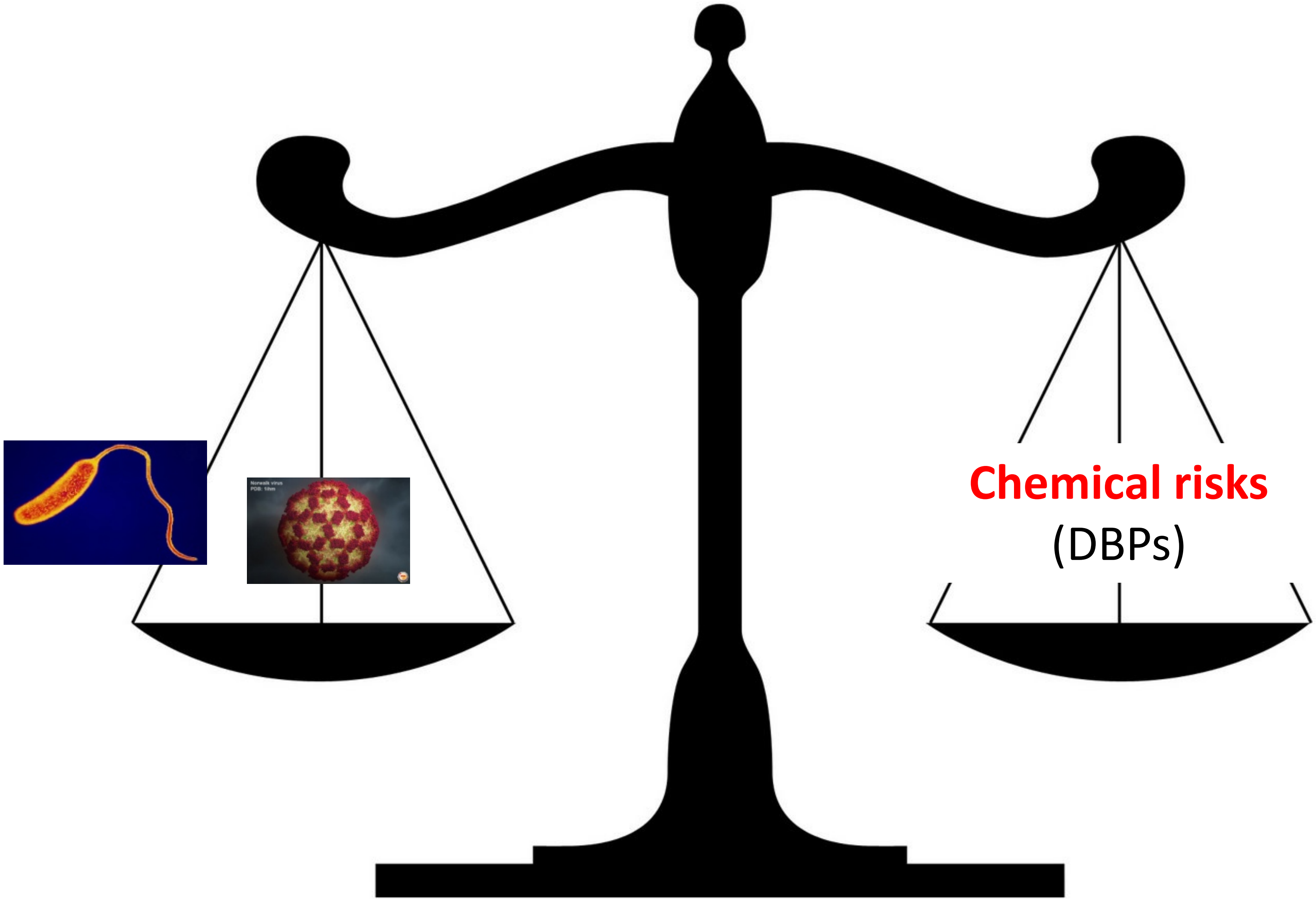
POOL WATER TREATMENT ADVISORY GROUP

Disinfection by-product exposure and health risks among swimmers

Cristina Villanueva, ISGlobal, Barcelona

13 November 2019

ISGlobal Barcelona Institute for Global Health


Chemical risks
(DBPs)

More than 100 DBPs identified in 2 swimming pools in Barcelona

Haloalkanes
- *Chloroform*[a]
- *Bromodichloromethane*
- *Dibromochloromethane*
- *Bromoform*
- Dibromomethane
- Bromotrichloromethane
- Dibromodichloromethane
- 1,1,2-Trichloroethane

Haloacetic acids
- *Chloroacetic acid*
- *Bromoacetic acid*
- *Dichloroacetic acid*
- *Bromochloroacetic acid*
- *Dibromoacetic acid*
- *Trichloroacetic acid*
- *Bromodichloroacetic acid*
- *Dibromochloroacetic acid*
- *Tribromoacetic acid*

Other haloacids
- 3-Bromopropenoic acid
- *2,2-Dichloropropanoic acid*
- *3,3-Dichloropropenoic acid*
- cis-*2,3-Bromochloropropenoic acid*
- trans-*2,3-Bromochloropropenoic acid*
- *2,3-Dibromopropanoic acid*
- cis-*2,3-Dibromopropenoic acid*
- *trans*-2,3-Dibromopropenoic acid
- 3,3-Dibromopropenoic acid
- *Trichloropropenoic acid*
- *2-Bromo-3,3-dichloropropenoic acid*
- *(E)-3-Bromo-2,3-dichloropropenoic acid*
- *(Z)-3-Bromo-2,3-dichloropropenoic acid*
- 2,2-Dichlorobutanoic acid
- *cis*-Bromobutenoic acid
- *trans*-Bromobutenoic acid
- 2,2-Dichlorobutenoic acid
- 2,3-Dibromobutenoic acid
- *2-Chloro-3-methylbutanoic acid*
- Chlorophenylacetic acid
- 3,5-Dibromobenzoic acid
- *Tribromopropenoic acid*

Halodiacids
- cis-*Bromobutenedioic acid*
- trans-*Bromobutenedioic acid*
- *cis*-Dichlorobutenedioic acid
- *trans*-Dichlorobutenedioic acid
- cis-*Bromochlorobutenedioic acid*
- trans-*Bromochlorobutenedioic acid*
- cis-*Dibromobutenedioic acid*
- *(E)-2-Chloro-3-methylbutenedioic acid*
- (*E*)-2-Bromo-3-methylbutenedioic acid

Haloaldehydes
- *Dichloroacetaldehyde*
- *Bromochloroacetaldehyde*
- *Dibromoacetaldehyde*
- *Trichloroacetaldehyde (chloral hydrate)*
- *Bromodichloroacetaldehyde*
- *Dibromochloroacetaldehyde*
- *Tribromoacetaldehyde*
- 3-Bromo-4-methoxybenzaldehyde

Halonitriles
- *Bromoacetonitrile*
- *Dichloroacetonitrile*
- *Bromochloroacetonitrile*
- *Dibromoacetonitrile*
- *Trichloroacetonitrile*

Haloketones
- *Bromopropanone*
- 1,1-Dichloropropanone
- *1-Bromo-1-chloropropanone*
- *1,1-Dibromopropanone*
- 1,3-Dibromopropanone
- *1,1,1-Trichloropropanone*
- *1,1,3-Trichloropropanone*
- 1-Bromo-1,1-dichloropropanone
- 1,1,1-Tribromopropanone
- *1,1,3,3-Tetrachloropropanone*
- 1,1-Dibromo-3,3-dichloropropanone
- Pentachloropropanone
- Dichlorofurandione
- 1-Chloro-2-butanone
- 1-Bromo-2-butanone
- Tetrachlorohydroquinone

Halonitromethanes
- *Dibromonitromethane*

Haloamides
- *Dichloroacetamide*
- *Bromochloroacetamide*
- *Dibromoacetamide*
- *Bromodichloroacetamide*
- *Dibromochloroacetamide*
- *Tribromoacetamide*

Haloalcohols
- 2,2,2-Trichloroethanol
- 1,1,1-Trichloropropanol

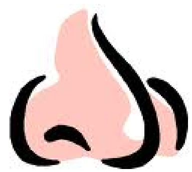
Other halogenated DBPs
- 3-Chlorobenzeneacetonitrile
- 2,6-Dichloro-4-methylphenol
- 2-Bromo-4-chlorophenol
- Trichlorophenol
- Bromodichlorophenol
- Tribromophenol
- 2-Bromo-4-chloro-6-methylphenol
- Dibromomethylphenol
- 2,4-Dibromo-1-methoxybenzene
- 2,3,4-Trichlorobenzeneamine
- Dibromochloroaniline
- 2-Bromo-4-chloroanisole
- 3,4,5-Tribromo-*1H*-pyrazole
- 2,6-Dibromo-4-nitrophenol
- 2,6-Dibromo-4-nitrobenzeneamine

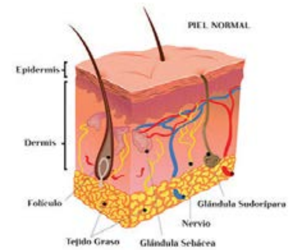
Nonhalogenated DBPs/contaminants
- Propionamide
- *Benzaldehyde*
- *Benzoic acid methyl ester*
- Benzeneacetonitrile
- *Phthalic acid*
- *Diethylphthalate*
- *Benzophenone*

Richardson et al. EHP 2010

Complex exposure scenario

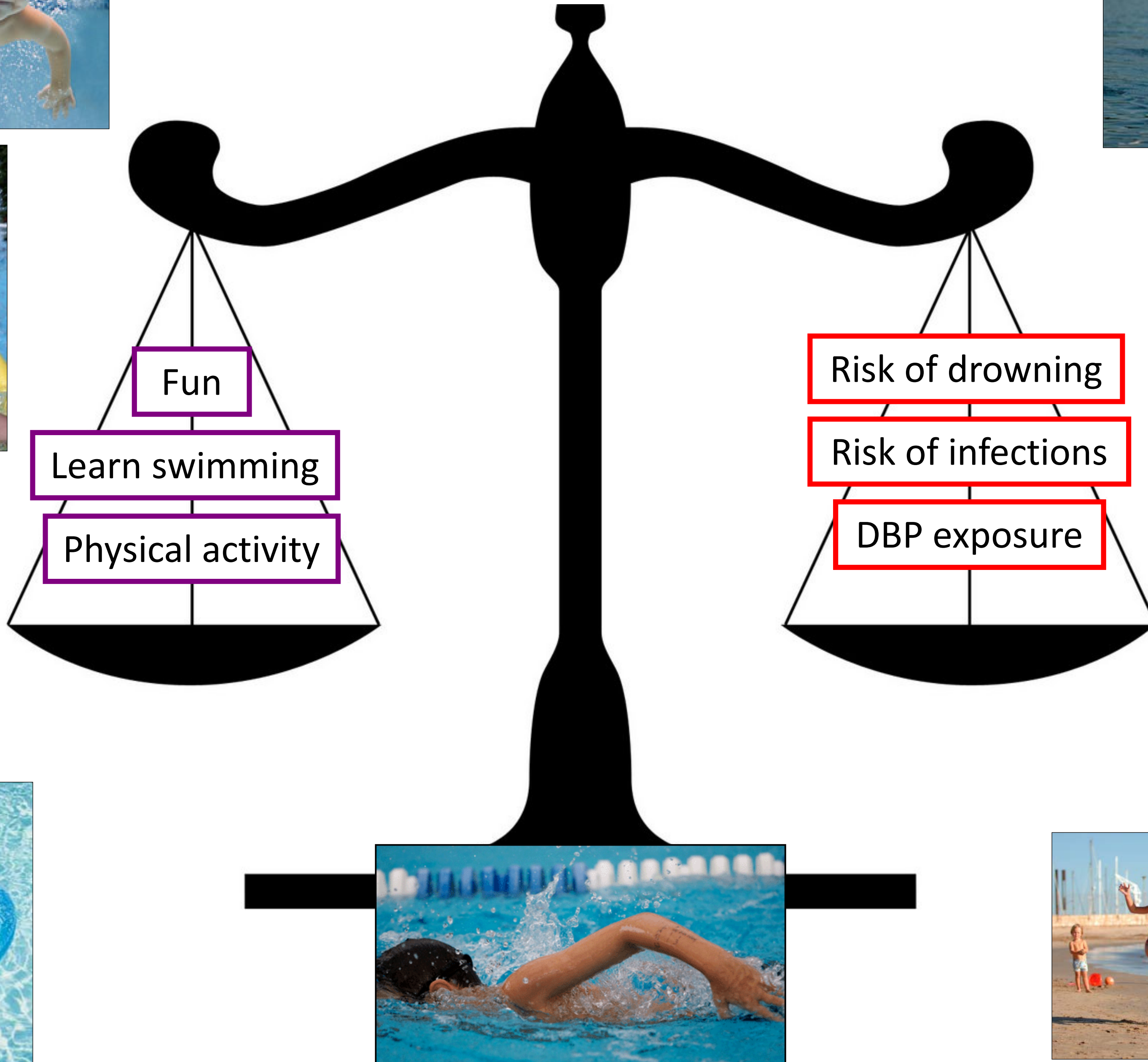
- Complex mixture (>700 substances)
- Heterogeneous physico-chemical properties (volatility/permeability)
- Multiple exposure pathways:
  - Inhalation
  - Skin absorption
  - (Ingestion)





TOTAL
INTERNAL DOSE

Swimming in pools



Professional swimmers

Pool workers

General population (recreational swimming)

Asthma diagnosis among elite swimmers vs. other elite athletes Meta analysis: **2.57 (1.87-3.54)**

Goodman & Hayes 2008, J Asthma

The prevalence of airway dysfunction in elite swimmers is among the highest in elite athletes.

Airway dysfunction…

- Does not prevent success in elite level swimming.
- Neither does it inhibit lung growth
- Might be partially reversible when elite swimmers retire from competition

Lomax 2016, Open Access J Sports Med

Indoor swimming pool environments and self-reported irritative and respiratory symptoms among lifeguards

Bureau 2017, Int J Environ Health Res

Symptoms, previous 12 months	Length of occupational exposure, previous 12 months (h)	Adjusted OR[a]	95 % CI
Cough[b–e]	0	1.00	
	1–500 vs. 0	0.97	0.60–1.55
	>500 vs. 0	2.54	1.51–4.25
Sputum[b–e]	0	1.00	
	1–500 vs. 0	1.51	0.84–2.73
	>500 vs. 0	1.82	0.96–3.45
Lung congestion[c,d]	0	1.00	
	1–500 vs. 0	0.84	0.34–2.07
	>500 vs. 0	1.51	0.62–3.68
Sneezing[c,e,f]	0	1.00	
	1–500 vs. 0	0.77	0.50–1.18
	>500 vs. 0	1.00	0.62–1.62
Throat irritation[c,g,i]	0	1.00	
	1–500 vs. 0	1.62	1.00–2.63
	>500 vs. 0	2.47	1.44–4.24
Eye irritation[c,h,i]	0	1.00	
	1–500 vs. 0	2.02	1.27–3.21
	>500 vs. 0	4.34	2.52–7.50
Hoarseness[c]	0	1.00	
	1–500 vs. 0	1.09	0.65–1.83
	>500 vs. 0	1.79	1.04–3.08
≥1 URT symptoms[c,g,h]	0	1.00	
	1–500 vs. 0	0.83	0.49–1.40
	>500 vs. 0	1.52	0.77–2.99
≥1 LRT symptoms[c–e,g]	0	1.00	
	1–500 vs. 0	0.81	0.52–1.27
	>500 vs. 0	1.62	0.96–2.72
≥1 Respiratory symptoms[c,e,g,h]	0	1.00	
	1–500 vs. 0	0.90	0.52–1.55
	>500 vs. 0	1.62	0.80–3.30

General population (recreational swimming)

Pregnant women

Babies

Children

Adults

Is swimming during pregnancy a safe exercise?

Juhl 2010, Epidemiology

	Swimming Measure of Association[b] (95% CI)	
Preterm birth (HR)[c]	0.80	(0.72 to 0.88)
Postterm birth (OR)	0.97	(0.90 to 1.04)
SGA (HR)[c]	0.97	(0.90 to 1.04)
Birth weight (g)[d]	7	(−3 to 16)
Length (cm)[d]	0.02	(−0.03 to 0.06)
Ponderal index ((g*100)/cm^3)[d]	0.002	(−0.004 to 0.008)
Head circumference (cm)[d]	−0.03	(−0.06 to 0.007)
Abdominal circumference (cm)[d]	0.02	(−0.02 to 0.07)
Placental weight (g)[d]	−1	(−4 to 2)
Congenital malformations, any (OR)	0.89	(0.80 to 0.98)
Circulatory system (OR)	1.01	(0.82 to 1.25)
Respiratory system (OR)	0.59	(0.29 to 1.17)
Cleft lip/palate (OR)	0.63	(0.35 to 1.13)

Baby swimming

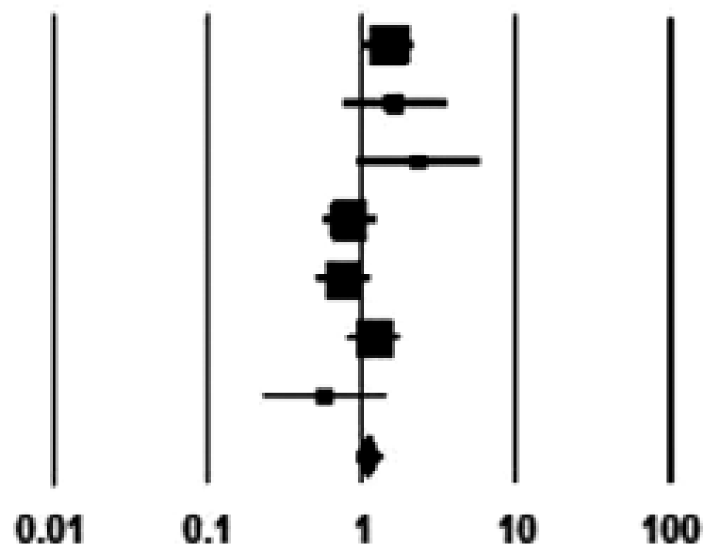
Study	Outcomes	Findings
Nyastad 2003	Recurrent respiratory tract infections, Otitis (<1 yr)	+ (if atopic parents)
Nyastad 2008	LRTI, wheeze, otitis (6-18 months)	+ (if atopic parents)
Bernard 2007	Lung damage biomarkers Asthma, bronchitis (10-13 yrs)	+
Schoefer 2008	Diarrhea, otitis, airway infections (<1 yr) Asthma (6 yrs)	+
Voisin 2010	Bronchiolitis (< 2 yrs)	+
Font-Ribera 2013	LRTI, wheezing, persistent cough, atopic eczema, otitis (1 yr)	-

Swimming attendance during childhood and development of asthma: Meta-analysis

Valeriani 2017 Pediatrics Int.

Study	OR	Lower limit	Upper limit	*Z*-value	*P*-value
Andersson (2015)[45]	1.490	1.019	2.178	2,058	0.040
Bernard (2006)[47]	1.630	0.729	3.645	1.190	0.234
Bernard (2007)[48]	2.300	0.906	5.839	1.752	0.080
Font-Ribera (2011)[44]	0.830	0.551	1.251	−0.890	0.374
Kohlhammer (2006)[42]	0.760	0.499	1.158	−1.278	0.201
Lévesque (2006)[46]	1.200	0.800	1.800	0.881	0.378
Schoefer (2008)[43]	0.579	0.230	1.454	−1.163	0.245
	1.084	0.89	1.31	0.841	0.401

OR and 95% CI



Evidence of animal carcinogenicity

THM	Chloroform	Sufficient
	Bromodichloromethane	Sufficient
	Dibromochloromethane	Limited
	Bromoform	Limited
HAA	Dichloroacetic acid	Sufficient
	Trichloroacetic acid	Limited
other	MX	Limited

Swimming in pools and adult cancer?

Bladder

+ Villanueva CM et al 2007
- Beane-Freeman et al. 2017

Melanoma

+ Nelemans et al. 1994

Breast ???

Physical activity is protective

Colorectal ???

Studies of biomarkers in adults

PISCINA - ***Projecte d'Investigació sobre Compostos Irritants i Natació***

PISCINA 1 – 2007
N=50

PISCINA 2 – 2013
N=116

Objectives

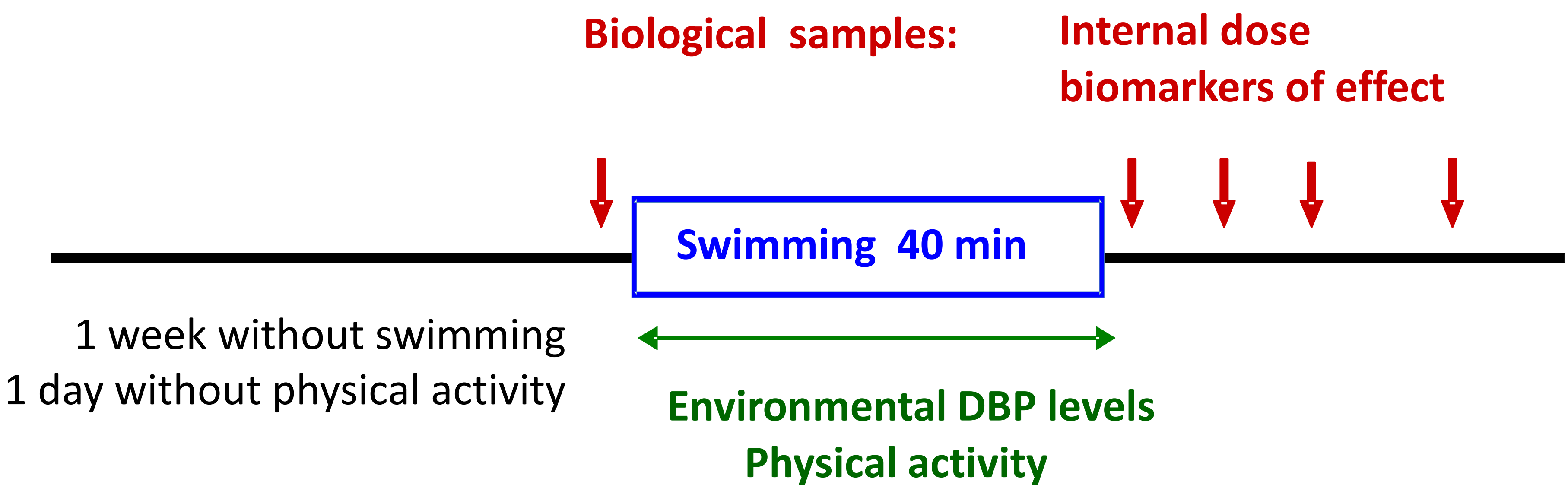
- Evaluate DBP exposure in adult volunteers swimming in a pool

- Evaluate the association with biomarkers of:

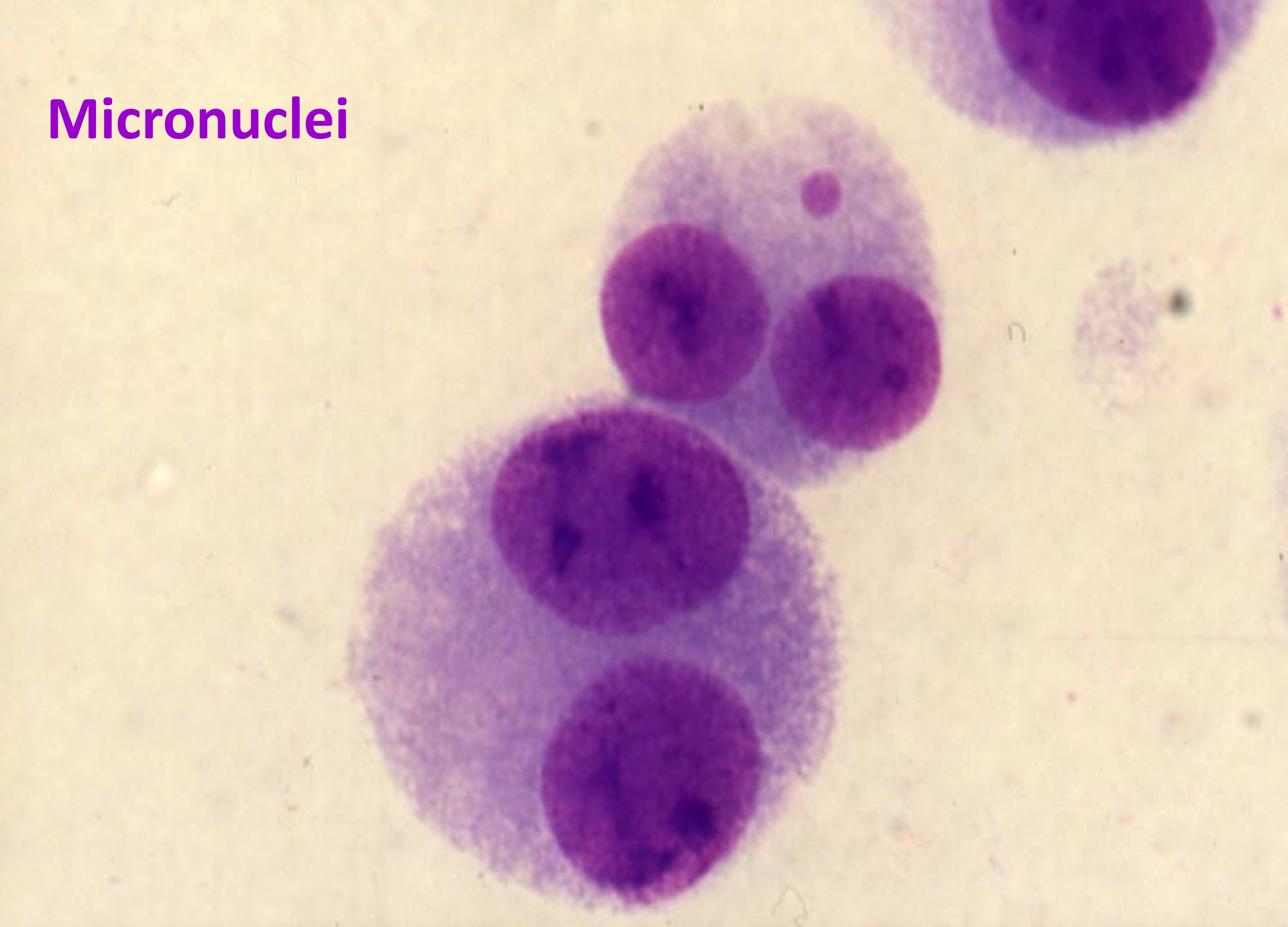
- **Genetic damage (Micronuclei, mutagenicity)**
- **Respiratory damage (Serum CC16)**
- **Omic profiles**

Study design

- Participants: 18 to 40 years old, non-smokers, non professional swimmers.

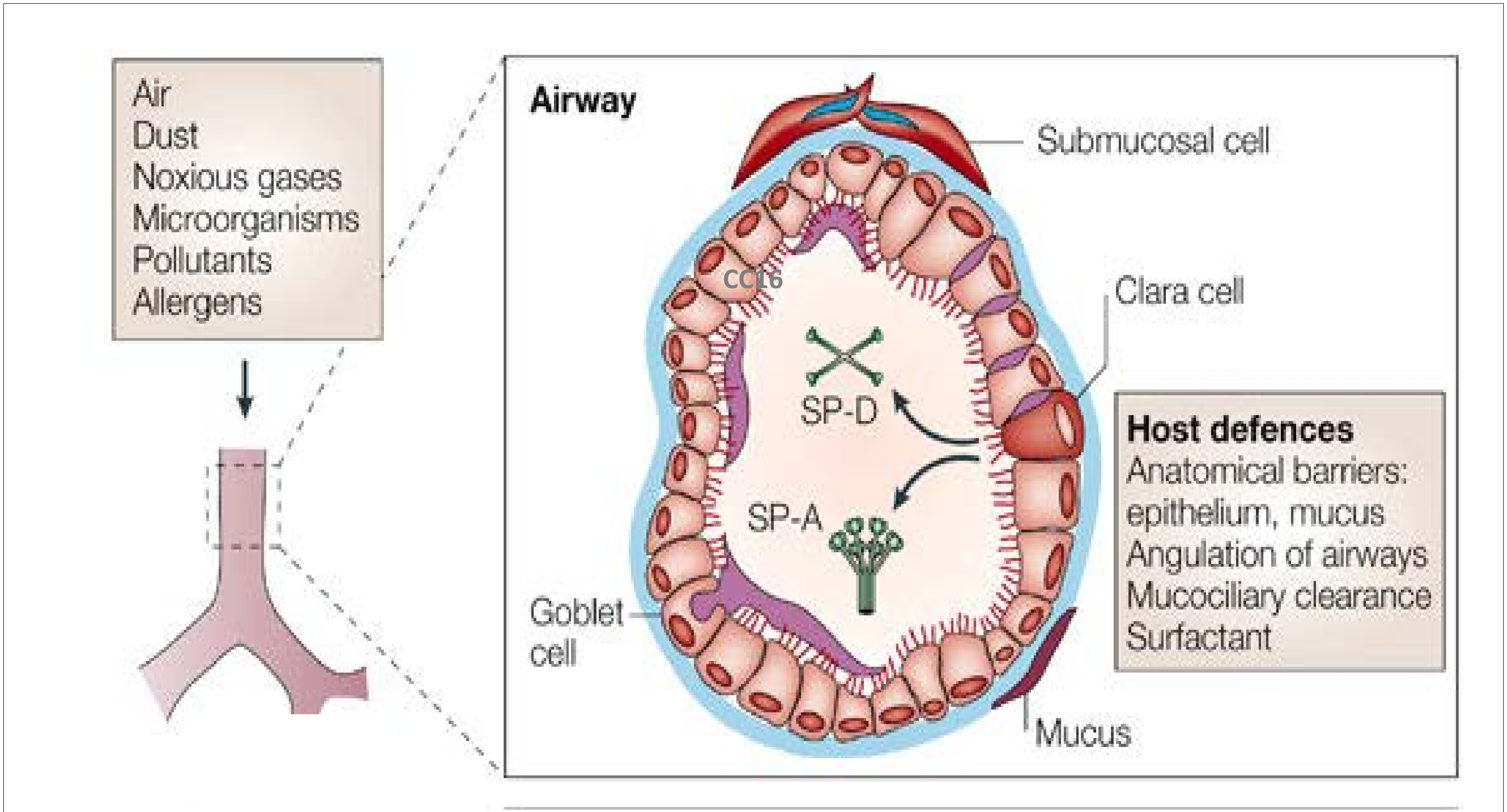


Micronuclei

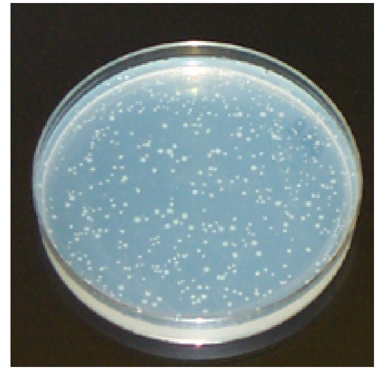


Serum CC16

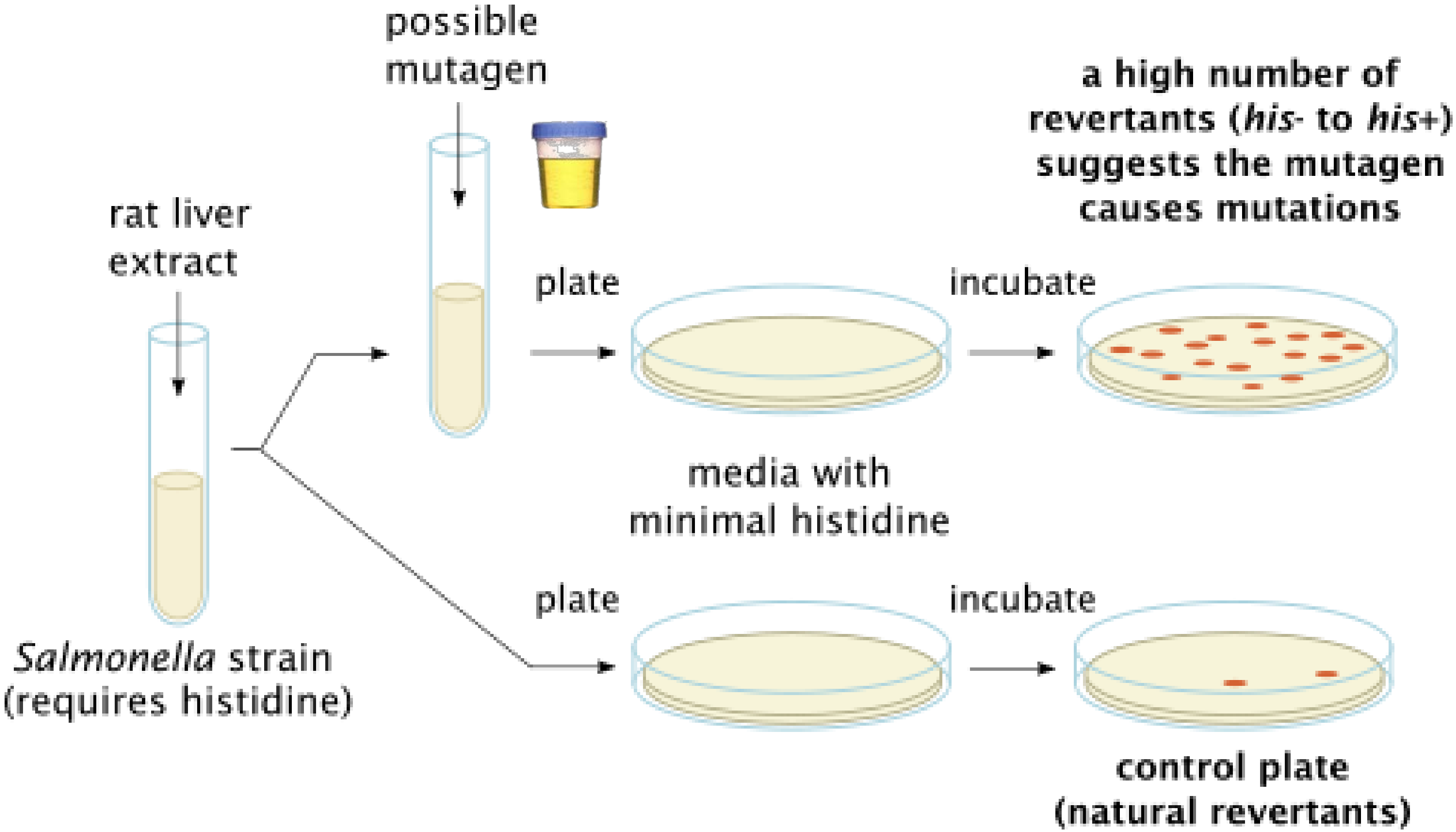
- Lung surfactant protein, secreted by club cells *(Clara)*
- Blood levels indicate lung epithelium damage



Urine mutagenicity



In vitro assay (*Salmonella* YG 1024 + S9) – Ames test



Physical activity

40 minutes at a **<u>relaxed</u>** pace

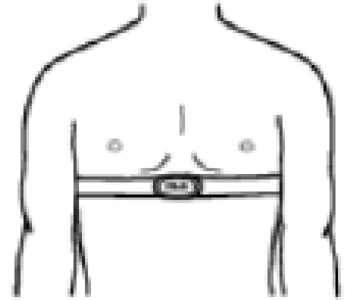
1 technician per participant

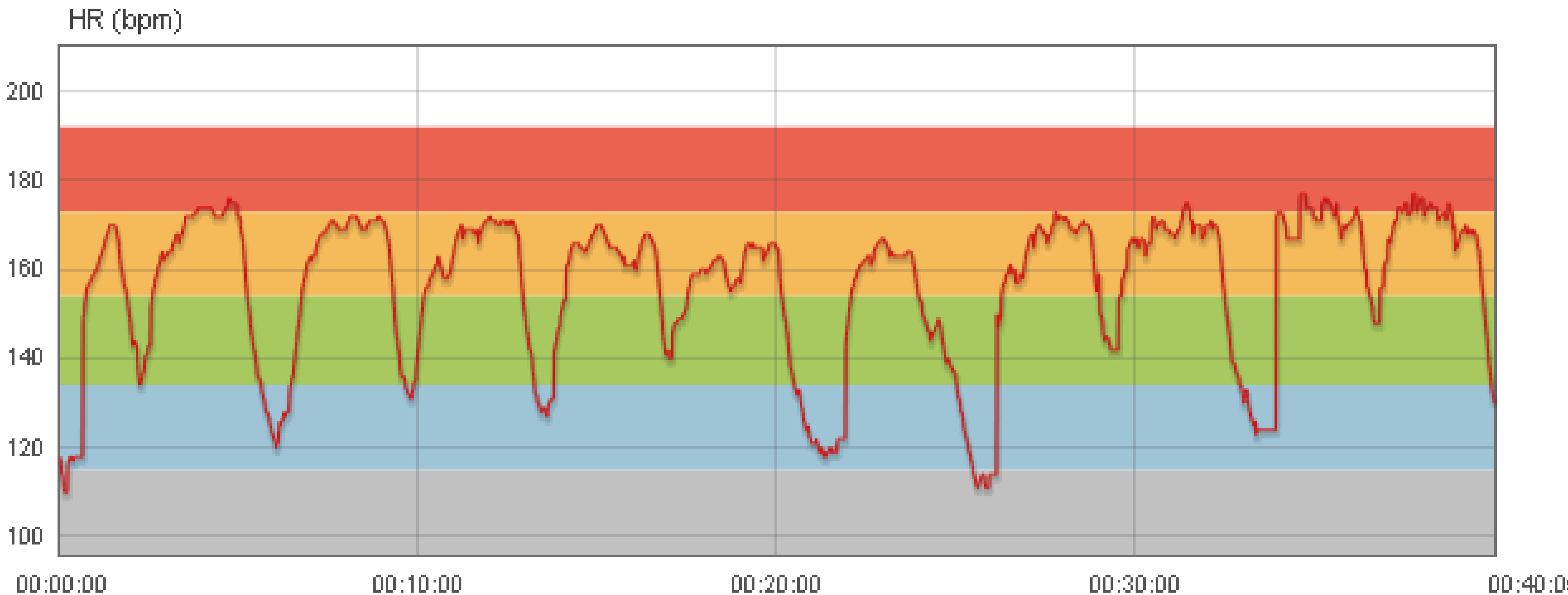
- **Distance** (number of pools)
- **Active time** (direct count)

Physical activity

Heart rate (pulsometer)

- Low (<50% maximum HR)
- Moderate (50-69%)
- Hard (>69%)





Results

More than 100 DBPs identified in 2 swimming pools in Barcelona

Haloalkanes
- Chloroform[a]
- Bromodichloromethane
- Dibromochloromethane
- Bromoform
- Dibromomethane
- Bromotrichloromethane
- Dibromodichloromethane
- 1,1,2-Trichloroethane

Haloacetic acids
- Chloroacetic acid
- Bromoacetic acid
- Dichloroacetic acid
- Bromochloroacetic acid
- Dibromoacetic acid
- Trichloroacetic acid
- Bromodichloroacetic acid
- Dibromochloroacetic acid
- Tribromoacetic acid

Other haloacids
- 3-Bromopropenoic acid
- 2,2-Dichloropropanoic acid
- 3,3-Dichloropropenoic acid
- cis-2,3-Bromochloropropenoic acid
- trans-2,3-Bromochloropropenoic acid
- 2,3-Dibromopropanoic acid
- cis-2,3-Dibromopropenoic acid
- trans-2,3-Dibromopropenoic acid
- 3,3-Dibromopropenoic acid
- Trichloropropenoic acid
- 2-Bromo-3,3-dichloropropenoic acid
- (E)-3-Bromo-2,3-dichloropropenoic acid
- (Z)-3-Bromo-2,3-dichloropropenoic acid
- 2,2-Dichlorobutanoic acid
- cis-Bromobutenoic acid
- trans-Bromobutenoic acid
- 2,2-Dichlorobutenoic acid
- 2,3-Dibromobutenoic acid
- 2-Chloro-3-methylbutanoic acid
- Chlorophenylacetic acid
- 3,5-Dibromobenzoic acid
- Tribromopropenoic acid

Halodiacids
- cis-Bromobutenedioic acid
- trans-Bromobutenedioic acid
- cis-Dichlorobutenedioic acid
- trans-Dichlorobutenedioic acid
- cis-Bromochlorobutenedioic acid
- trans-Bromochlorobutenedioic acid
- cis-Dibromobutenedioic acid
- (E)-2-Chloro-3-methylbutenedioic acid
- (E)-2-Bromo-3-methylbutenedioic acid

Haloaldehydes
- Dichloroacetaldehyde
- Bromochloroacetaldehyde
- Dibromoacetaldehyde
- Trichloroacetaldehyde (chloral hydrate)
- Bromodichloroacetaldehyde
- Dibromochloroacetaldehyde
- Tribromoacetaldehyde
- 3-Bromo-4-methoxybenzaldehyde

Halonitriles
- Bromoacetonitrile
- Dichloroacetonitrile
- Bromochloroacetonitrile
- Dibromoacetonitrile
- Trichloroacetonitrile

Haloketones
- Bromopropanone
- 1,1-Dichloropropanone
- 1-Bromo-1-chloropropanone
- 1,1-Dibromopropanone
- 1,3-Dibromopropanone
- 1,1,1-Trichloropropanone
- 1,1,3-Trichloropropanone
- 1-Bromo-1,1-dichloropropanone
- 1,1,1-Tribromopropanone
- 1,1,3,3-Tetrachloropropanone
- 1,1-Dibromo-3,3-dichloropropanone
- Pentachloropropanone
- Dichlorofurandione
- 1-Chloro-2-butanone
- 1-Bromo-2-butanone
- Tetrachlorohydroquinone

Halonitromethanes
- Dibromonitromethane

Haloamides
- Dichloroacetamide
- Bromochloroacetamide
- Dibromoacetamide
- Bromodichloroacetamide
- Dibromochloroacetamide
- Tribromoacetamide

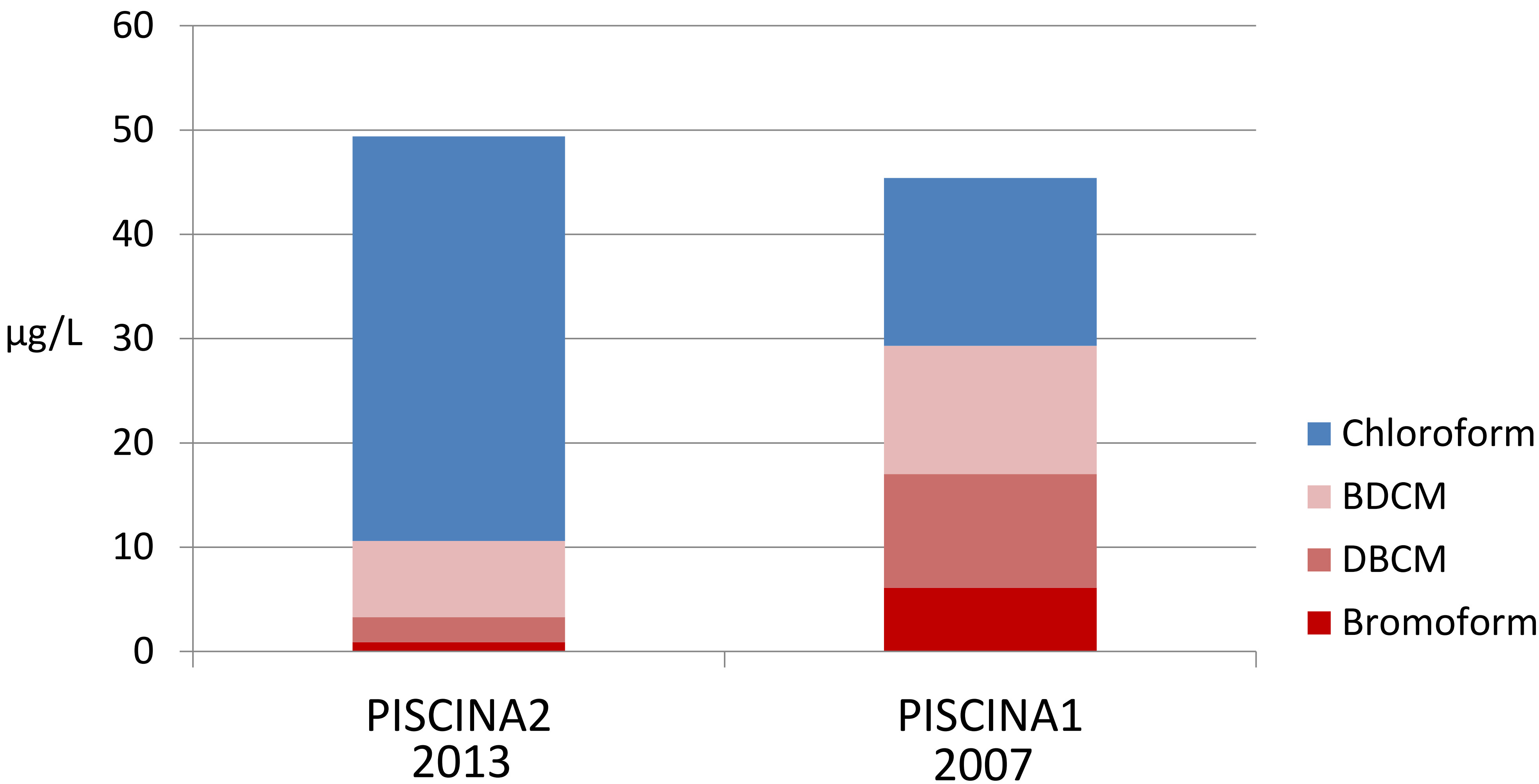
Haloalcohols
- 2,2,2-Trichloroethanol
- 1,1,1-Trichloropropanol

Other halogenated DBPs
- 3-Chlorobenzeneacetonitrile
- 2,6-Dichloro-4-methylphenol
- 2-Bromo-4-chlorophenol
- Trichlorophenol
- Bromodichlorophenol
- Tribromophenol
- 2-Bromo-4-chloro-6-methylphenol
- Dibromomethylphenol
- 2,4-Dibromo-1-methoxybenzene
- 2,3,4-Trichlorobenzeneamine
- Dibromochloroaniline
- 2-Bromo-4-chloroanisole
- 3,4,5-Tribromo-1H-pyrazole
- 2,6-Dibromo-4-nitrophenol
- 2,6-Dibromo-4-nitrobenzeneamine

Nonhalogenated DBPs/contaminants
- Propionamide
- Benzaldehyde
- Benzoic acid methyl ester
- Benzeneacetonitrile
- Phthalic acid
- Diethylphthalate
- Benzophenone

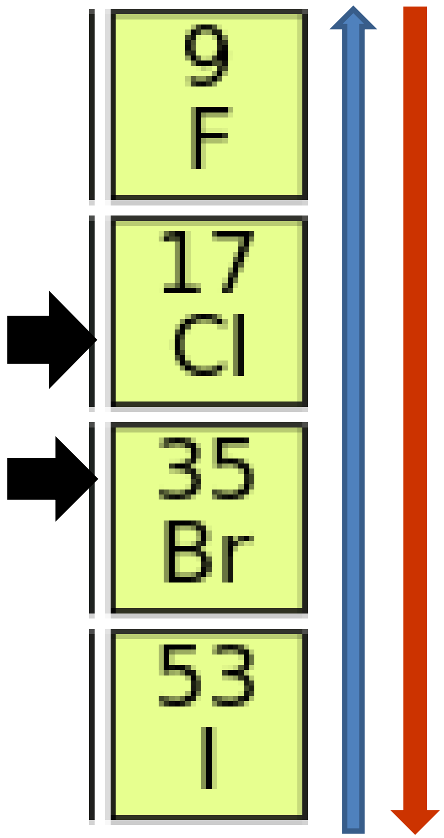
Richardson et al. EHP 2010

Levels of THMs in the pool water



Trihalomethanes = Tri + halo + methane

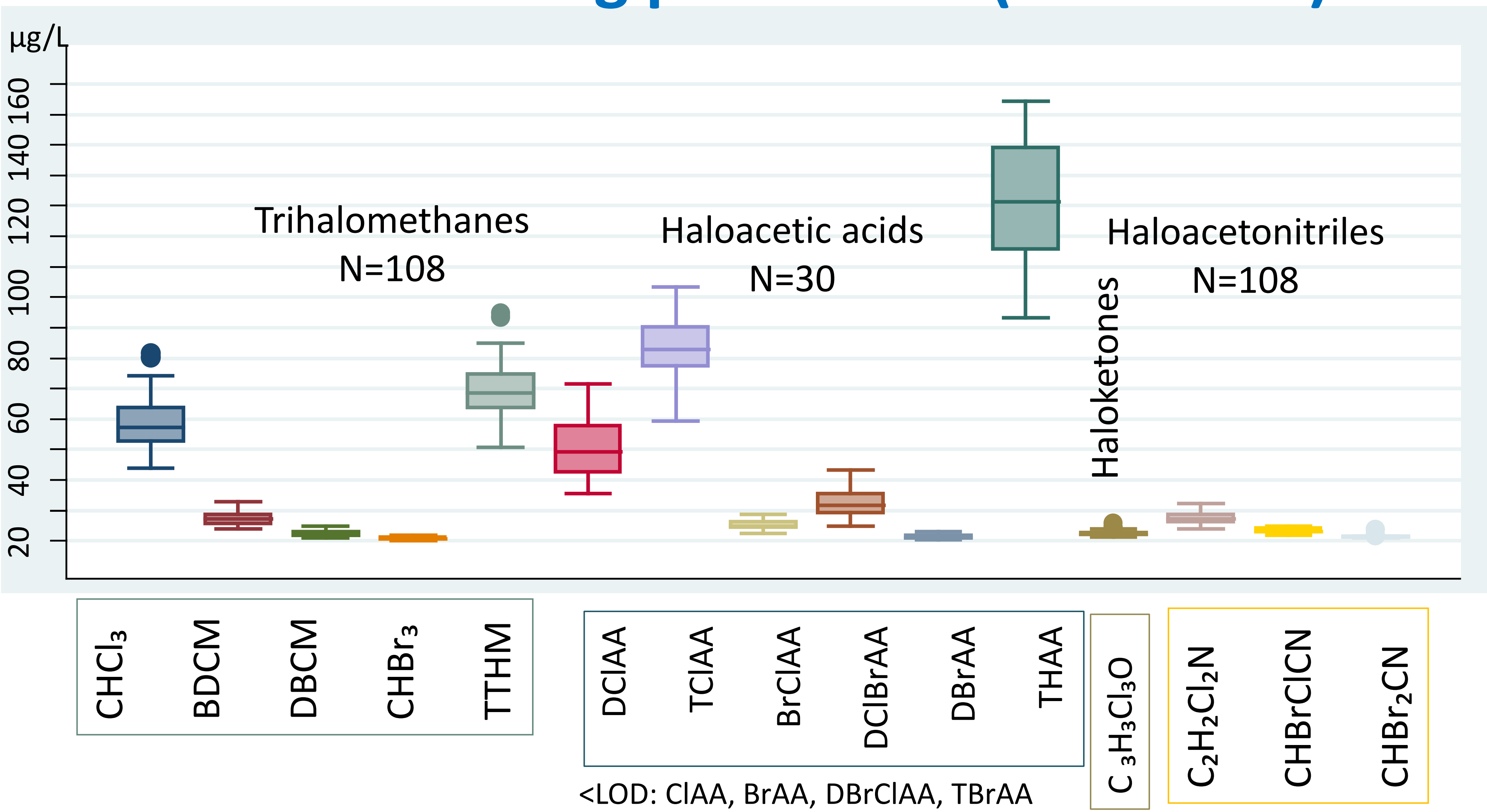
Halogens



- Disinfectant capacity
- Volatility

- Density
- Lipophilicity, skin permeability
- Genotoxicity

DBPs in swimming pool water (PISCINA 2)



<LOD: CCl3NO₂, 11DCPAONE

<LOD: CCl_3CN

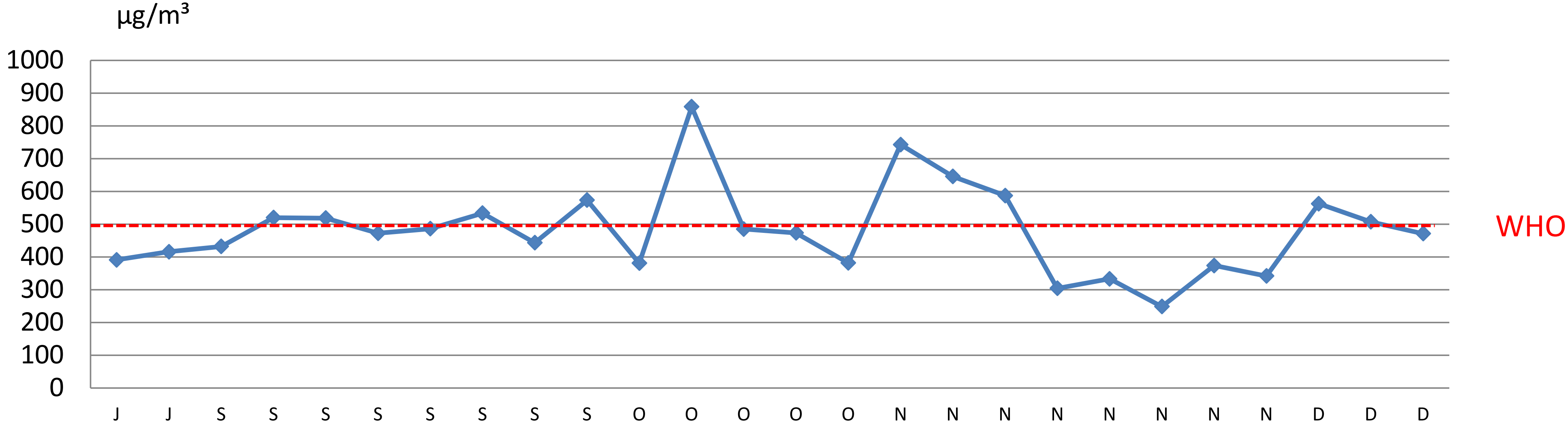
Font-Ribera 2016 Environ Res

Trichloamine in air (PISCINA 2)

N=26 days Mean=480 µg/m³ (SD=133.5)

Range= 248 - 858.3

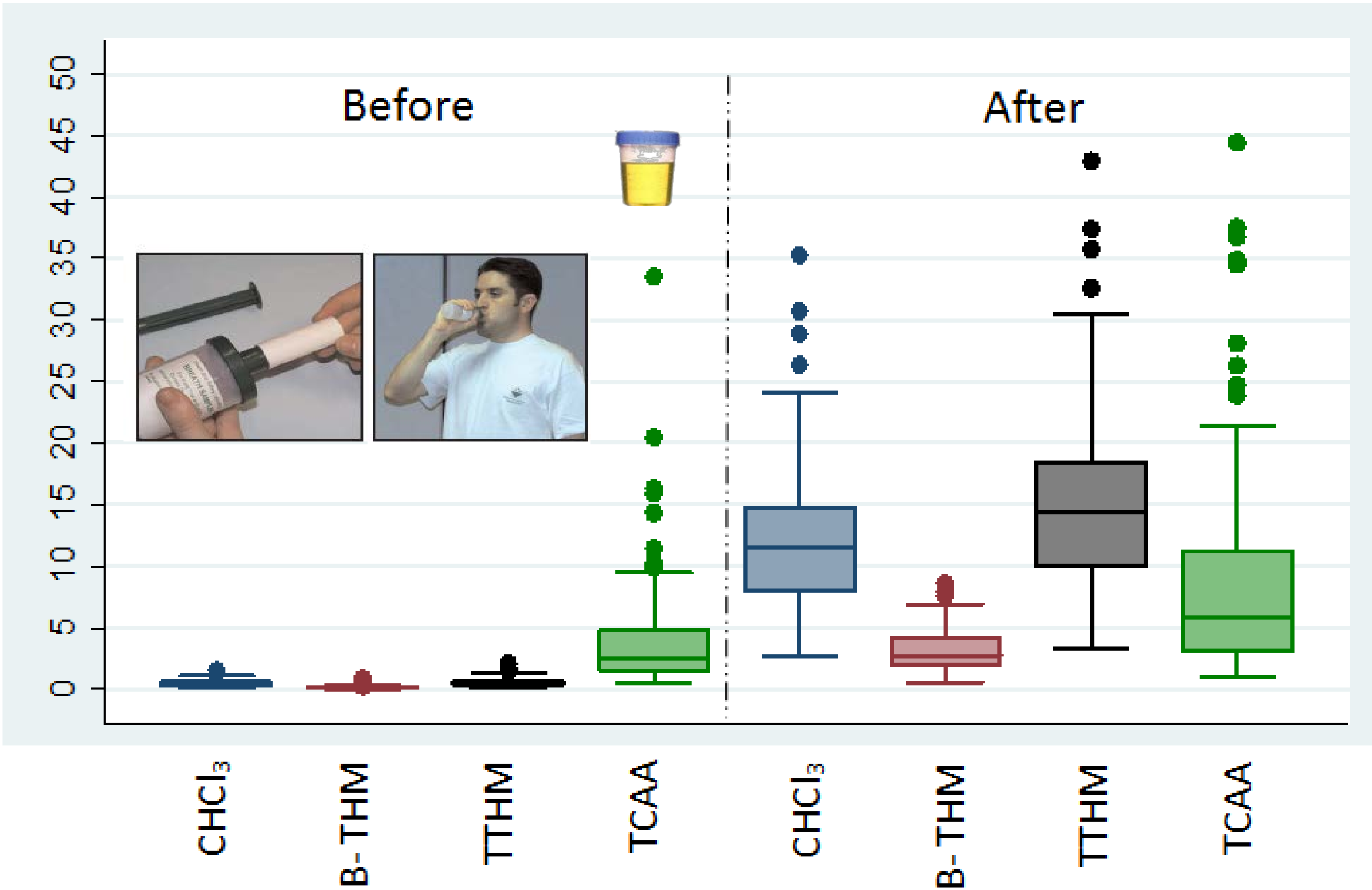
In PISCINA-1, 6 days measured:
mean= 290 µg/m³
Range= 170 – 430



As$_2$O$_3$ impregnated filters - IRAS

Internal dose biomarkers (PISCINA 2)

THM in exhaled breath (µg/m³) and creat. adj. TCAA in urine (µmol/mol)

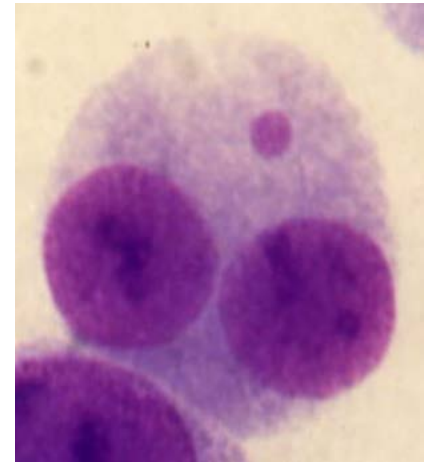


Spearman correlation

	Median (IQR)	Chloroform exhaled breath	B-THM exhaled breath	C. adj TCAA urine
Number of pools	42 (34-49)			
Distance swam (km)	1.0 (0.85, 1.22)	0.26**	0.21*	-0.32*
Energy expenditure (Kcal)	204.5 (166.9-254-6)	0.27**	0.24**	-0.33**
Active time (min)	35.3 (29.8-38.7)	0.05	0.14	0.03

* pvalue<0.05 **pvalue<0.01

MN in lymphocytes (pre - 1 hour post) N=115



MN per 1000 binucleated lymphocytes

	mean	pvalue
Before	4	
1h after	4	
Difference	0	0.185

Correlation between change in MN and increase in exposure

	Spearman ρ	95%CI
Chloroform breath	-0.16	-0.35, 0.04
Bromodichloromethane		
Dibromochloromethane		
Bromoform		
TTHM breath	-0.15	-0.34, 0.05
Br-THM	-0.05	-0.25, 0.14
TCAA urine	-0.02	-0.21, 0.17
Kcalories	0.0003	-0.19, 0.18

Villanueva CM 2019

MN in reticulocytes (pre - 4 days post) N=19

- Assay performed in the second half of the study sample (Oct-Dec 2013)
- 47.7% with undetectable isolated cells
- 19 subjects with detectable levels in both pre and post sample
- Mean number of cells counted = 27,251.2 / sample

MN per 1.000 reticulocytes

	median	pvalue
Before	0.9	
4 days after	1.1	
Difference	0.4	0.277

Villanueva CM 2019

MN in reticulocytes (pre - 4 days post) N=19

Spearman correlation coefficient between change in MN and increase in exposure:

	ρ	(95%CI)
Chloroform breath	**0.50**	**0.07, 0.93**
Brominated THM breath	**0.55**	**0.13, 0.97**
TTHM breath	**0.56**	**0.14, 0.97**
TCAA urine	**-0.59**	**-0.94, -0.24**
Distance swam	-0.01	-0.47, 0.45

Villanueva CM 2019

Serum CC16

Serum CC16 (ng/mL)	N=105	
	Median	pvalue
Before	11.1	
1h After	10.5	
Difference	-0.5	0.289

Villanueva CM 2019

Serum CC16

Correlation between change in CC16 and increase in exposure (N=105):

	Spearman ρ (95%CI)
Trichloramine air	-0.03 (-0.18, 0.25)
Cl3CH breath	-0.01 (-0.20, 0.18)
BDCM breath	
DBCM breath	
Br3CH breath	
TTHM breath	-0.01 (-0.21, 0.18)
Br-THMs	-0.05 (-0.25, 0.14)
Kcalories	

Urine mutagenicity (n=88)

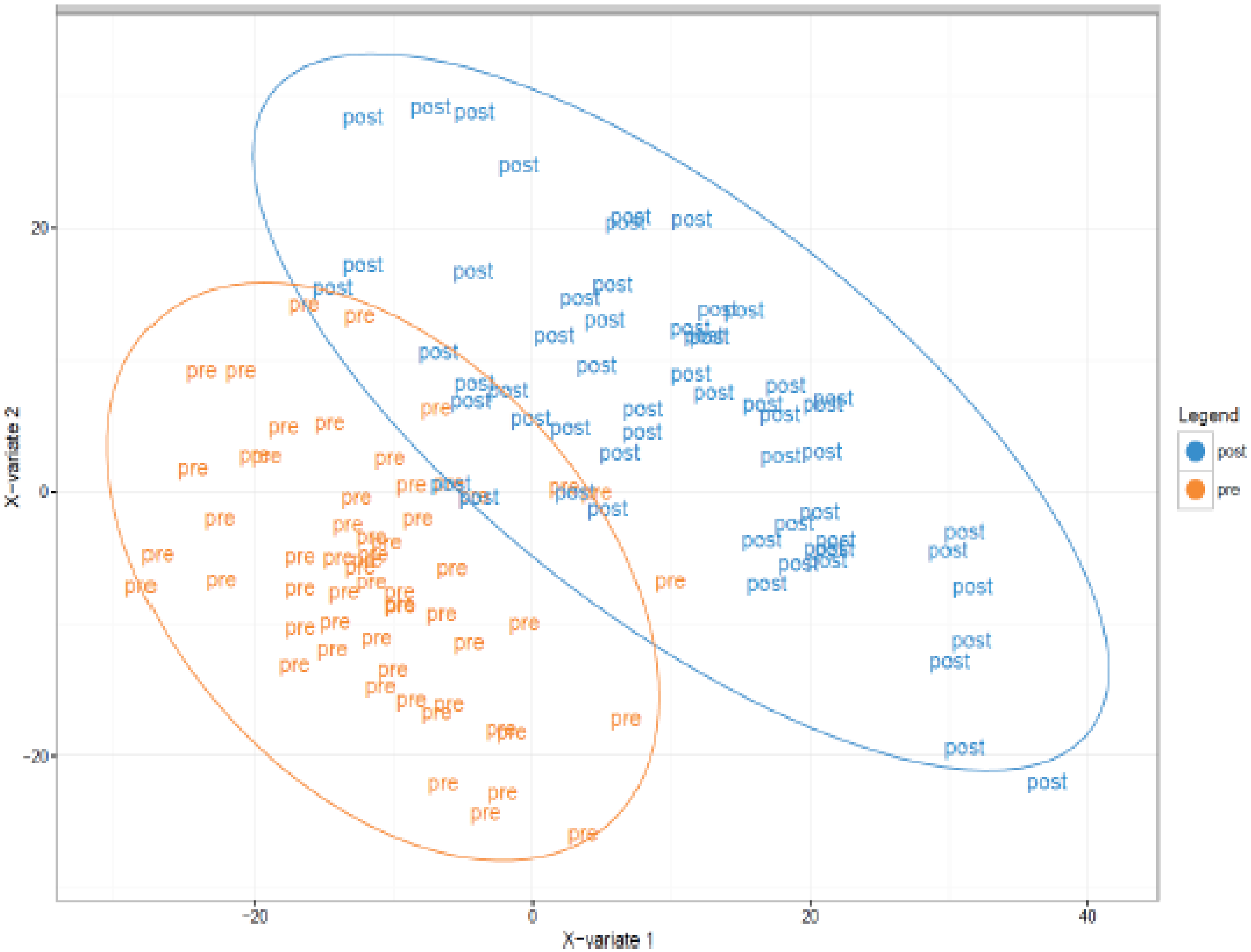
Salmonella YG 1024 + S9

Correlation between change in mutagenicity and an IQR increase in exposure:

	Spearman ρ (95%CI)	**PISCINA-1**
Cl3CH breath	-0.13 (-0.33, 0.08)	0.33 (-0.22, 0.89)
BDCM breath		0.61 (-1.12, 2.35)
DBCM breath		0.92 (-0.75, 2.59)
Br3CH breath		**5.27 (1.80, 8.75)**
TTHM breath	-0.14 (-0.36, 0.07)	0.24 (-0.11, 0.58)
Br-THM	-0.14 (-0.36, 0.07)	

Villanueva CM 2019

Metabolomic pathways suggest potential mechanisms by which swimming in chlorinated water may affect health



PLS-DA score plot of the plasma metabolic profiles obtained from volunteers PRE (red) and POST (blue) the experiment

(Karin van Veldhoven, Env Int 2018)

Acute changes in serum immune markers due to swimming in a chlorinated swimming pool

- Significant decrease from before to after swimming in serum concentrations of :
  - IL-8 (−12.53%; q =2.00e-03),
  - CCL22 (−7.28%; q =4.00e-04),
  - CCL11 (−7.15%; q =9.48e-02),
  - CRP (−7.06%; q =4.68e-05)
  - CXCL10 (−13.03%; q = 6.34e-14)
- Significant increase:
  - IL-1RA (20.16%; q =4.18e-06).
- Associations with quantitative measurements of DBPs or physical activity were similar in direction and strength.

(Jelle Vlaanderen, Environ Int 2017)

Some concluding ideas

- Swimming in pools is related to adverse health outcomes in specific populations (e.g. pool workers, elite swimmers)

- Among the general population, swimming in pools involve exposure to potentially harmful chemicals (DBPs) but also to the positive effects of physical exercise

- Recommendations should be tailored to specific target populations

- Reduction of brominated THMs have shown to reduce biological responses in swimmers at the molecular level (genotoxicity, lung permeability)

- Most studies are focused on chlorinated pools. There is limited scientific evidence on alternative disinfection methods.

PISCINA2 - PARTNERS

	Center	Role in the study
Christian Zwiener	Univ. Tübingen	THM, HAA, HAN, HK water
Joan Grimalt, Esther Marco	IDAEA-CSIC, Barcelona	THM exhaled breath
Dick Heederik, Jack Spithoven	Univ. Utrecht	Trichloramine air
Bill Mitch	Stanford University	NDMA water
Xiangru Zhang, Jiaqi Liu	Hong Kong University	TOx water
Susan Richardson, Cristina Postigo	Univ. South Carolina	DBP screening water
David DeMarini	EPA, US	Mutagenicity water
Michael Plewa	Univ. Illinois	Cytotoxicity water
Tamara Grummt, Ralf Junek	German FEA	Mutagenicity urine
Susana Pastor, Ricard Marcos	UAB, Barcelona	Micronuclei PBL
Lilliane Abramsson	NFA, Uppsala	Micronuclei reticulocytes
Marie Pedersen	DCRC, Denmark	MN assessment
Esther Barreiro	IMIM, Barcelona	CC16 serum
Dinesh K. Barupal	IARC, Lyon	Metabolomics, lab
George Preston, David Philips	KCL, London	Adductomics, lab, stat analysis
Almudena Espín, Jos Kleinjans	Maastricht Univ.	Transcriptomics, lab, stat analysis
Alessio Naccarati	HuGeF	Genotying
Paolo Vineis, Karin van Veldhoven	ICL, London	EXPOsOMICS PI, metab. Stat. analysis
Laia Font, **Cristina Villanueva**, Lourdes Arjona, Jeroen de Bont, Lluïsa Tarès, **Manolis Kogevinas**	CREAL, Barcelona	Design, field work, stat. analysis, coordination

Manolis Kogevinas

Laia Font

expos omics

www.isglobal.org

Una iniciativa de:

Obra Social "la Caixa"

CLÍNIC BARCELONA Hospital Universitari · Parc de Salut MAR · UNIVERSITAT DE BARCELONA · upf. Universitat Pompeu Fabra Barcelona · Generalitat de Catalunya · GOBIERNO DE ESPAÑA · Ajuntament de Barcelona · FUNDACIÓN RAMÓN ARECES