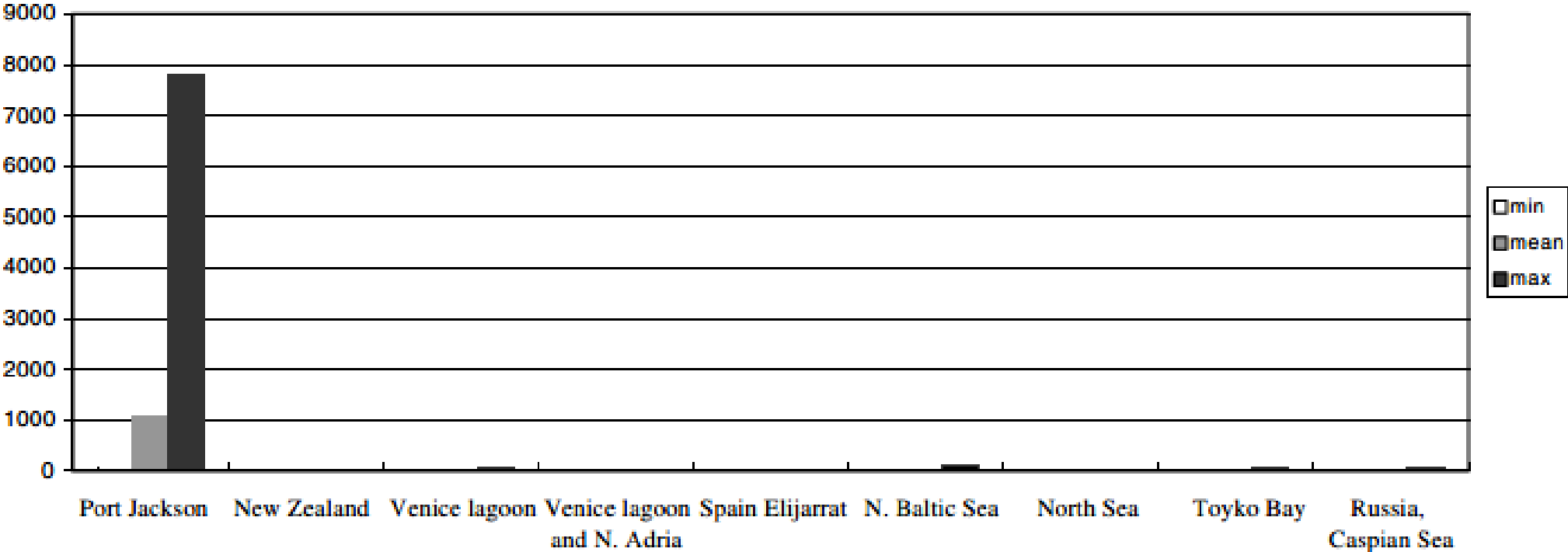
Fishing ban in Homebush Bay in 1989

Commercial fishing ban area extended in 1990

Commercial fishing banned throughout Sydney Harbour 2006

Recreational fishing advice 2006:

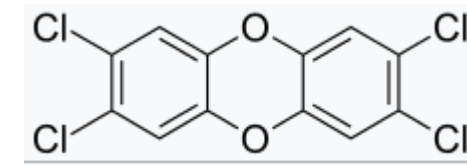
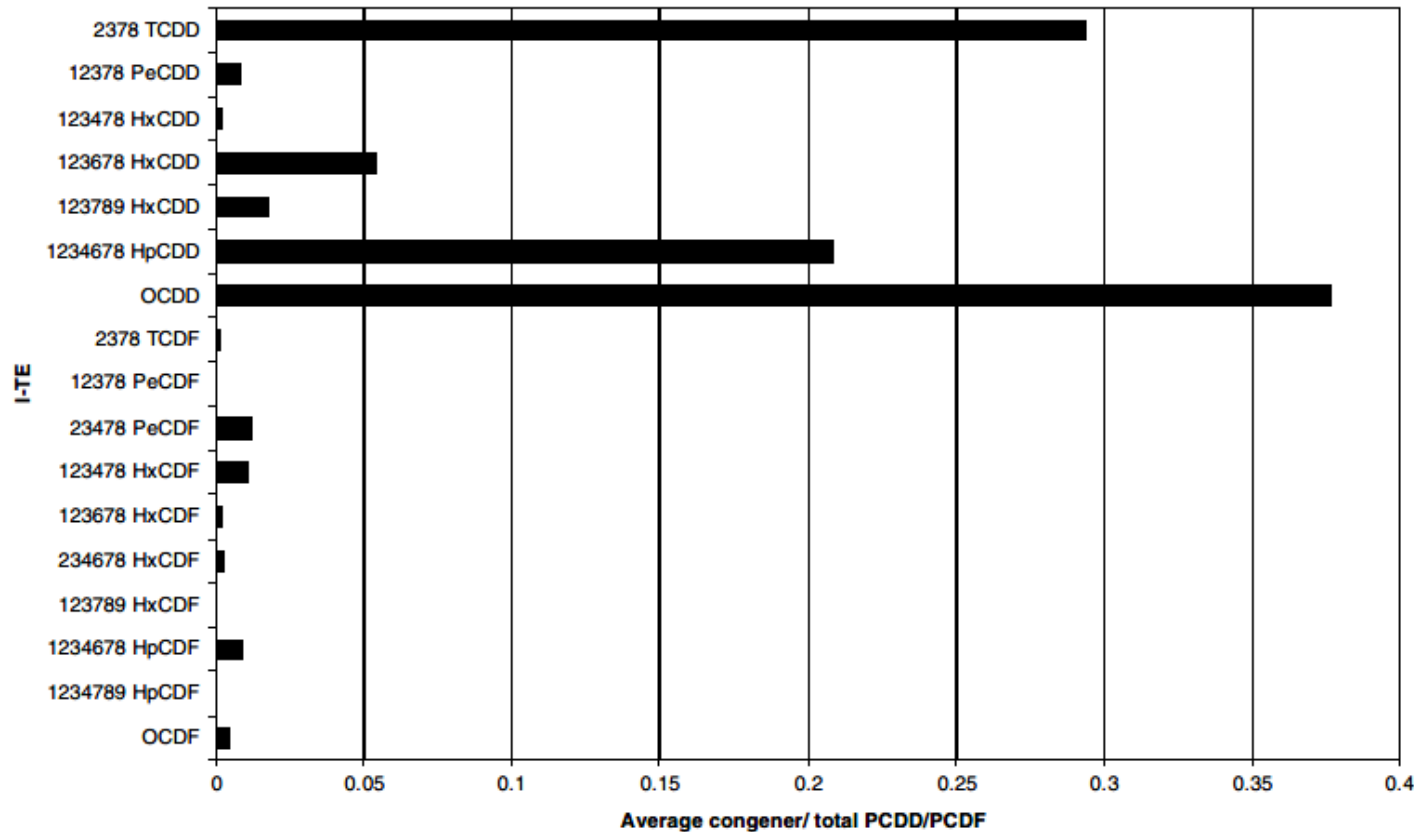
- Don't eat fish caught west of Harbour Bridge
- Limit consumption east of Harbour Bridge



International dioxin concentrations in I-TE  
Figure 3b from Birch et al 2005

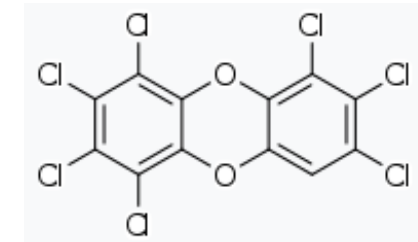
## Homebush Bay averaged congener profile

Figure 3a from Birch et al 2005

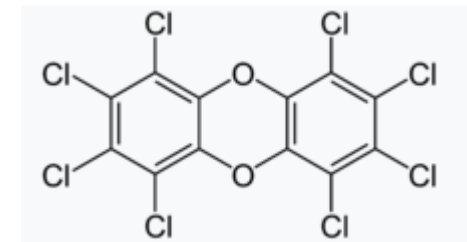


TEF (WHO 2005)

TCDD 1



HpCDD 0.01



OCDD 0.0003

Homebush Bay dominated by OCDD, TCDD and higher chlorinated PCDD, hepta- and hexa-CDDs



# 02 Project Plan





**Objective 1:** Characterise the current dioxin concentrations and corresponding microbial community composition in Sydney Harbor sediments.

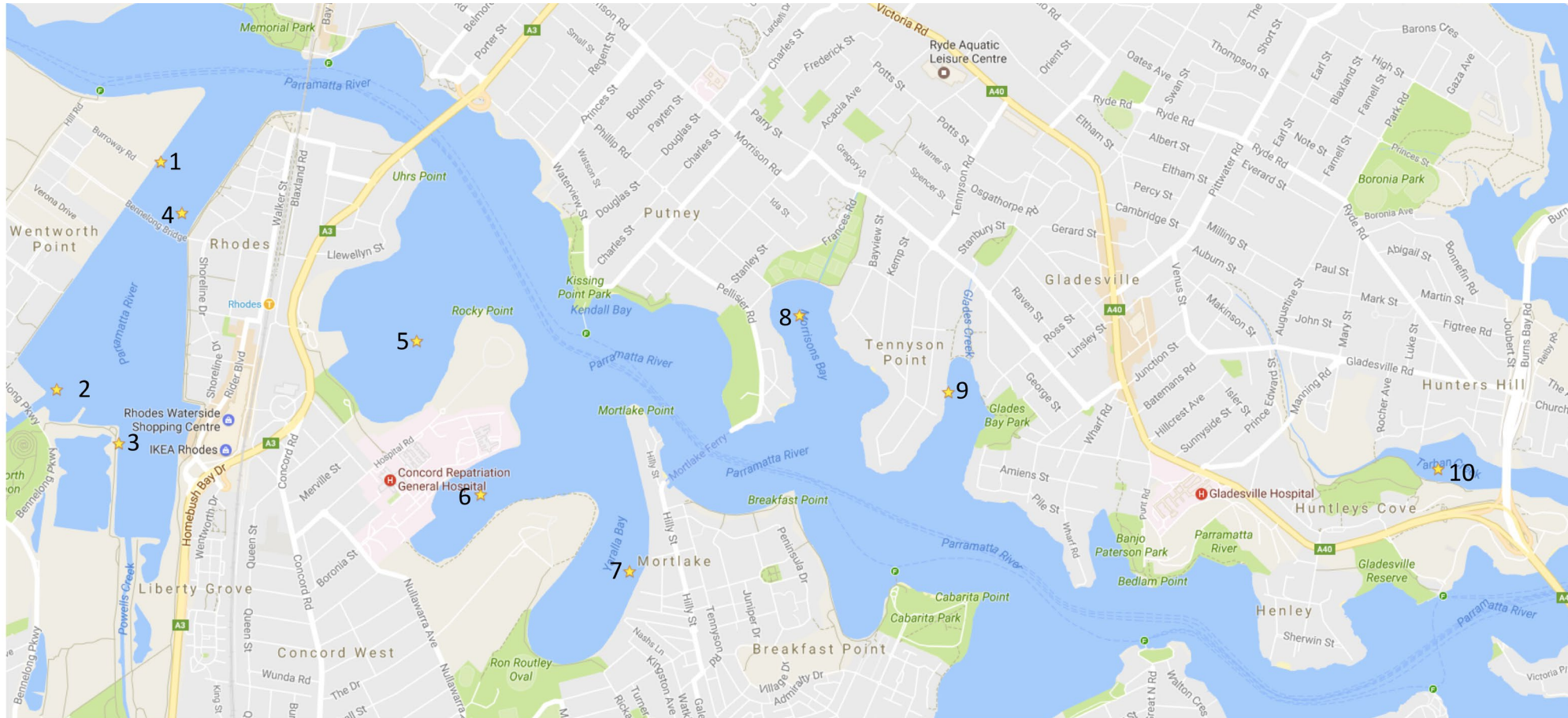
**Objective 2:** Determine if dioxin degrading microorganisms are present in Sydney Harbour sediments.

**Objective 3:** To obtain and describe dioxin degrading microorganisms.

**Objective 4:** To demonstrate biological dioxin degradation in harbor sediments *in situ*.

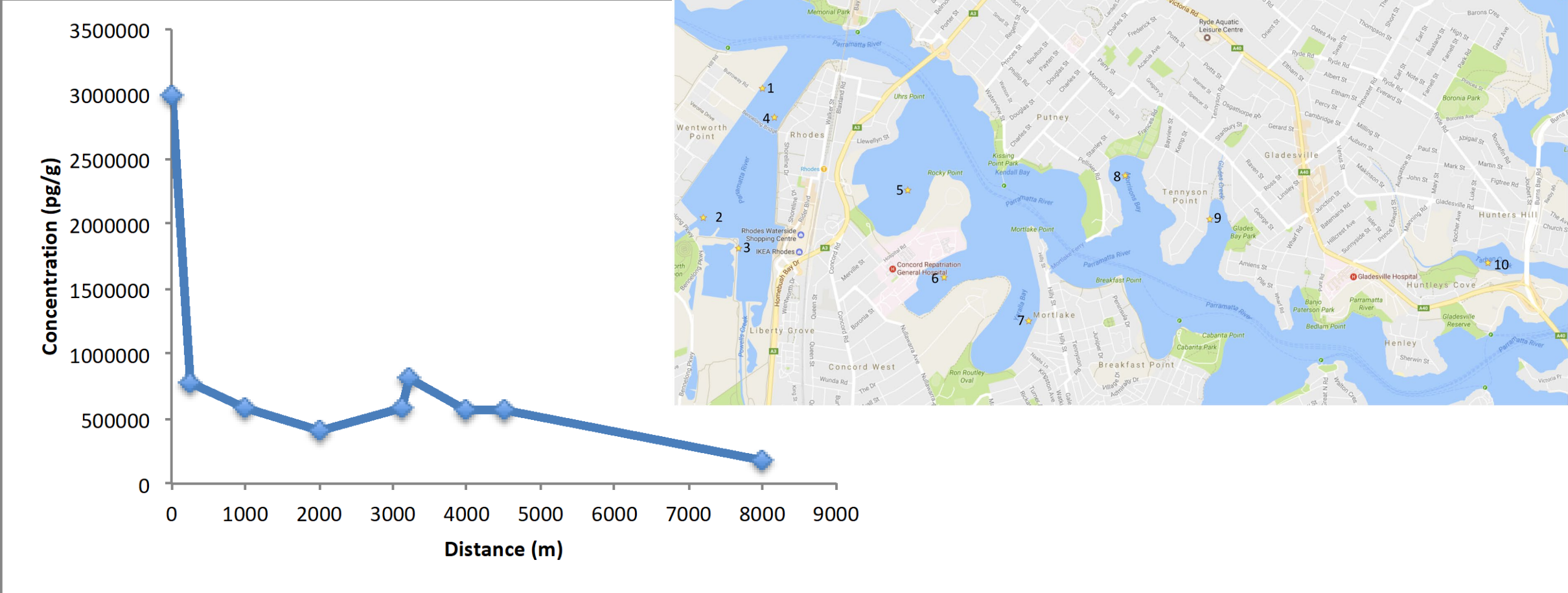


# Sample locations from Homebush Bay (1-4) and Westward to Tarban Creek (10)

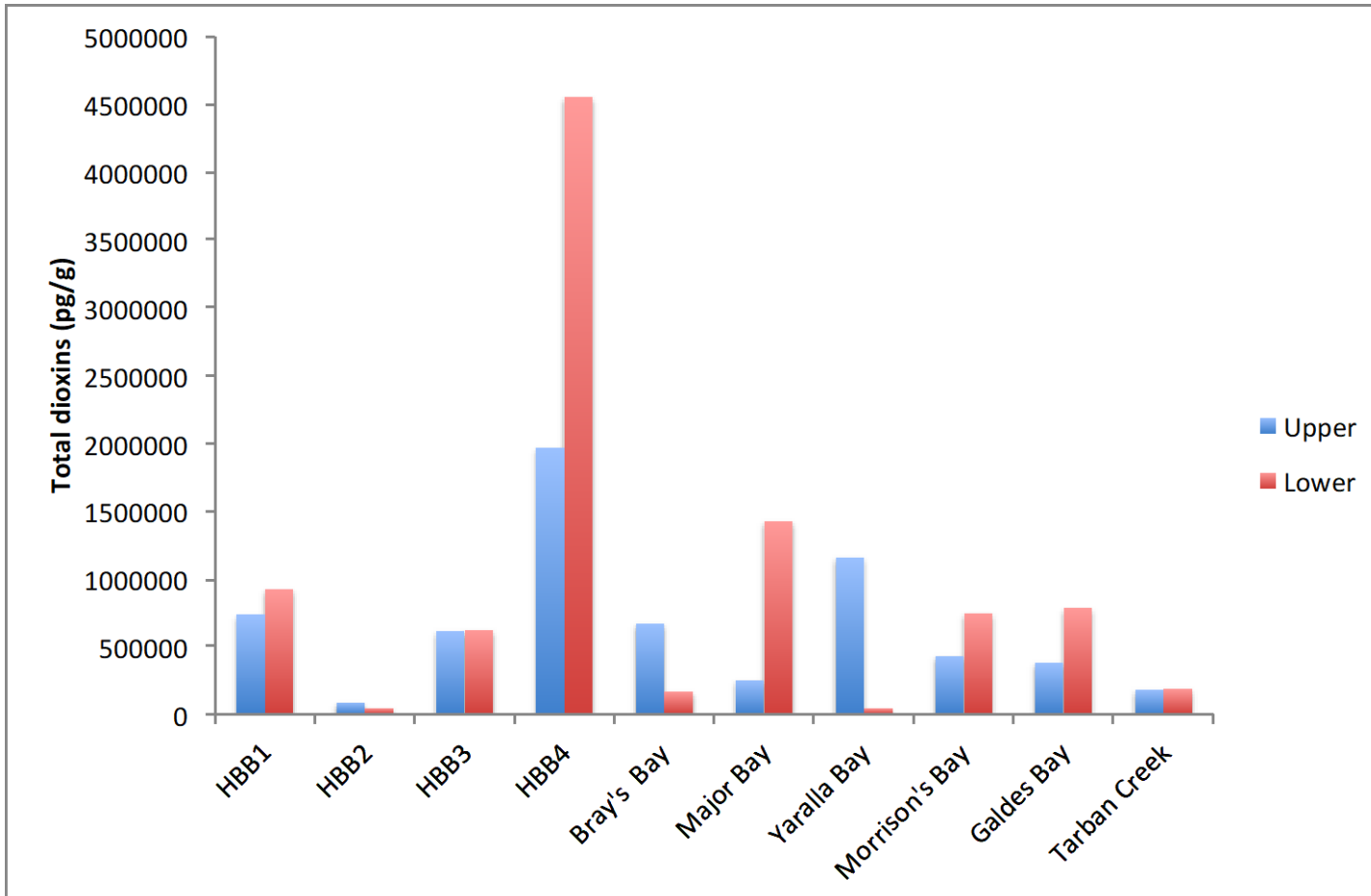


50 cm sample cores were taken from each location and then divided into 25 cm upper and lower fractions.  
Location 4 is adjacent to the former Union Carbide facility.

# Total dioxin concentration (pg/g) versus distance from Homebush Bay (HBB 4)



# Sum of all dioxins (pg/g) at the 10 sample locations



Averaged % contribution of each dioxin congener (n=20)

	mean	$\sigma$
TCDD	0.02	0.01
PCDD	0.01	0.02
HCDD	0.19	0.49
heptCDD	3.44	8.03
OCDD	96.34	8.40

**In absolute terms OCDD was the most abundant dioxin congener**

TCDD represents ~ 25% of the total dioxin load (WHO-TEFs)

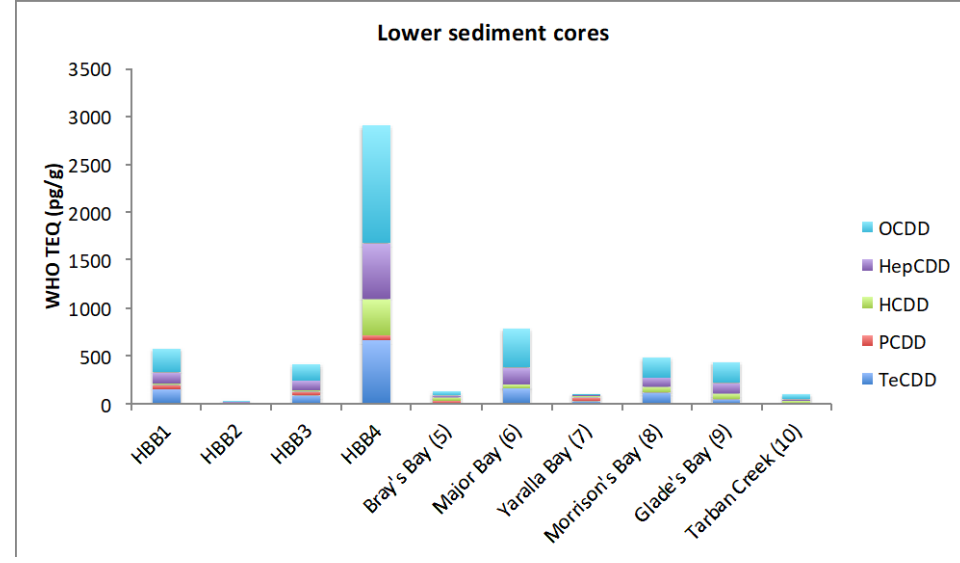
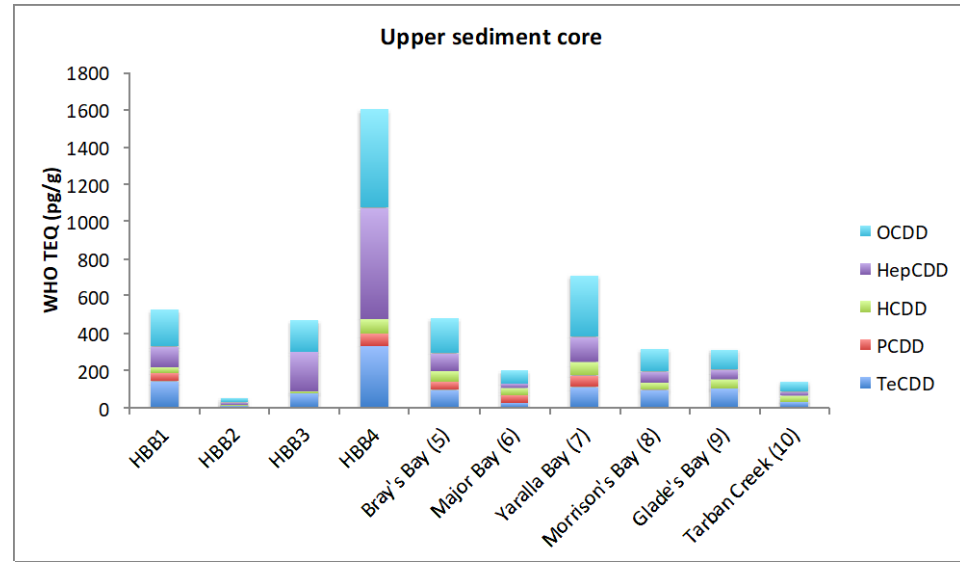


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**WHO Toxicological equivalence factors (WHO-TEF)**

<b>2378TetraF</b>	<b>0.1</b>
<b>2378TetraD</b>	<b>1</b>
<b>12378PCDF</b>	<b>0.03</b>
<b>23478PCDF</b>	<b>0.3</b>
<b>12378PCDD</b>	<b>1</b>
<b>123478HCDF</b>	<b>0.1</b>
<b>123678HCDF</b>	<b>0.1</b>
<b>234678HCDF</b>	<b>0.1</b>
<b>123478HCDD</b>	<b>0.1</b>
<b>123678HCDD*</b>	<b>0.1</b>
<b>123789HCDD</b>	<b>0.1</b>
<b>123789HCDF</b>	<b>0.1</b>
<b>1234678HepCDF*</b>	<b>0.01</b>
<b>1234678 HepCDD</b>	<b>0.01</b>
<b>1234789HepCDF</b>	<b>0.01</b>
<b>OctaD</b>	<b>0.0003</b>
<b>OctaF</b>	<b>0.0003</b>

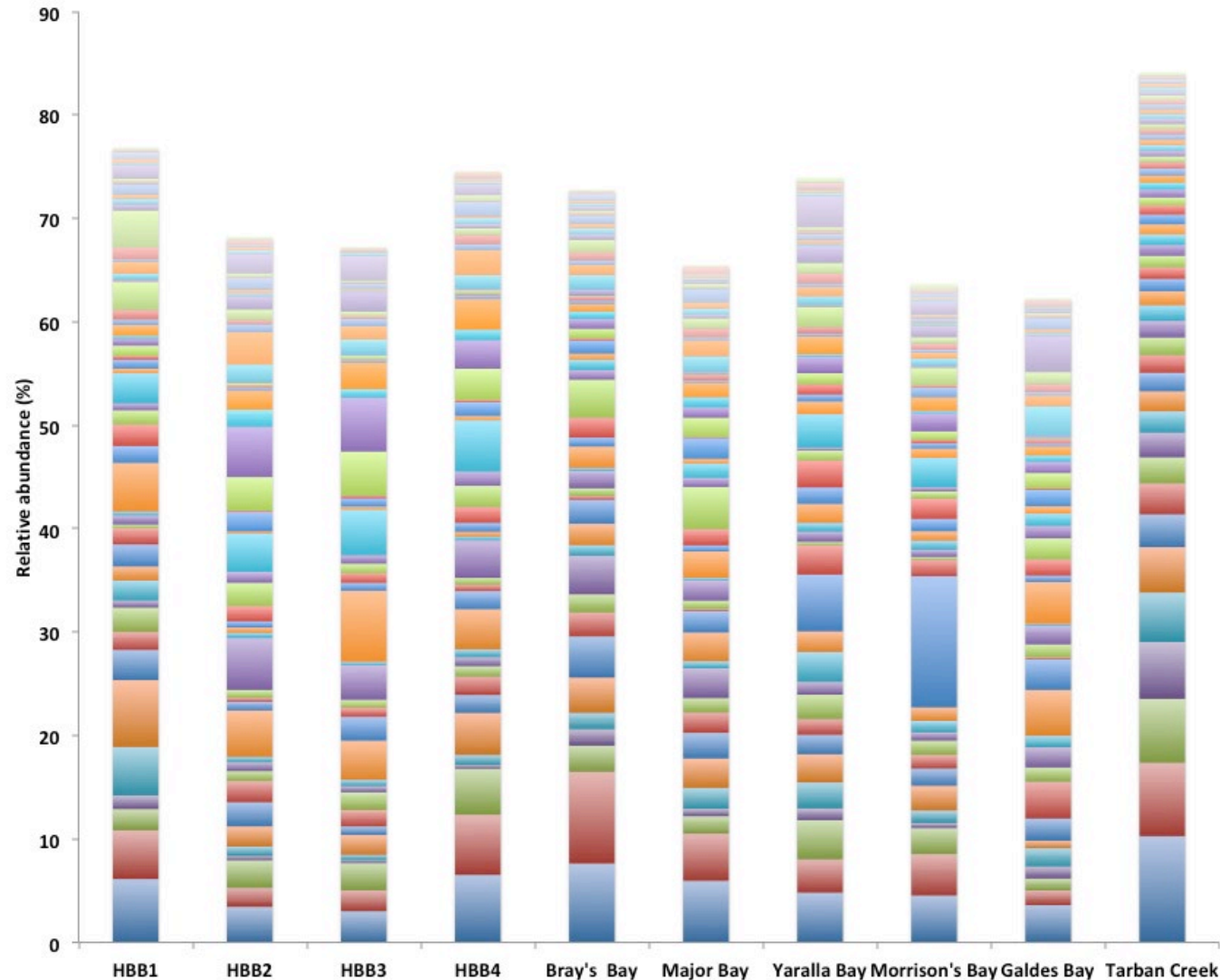




- The sediment samples contained an average of  $5.8 \times 10^8$  bacterial cells per gram.
- DNA profiling: average of  $70,000 \pm 26,000$  sequences representing over 770 genera
- *Dehalococcoides* was only obligate organohalide respiring bacteria, ~ 1% of total microbial population.
- Quantitative PCR of sediment cores for 16S rRNA gene:  $7 \pm 2 \times 10^6$  *Dehalococcoides* cells/gram



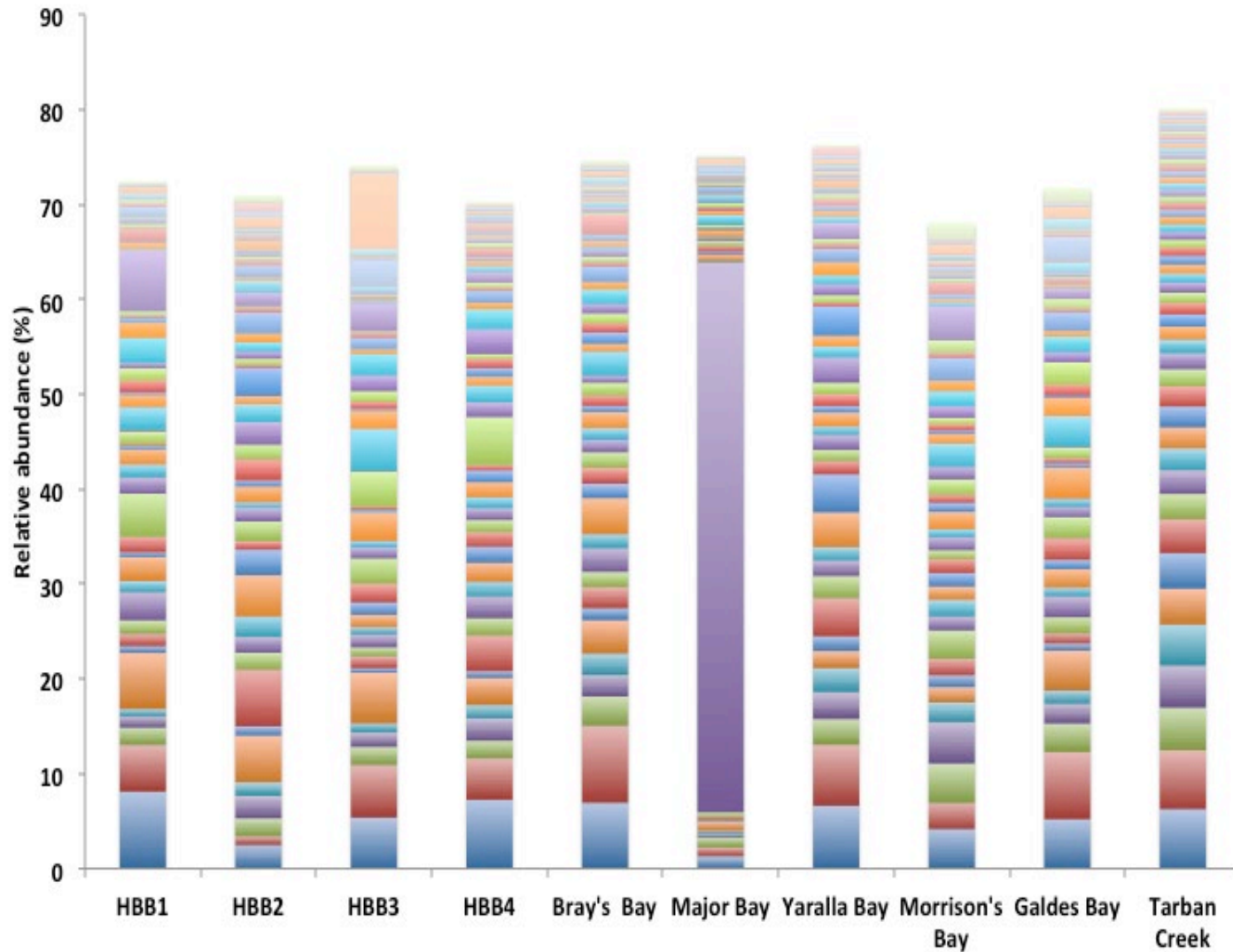
# Bacterial diversity in upper sediment samples



- Cystobacter
- Geobacillus
- Knoellia
- Leptospira
- Thermoanaerobacter
- Desulfobulbus
- Desulfacinum
- Phenylobacterium
- Chondromyces
- Candidatus Contubernalis
- Thermogemmatispora
- Desulfotalea
- Treponema
- Planctomyces
- Desulfomonile
- Thiorhodococcus
- Legionella
- Actinoallomurus
- Chromatium
- Kineosporia
- Candidatus Liberibacter
- Thiorhodospira
- Geobacter
- Desulforhopalus
- Ectothiorhodospira
- Candidatus Tammella
- Caldithrix
- Rickettsiella
- Thiocapsa
- Desulfobacterium
- Thermodesulfobivrio
- Bifidobacterium
- Candidatus Methylocidiph
- Sulfurospirillum
- Ferrimicrobium
- Desulfococcus
- Mycobacterium
- Blautia
- Clostridium
- Desulfosarcina
- Nocardia
- Saccharopolyspora
- Streptosporangium
- Desulfobivrio
- Chlorobaculum
- Hymenobacter
- Actinopolyspora
- Campylobacter
- Desulfobacter
- Anaerolinea
- Bellilinea



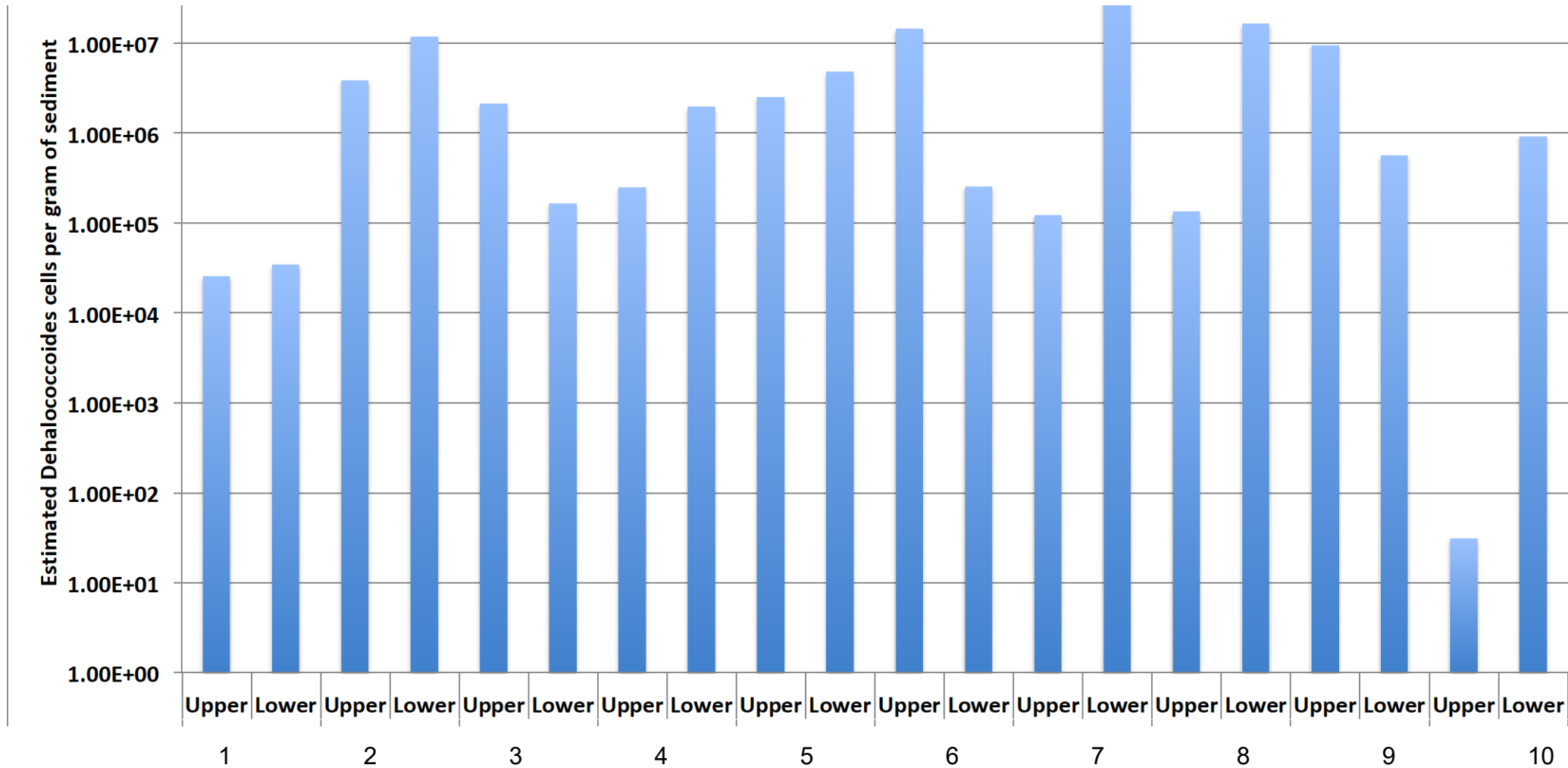
# Bacterial diversity in lower sediment samples



- Bellilinea
- Desulfotalea
- Desulfotomaculum
- Candidatus Contubernalis
- Desulfobulbus
- Caldilinea
- Arthronema
- Leptospira
- Geobacillus
- Blautia
- Solirubrobacter
- Ferrimicrobium
- Desulfacinum
- Rubritalea
- Campylobacter
- Pelobacter
- Thermogemmatispora
- Thermobaculum
- Sulfurospirillum
- Rickettsiella
- Desulfomonile
- Desulforhopalus
- Treponema
- Thiorhodospira
- Legionella
- Candidatus Methylocidiphilum
- Planctomyces
- Nitrosococcus
- Nocardia
- Thiocapsa
- Dehalogenimonas
- Ectothiorhodospira
- Geobacter
- Mycobacterium
- Bifidobacterium
- Chondromyces
- Thiorhodococcus
- Actinopolyspora
- Streptosporangium
- Saccharopolyspora
- Chlorobaculum
- Caldithrix
- Clostridium
- Desulfovibrio
- Thermodesulfovibrio
- Desulfobacterium
- Hymenobacter
- Desulfococcus
- Desulfosarcina
- Desulfobacter
- Anaerolinea



# Dehalococcoides cells per gram of sediment across sample locations





# 03

## What the current microbial consortia can and can't do

Anaerobic enrichment cultures of HBB sediment + TCDD and OCDD

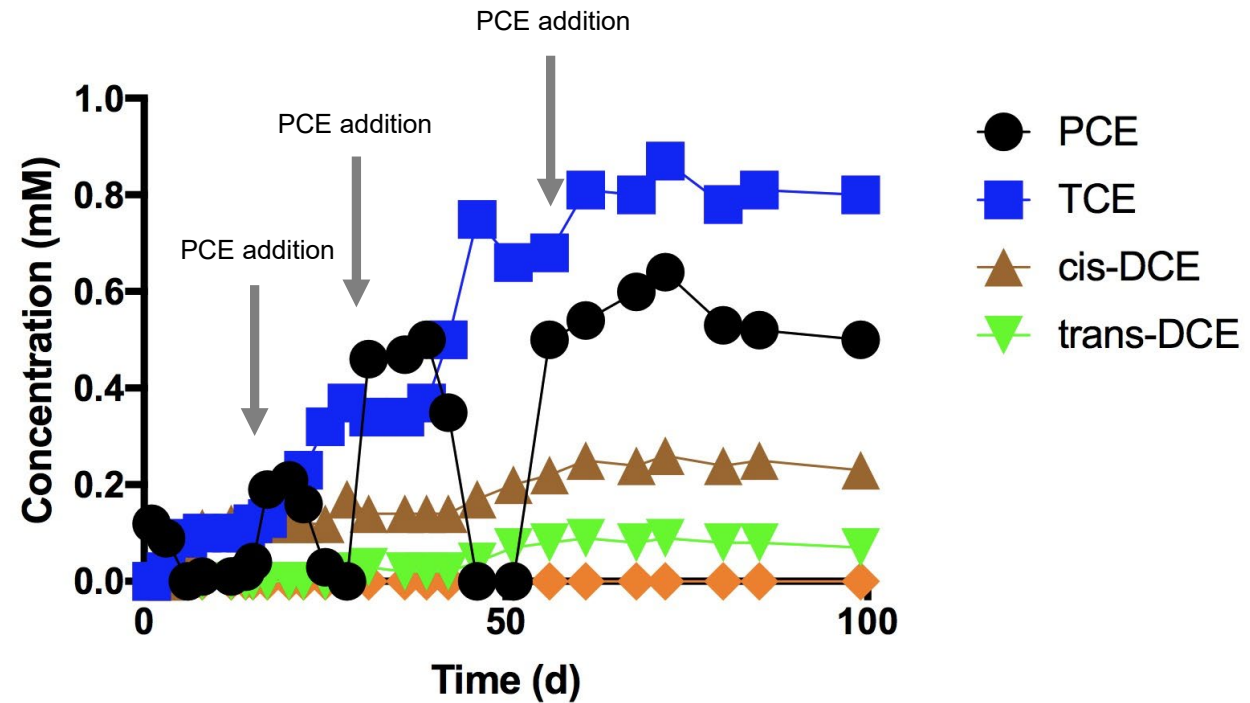
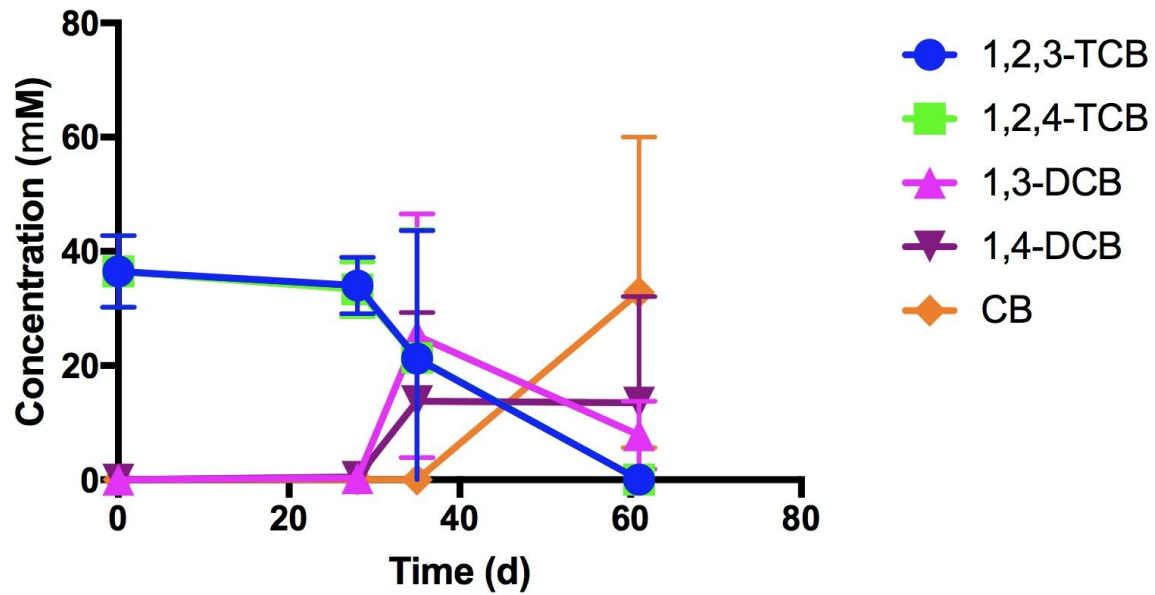
Aerobic cultures + TCDD.

**After 12 months none of the cultures showed dioxin degrading activity**

Anaerobic cultures were also prepared with perchloroethene (PCE) and 1,2,3- and 1,2,4-trichlorobenzene (TCBs).

TCBs were dechlorinated to chlorobenzene (CB), and PCE was dechlorinated to cis- and trans-dichloroethene (DCE)

# Transformation of 1,2,3- and 1,2,4-TCB to monochlorobenzene (CB) or PCE to cis- and trans-DCE with *in situ* (HBB) microorganisms



# What the current microbial consortia can and can't do



Microbial community analysis (Illumina 16S rRNA sequencing) revealed the enrichment of *Dehalococcoides* with TCBs and *Desulfovibrio* with PCE.

*Desulfovibrio* has been shown previously to transform PCE to DCE.

Genus	PCE Enrichment		TCB Enrichment	
	Day 0	Day 90	Day 0	Day 120
<i>Dehalococcoidaceae</i>	5.86	0.00	0.00	<u>10.9</u>
<i>Thermacetogenium</i>	0.00	0.00	0.00	10.7
<i>Thermovirgaceae</i>	0.00	0.00	0.00	7.76
<i>Desulfovibrio</i>	0.00	<u>43.4</u>	0.00	7.13
<i>Christensenellaceae (family)</i>	0.00	21.3	0.00	3.78
<i>Stenotrophomonas</i>	0.00	0.00	0.00	3.35
<i>Ruminococcaceae(family)</i>	0.00	0.00	0.00	3.14
<i>Candidatus Koribacter</i>	0.00	0.00	1.68	0.00
<i>Anaerolinaceae (family)</i>	0.00	0.00	1.47	0.00
<i>Gracilibacteraceae (family)</i>	0.00	0.00	8.00	0.00
<i>Rhodobacteraceae</i>	0.00	6.5	0.00	0.00
<i>Desulfocapsa</i>	3.35	0.00	0.00	0.00
<i>Helicobacteraceae</i>	7.74	7.52	13.7	0.00
<i>Marinobacter</i>	40.2	0.00	0.00	0.00
<i>Enterobacteriaceae (family)</i>	0.00	0.00	7.79	0.00
<i>Amphritea</i>	21.3	0.00	0.00	0.00
<i>Mariprofundus</i>	1.88	0.00	0.00	0.00
<i>Unclassified bacteria</i>	19.6	21.3	67.3	53.2



# 04

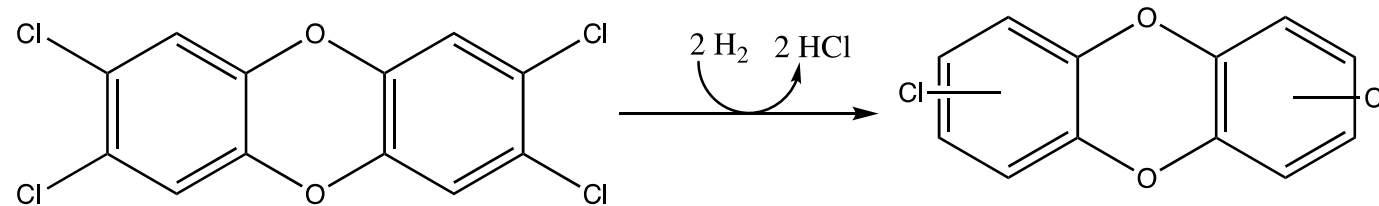
## Testing a candidate: *Dehalococcoides mccartyi* CBDB1



- 10 ml of Anaerobic medium was amended with Tarban Creek (0.1 g) sediment spiked with TCDD and OCDD (50 ppm)
- Quadruplicate flasks were inoculated with 1ml of either *Dehalococcoides mccartyi* strain CBDB1 or HBB/TCB enrichment culture and supplied hydrogen as the electron donor.
- Analysis by GC-TQMS after 1 month revealed that neither CBDB1 or HBB enrichment cultures had dechlorinated OCDD.
- However, CBDB1 cultures had dechlorinated TCDD ( $56 \pm 22$  ppm) to 2,3,7-TriCDD ( $2.4 \pm 1.2$  ppm) and DiCDD ( $3.1 \pm 1.4$  ppm).



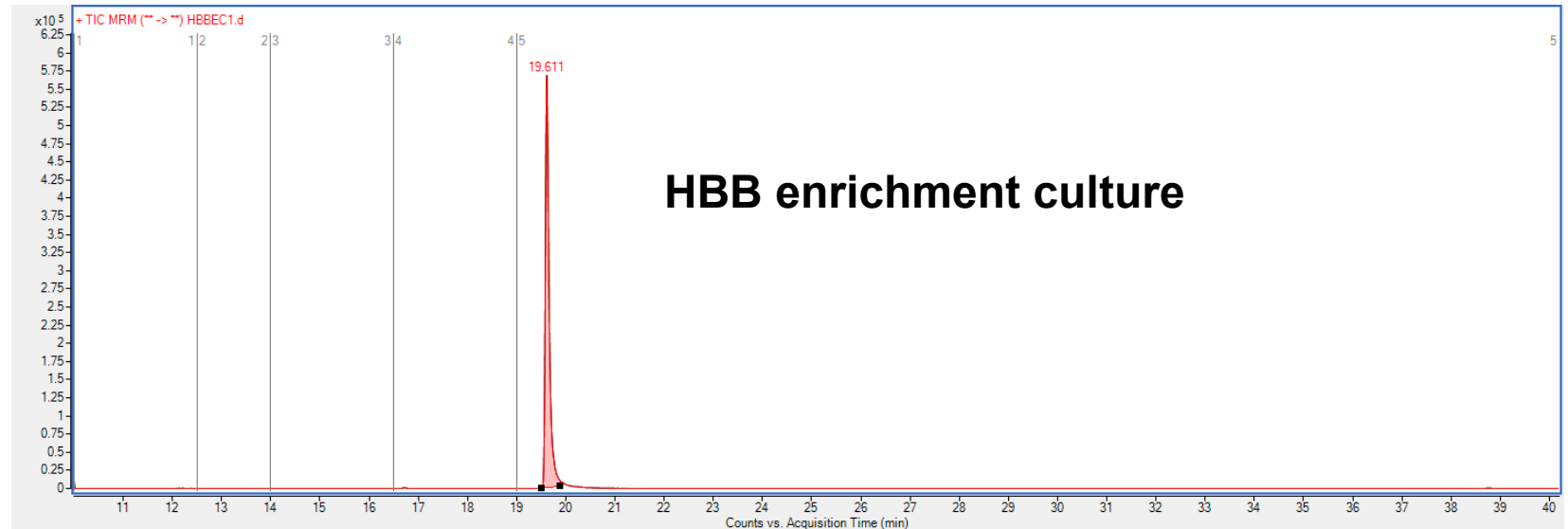
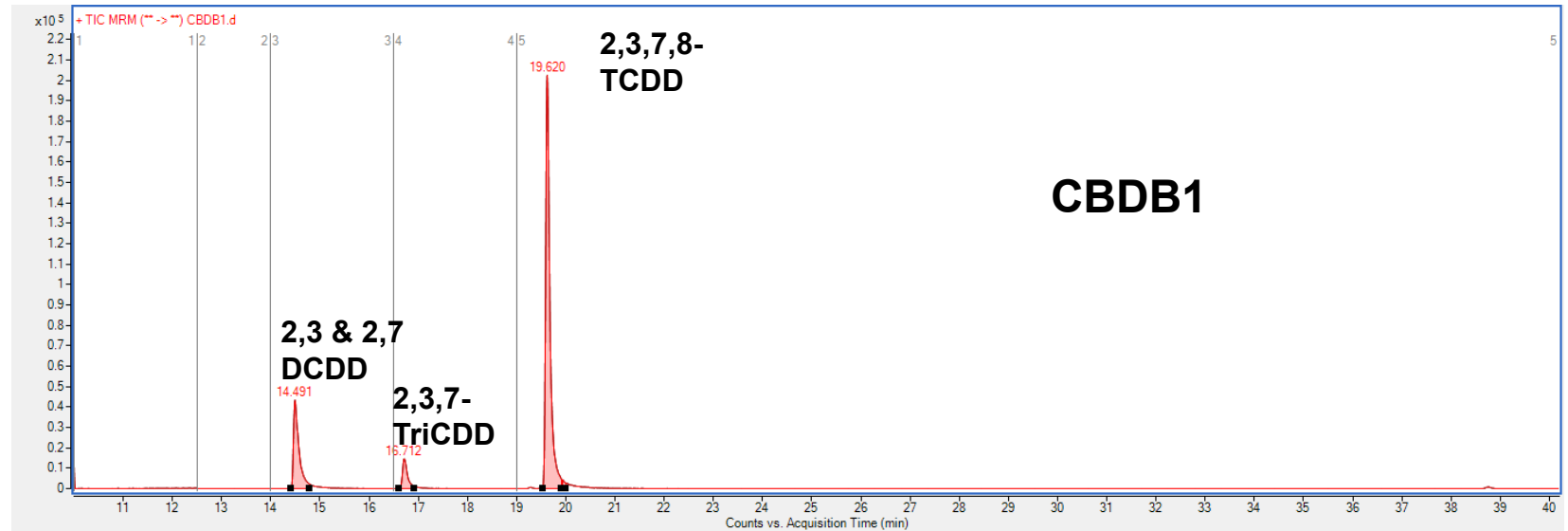
# Dehalococcoides CBDB1 can transform TCDD to DCDD



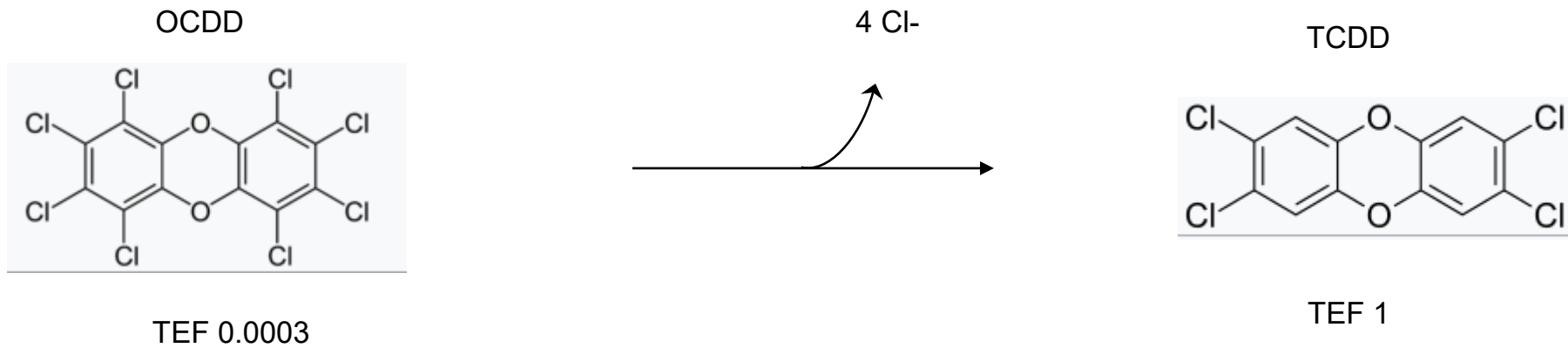
Bunge et al., 2003 Nature 421 (23) 357

GC-TQMS:

CBDB1 production of di- and tri- dibenzo dioxin from 2,3,7,8-TCDD



OCDD degradation to TCDD could make problem much worse



**➔** BAD ☹️



# 05 Ongoing studies



- Will CBDB1 work in a marine environment (i.e high salinity etc).
- Are there *in situ* bacteria that can dechlorinate 2,3 and 2,7-DiCDD.
- Does S-nZVI react with DiCDDs
- Can S-nZVI be used in place of titanium (III) citrate as the redox reductant, and as an *in situ* source of hydrogen.
- Can HBB enrichment cultures dechlorinate 2,4,5-T and 2,4-D?